Emerging technologies for safe, healthy and sustainable fresh-cut produce

A Ph.D. dissertation presented by

Alexandra Ignat

to the

University of Udine

for the degree of Ph.D. in the subject of

Food Science (Cycle XXVII)

Department of Agricultural, Food, Environmental and Animal Sciences

UNIVERSITY OF UDINE

Italy

March 2016

Coordinator:

Mara Lucia Stecchini, Professor
Department of Agricultural, Food,
Environmental and Animal Sciences
University of Udine, Italy

Supervisors:

Maria Cristina Nicoli, Professor
Department of Agricultural, Food,
Environmental and Animal Sciences
University of Udine, Italy

Lara Manzocco, Professor
Department of Agricultural, Food,
Environmental and Animal Sciences
University of Udine, Italy

Reviewers:

James G. Lyng, Professor
School of Agriculture and Food Science
University College Dublin, Ireland

Giuseppe Muratore, Professor
Department of Agricultural and Food Science
University of Catania, Italy

Amalia Conte, PhD
Department of the Sciences of Agriculture, Food and Environment
University of Foggia, Italy

I declare that my PhD thesis has been amended to address all the Referee's comments.

Acknowledgments

This PhD thesis is the final step of a long journey shared with someone who believed in me, with my ups and downs. I really thank Prof. Maria Cristina Nicoli and Prof. Lara Manzocco for these years.
Thanks to Prof. Monica Anese and Dr. Sonia Calligaris for sharing their time and work with me.
I am grateful to Dr Michela Maifreni for the opportunity to join the Laboratory of Food Microbiology and to Dr Francesca Frigo and Dr Ingrid Bartolomeoli for their huge support.
Most of the work regarding high pressure carbon dioxide had the important contribution of Prof. Sara Spilimbergo and I thank her for the scientific support and the possibility to perform initial trials at the University of Trento.
I would also like to thank Prof. Giovanna Lippe and Dr Astrid Fabbro for the great support to perform electrophoresis and interpret data.
Special thanks go to Prof. James Lyng and Dr. Nigel Brunton for welcoming me in their group during my visiting period at the UCD School of Agriculture and Food Science.
Finally, this research and my training activities were possible thank to the support of Fondazioni in Rete per la Ricerca Agroalimentare, "AGER - Agroalimentare e Ricerca". Project 2010 2370.

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List of notations

List of abbreviations

Abs	Absorbance
AEW	Acidic Electrolyzed Water
DL	Detection limit
EW	Electrolyzed water
HP-CO_2	High pressure carbon dioxide
NEW	Neutral Electrolyzed Water
ORP	Oxidation Reduction Potential
PEF	Pulsed electric fields
PPO	Polyphenoloxidase
SD	Standard Deviation
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
UV	Ultraviolet light
UV-C	Short-length ultraviolet light

List of symbols

δ	Penetration depth
D_p	Decimal reduction time
I	Light irradiance inside wastewater
I_0	Irradiance of incident light at wastewater surface
k	Apparent inactivation rate constant
Q	Total energy
z_p	Pressure sensitivity

Preface

In the last decade the food market has experienced the establishment of products meeting consumer needs of healthy commodities with improved fresh-like appearance and convenience. In this context, fresh-cut fruit and vegetables combine their fresh-like and healthy characteristics with a minimal time of preparation before consumption. Fresh-cut produce is thus a growing sector and there are still large opportunities for further development and improvement both at industrial level and in market shares. Appropriate solutions could overcome the critical aspects regarding product safety, quality and shelf life, production costs and environmental impact. In particular, suitable techniques should be employed to increase fresh-cut produce safety, affected by both chemical and microbiological contaminations. In addition to the latter, plant senescence together with enzymatic reactions should be controlled in order to guarantee product quality and extend the shelf life. Furthermore, proper technologies could be exploited to reduce the production costs and the environmental load of these produce. To this regard, process optimization as well as the implementation of novel technologies, could decrease energy, water and chemical compounds consumption, making fresh-cut production more sustainable in terms of economical, ethical and environmental aspects.

Driven by these needs from the fresh-cut production chain, a partnership among 8 Italian universities and research centres investigated the entire fresh-cut production chain *from farm to fork*. The project "AGER 2010 2370 Stayfresh: Novel strategies meeting the needs of the fresh-cut vegetable sector" aimed to find out integrated solutions able to optimize and innovate the fresh-cut produce from the agronomic aspects up to the ready-to-eat product.

This PhD thesis is therefore part of the abovementioned wide project and aimed to investigate whether selected novel non-thermal technologies can be exploited to enhance safety, quality and sustainability of fresh-cut produce, such as lettuce, apple and potato. The research was divided in two parts and the attention was focused on two processing steps, namely washing and stabilization treatments, which are critical in fresh-cut production (Figure 1). In the first part of the work, the application of

promising novel technologies such as electrolyzed water and light technologies (i.e. UV-C and pulsed light) were studied as alternatives to highly chlorinated solutions to avoid cross-contamination during fresh-cut lettuce washing, guaranteeing product hygienic quality as well as the reduction of chlorinated compounds, water use and wastewater discharges. The second part addressed to fruit and vegetables stabilization by studying the effect of light technologies, dense phase carbon dioxide and pulsed electric fields. The latter were considered as potential techniques for microbial decontamination, enzymatic inactivation and quality enhance of fresh-cut lettuce, apple and potato. In particular, the experimental work regarding the application of pulsed electric fields on potato cubes was performed at the Laboratory of Food Technology of the UCD School of Agriculture and Food Science, Dublin, Ireland.

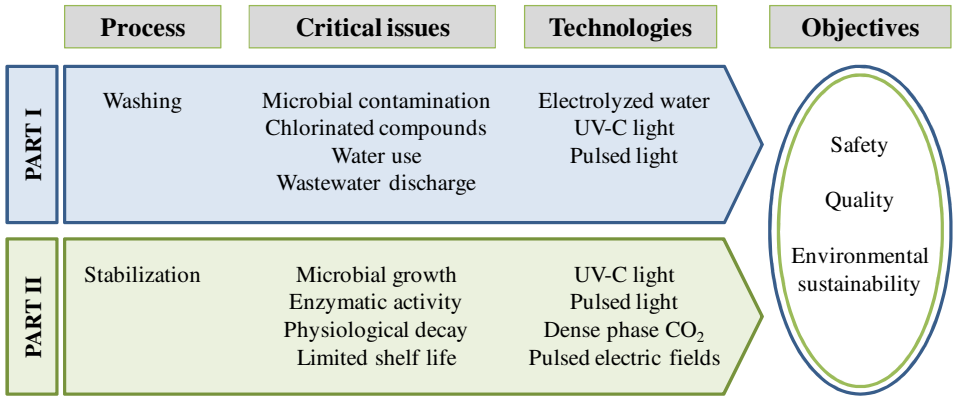

Figure 1. Overall research strategy of this PhD thesis.

Chapter 1 Introduction

1.1 Fresh-cut fruit and vegetables

Fresh-cut products are defined as any fresh fruit and vegetable that has been washed, trimmed, peeled and/or cut into 100% edible product and subsequently packed and stored under refrigeration conditions (IFPA, 2005). During the last decade, the market of fresh-cut fruit and vegetables has experienced a remarkable growth due to consumer preference for healthy, fresh-like and ready-to-eat products. Consumers are moving towards convenient and fresh ready-to-eat products, in the attempt to meet the objective of consuming vitamins, minerals and fiber through a daily intake of at least 400 g of vegetables, as recommended by World Health Organization (WHO, 2008; Freshfel, 2013). Fresh-cut sector had thus become a multi-billion dollar market with an estimated $27 billion sales in the sole US (PMA, 2014). The overall consumption regards packaged salad (61%), other fresh-cut vegetables (27%) and ready-to-eat fruit (12%).

Although this market is considered well-established, some weakness and concerns about fresh-cut produce still need to be addressed. In particular, microbial contamination can represent a serious risk for consumers since fruit and vegetables are only subjected to minimal processing treatments. The latter may be also not sufficient to ensure quality during storage, limiting fresh-cut product shelf life which difficultly exceeds 7-10 days. In addition, consumers are becoming more and more aware about the environmental impact of their food choices, so that industry should meet their demand for sustainable products. On the basis of these consideration, an overview on safety, quality and environmental issues regarding fresh-cut produce is presented.

1.2 Safety issue

Microbial contamination of fresh-cut products can occur during any of the steps in the farm-to-consumer chain and can derive from environmental, animal or human sources (WHO/FAO, 2008). Typical transmission route in primary production are water, soil and manure and it has been demonstrated that contaminated bacteria have the ability to internalize

and grow in plant tissue (Hou et al., 2013). These circumstances can lead to raw vegetables having high initial contamination levels which can easily exceed 6 Log of viable microorganisms (Alegria et al., 2010). Furthermore, the operations required for fresh-cut vegetables production as cutting, slicing or peeling cause tissue damage which release nutrients and facilitate microbial growth with effects on safety and quality during storage (Harris et al, 2003; Ragaert et al., 2007). In particular, microorganisms can easily gain access to the surface of the fresh-cut produce and, in the absence of adequate sanitation processes, they can reach the infective dose, becoming the cause of food poisoning outbreaks. Although the wide application of GAP (Good Agricultural Practices) and HACCP (Hazard Analysis and Critical Control Points) systems, documented cases of foodborne illness are currently increasing. Illness outbreaks associated with the consumption of ready-to-eat vegetables were mainly attributed to the presence of pathogens such as *Listeria monocytogenes, Salmonella* spp., *Escherichia coli* and *Shigella* spp. in fresh-cut lettuce, melon and alfalfa sprouts among others (CDC, 2011; EFSA, 2011; FDA, 2013). Further causes of disease can be attributable to fresh-cut products contaminated with viruses, such as hepatitis A and norovirus, or parasites as *Cryptosporidium* spp. and *Cyclospora* spp. (EFSA, 2013).

Microbiological criteria for fresh-cut produce have been included in several regulations making a step forward for food safety. For instance, European regulation 1441/2007 established the absence in 25 g for *Salmonella* spp. and set the maximum at 100 CFU/g for *E. coli* and *L. monocytogenes,* while, for the latter, US and Canadian legislation introduced a zero tolerance (Ragaert et al., 2011).

Preservation of fruit and vegetables intended for fresh-cut produce is accomplished by minimal processing, to replace traditional thermal methods in order to retaining nutritional and sensory quality. However, while conventional processing efficaciously guarantee product decontamination and extend the shelf life of vegetables, a single minimal processing difficultly can ensure product safety. The common strategy to assure the safety of fresh-cut vegetables is the combined use of different barriers to delay the microbial growth by means of “hurdle technology” (Leistner, 2000). In particular, post-harvest washing of fresh produce can

be an important measure to reduce pathogen contamination, followed by packaging in modified atmosphere and refrigerated storage to decrease microbial growth. Microbial load reduction is thus achieved by using washing solution containing various chemical sanitizers. The latter are chlorine-based (e.g. sodium hypochlorite, chlorine dioxide) or chlorine-free (e.g. ozone, peracetic acid, organic acids) compounds and are used primarily to avoid cross-contamination in washing tank rather than completely remove vegetable contamination (Allende et al., 2008; Artés et al., 2009; Siroli et al., 2015). To this regard, Allende et al. (2008) demonstrated that, independently on the presence or not of chemical sanitizer, an average bacterial reduction of 1-2 Log is generally achieved upon washing. This is due to the sole mechanical removal of microorganisms from vegetable surface by water turbulent flow. In addition, Danyluk and Schaffner (2011) estimated that when a pathogen as *Escherichia coli* O157:H7 is present on 0.1% of product samples at harvest, cross-contamination at washing in the absence of any sanitizer could be responsible for up to 99% of illness caused by the affected product.

To meet fresh-cut industry demand for an effective antimicrobial treatment to limit the risk associated with pathogen contamination, research has moved towards novel non-thermal technologies. Promising bactericidal effects on fresh-cut fruit and vegetables were obtained by applying UV-C light, irradiation and cold plasma (Fan, 2010; Gómez et al., 2010; Manzocco et al., 2011a; Ziuzina et al., 2015). The latter could provide a killing step to enhance safety of fresh-cut produce, but their efficacy is tightly linked to the type of contamination, vegetable characteristics and treatment parameters. In addition, the antimicrobial treatment should not damage the delicate and fragile structure of the product, impairing its fresh-like appearance and reducing the shelf life.

In addition to microbial risk, other aspects related to the safety of fresh-cut produce include the potential presence of external chemical contaminants, naturally occurring toxic compounds and foreign bodies (Francis et al., 2012). The potential presence of foreign bodies, such as insects or material of human origin as glass or plastic, is a very important issue in the fresh-cut produce industry. In addition, chemical contaminants from external origin may include residues of pesticides and

other pre-harvest and/or postharvest chemicals. Finally, degradation of sanitizing agents (i.e. chlorine) may lead to the accumulation of chlorinated by-products which are recognized as carcinogenic (Legay et al., 2010).

1.3 Quality issue

Quality of fresh-cut fruit and vegetables determines the value to the consumer and is a combination of parameters including appearance, texture, flavour and nutritional value (Kader, 2002). Quality parameters are closely related to physiological changes that occur in the vegetable tissue. In fact, any stress and wound caused to a living tissue induces a cascade of metabolic reactions that can result in texture changes, accelerated ripening and senescence, water loss, off flavour formation, enzymatic browning, discoloration, susceptibility to microbiological spoilage and other undesirable events that can limit the shelf life and render the product unmarketable (Toivonen and De-Ell, 2002). Further handling and processing to produce fresh-cut vegetables increase ethylene production which results in genetic signals that promote ripening and senescence (Karakurt and Huber, 2007). Processing also increases the respiration rate, using up sugar and acid substrates and shortening shelf life.

As previously mention, consumers take product appearance, in particular colour, into consideration as a primary criterion for food choice, preference and acceptability (Kays, 1999). Colour is one of the main attributes, along with texture, that characterises the freshness of most vegetables. Depending on the product, fresh-cut fruit and vegetables can undergo changes in colour due to different biochemical processes, mainly chlorophyll degradation, enzymatic browning due to polyphenoloxidase (PPO) and phenol peroxidase (POD) activity, carotene degradation and whiteness associated with lignin formation (Howard et al., 1994; Martinez and Whitaker, 1995; Cisneros et al., 1997; Ihl, et al., 2003; Degl'Innocenti et al., 2005). These effects are attributable to product wounding which produces a signal that migrates through the tissue and induces the synthesis of enzymes and metabolites which act in the defence of plant tissue from microbial and oxidative damage.

Texture is also an index of freshness and quality decline and is very tightly linked to tissue deterioration (Cantwell and Suslow, 2002). Consumers expect fresh-cut products to maintain firm and crunchy texture while tissue softening is one of the most important factors limiting shelf-life (Fillion and Kilcast, 2002; Karakurt and Huber, 2003). Firmness losses in fruit and vegetables are primarily caused by enzymatic degradation of pectins catalyzed by pectin methylesterase (PME) and polygalacturonase (PG) (Karakurt and Huber, 2003). In addition, wounding and any other stress induced to the product, result in increased ageing processes, senescence and water losses causing texture quality decay (Escalona et al., 2003).

Senescence is also responsible for flavour and nutrient compounds loss during fresh-cut storage (Forney, 2008; Beaulieu, 2011). Flavour volatile as alcohols, aldehydes, esters, ketones, lactones, terpenes and other compounds and nutrients such as vitamins, carotenoids or polyphenols usually degrades rapidly and can be used as an index of product freshness (Beaulieu, 2011).

Aside from physiological modification of vegetable tissue, naturally occurring spoilage bacteria in the product may also lead to quality changes and impair shelf life. In fact, where the protective peel of fresh produce has been removed or on cut and damaged product surfaces, nutrients are more available for microbial activity. Thus, spoilage microorganisms are able to grow and impair product quality (King and Bolin, 1989). In particular, spoilage activity is characterized by different processes such as production of enzymes and metabolites, resulting in visual and textural defects and off-odours. With regard to visual defects, different publications mention that these defects become visible from a microbiological count of 8 Log CFU/g (Li et al., 2001; Giménez et al., 2003). Composition of fruit and vegetables will also determine the type of spoilage. In the case of sugar-rich products such as carrots, bell peppers and most of the fresh-cut fruits, growth of yeasts and lactic acid bacteria is favoured, resulting in off-odours caused by microbial proliferation and the production of organic acids, ethanol and other organic compounds (Barry-Ryan and O'Beirne, 1998; Jacxsens et al., 2003; Ragaert et al., 2006). Moulds can also determine quality decay, resulting in visual

spoilage before yeasts or bacteria can produce off-odours (Nielsen and Leufven, 2008).

Products with lower sugar concentrations such as leafy vegetables, will mainly be spoiled by soft-rot bacteria such as *Pseudomonas* spp. resulting in both textural and visual defects, although these bacteria could also produce metabolites, resulting in off-odours as described above. Texture degradation is attributable to different microorganisms producing pectinolytic enzymes degrading the pectin molecule in the middle lamella and the primary cell wall (Artés et al., 2007). The most frequently isolated pectinolytic bacteria are species of *Erwinia* and *Pseudomonas* which are psychrotrophic and grow in the refrigeration conditions used in fresh-cut produce storage. Based on the extensive evidence that spoilage bacteria highly influence quality and shelf life of fresh-cut fruit and vegetables, Ragaert et al. (2011) proposed specific microbiological guidelines. In particular, when total aerobic psychrotrophic count and moulds and yeasts exceed 10^8 and 10^5 CFU/g respectively, notable spoilage occurs and the product will be rejected.

On the basis of the quality issues described, many processing techniques have been developed to counteract the reactions of the cut produce to ethylene production and other wound responses and extend the shelf life of these very perishable products. To reduce respiration and ethylene production and retard ripening and senescence, modified atmosphere packaging (MAP), use of ethylene absorbents as well as the addition of 1-methylcyclopropene (1-MCP) were applied (Artés and Allende, 2005; Vigneault et al., 2008; Toivonen, 2008). Surface treatments and edible coatings were used to reduce water loss, dehydration, browning and firmness modifications (Baldwin et al., 1995; Martin-Diana et al., 2007; Vargas et al., 2008). Finally, various sanitation or decontamination procedures were used not only against pathogens but also to reduce spoilage contamination (Artés et al., 2007; Gómez-López et al., 2009).

As already considered in the case of the safety issues, novel non thermal technologies could also be used to improve fresh-cut quality, acting on the physiological and biochemical aspects which influence product decay and shelf life. However, available data on the effect of emerging technologies (e.g. UV-C and pulsed light, irradiation, cold plasma and pulsed electric fields among others) on quality attributes of fresh-cut

products are still limited. Thus, the most crucial challenge of novel technologies is to optimize the processing conditions to extend shelf-life and guarantee safety of fresh-cut products, while assuring appropriate quality.

1.4 Environmental issue

Food safety and quality issues aside, food manufacturers and consumers, are also increasingly aware of the environmental impact of food production and consumption. Consequently, the demand for products produced in an environmentally friendly fashion has been increasing (Imkamp, 2000). Even if the main food environmental load arises in the primary production stage through factors such as land usage, water consumption and pollution, an improvement towards environmental friendly productions at industrial level can be pursued. Considering that within the EU, food manufacturing is the second largest industry, its environmental impact is significant and the activities of the food industry can degrade the environment in different ways, including the overuse of resources such as water, energy, packaging materials and the generation of food waste, wastewater and greenhouse gases (EC, 2011; Reisch et al., 2013). In addition, keeping into consideration that the adoption of new consumption habits as out-of-home and ready-to-eat products accounts for a significant proportion of food intake in developed countries, these products have a clear impact on the environment (Saarinen et al., 2012).
Thus, in recent years there has been a growing interest in sustainable manufacturing in the effort to move towards a green and resource-efficient global economy (UNEP, 2011). In this context, fresh-cut produce manufacturing may benefit from the implementation of sustainable strategies to better use resources such as energy and water. The rise in industrial energy price over the past decade and interest in environmental sustainability has seen many food manufacturers focusing on energy reduction as a top priority. There are various options open to food processing factories via the energy reduction, energy efficiency and renewable energy (Miah et al., 2015). To this regard, the adoption of novel technologies, not only can satisfy consumer demand for minimally processed foods with enhanced safety and quality attributes,

but have been also proven to be energy-saving and consequently reducing greenhouse gases emissions (Wang, 2014). In addition, preservation methods as UV-C light or pulsed electric fields among others, were demonstrated not to generate contaminants and thus have the potential to be considered clean technologies (Pereira and Vicente, 2010).

Among the environmental problems linked to food production and manufacturing, water is known to be a critical issue. In the food sector, the fresh-cut industry is one of the major water-intensive with washing being the sole technological step to remove dirt and decrease microbial load of vegetables intended for fresh-cut produce. Water consumption and wastewater volumes are generally in the range of 2-11 m^3/t and 11-23 m^3/t of fresh-cut product, respectively (FDM-BREF, 2006; Ölmez, 2014). These huge amounts of water are mainly discharged to surface water and make the fresh-cut industry difficultly fitting with nowadays global water scarcity and the stricter water regulations. Nowadays, to accomplish the requirements of water saving, water used for vegetables washing is used in multiple washing cycles. However, wash water may become not only a vehicle for spoilage microorganisms but also for pathogenic ones. The risk of cross-contamination is conventionally reduced by adding disinfection chemicals, such as chlorine and its related compounds to decrease the concentration of water-borne pathogens below the regulatory limits (Codex Alimentarius Commission, 2001; EC, 1998; Gil et al., 2009; Sommer et al., 2000). However, concern is growing about the possibility of chlorine reacting with organic matter and beget carcinogenic and/or mutagenic by-products such as trihalomethanes and haloacetic acids (Krasner et al., 2006; Richardson and Ternes, 2005). The minimization of water and chemical use and wastewater discharges are thus big challenges for the fresh-cut industry that will be increasingly required to implement sustainable strategies for water saving (Ölmez and Kretzschmar, 2009; Gómez-López et al., 2013).

The fresh-cut sector as well as the entire food industry are thus facing new issues that can stimulate the adoption of strategies and technologies able to guarantee a sustainable and cost-effective improvement of their productions. In order to be more eco-efficient, the European Commission promotes a Sustainable Consumption and Production action plan (EC, 2012) which fosters to standardize voluntary and mandatory tools to

reduce environmental load of products throughout their entire life cycle. In particular, the adoption of Life Cycle Assessment (LCA) method can support food manufacturers in making decisions concerning sustainable alternatives for production and industrial processes (ISO 14040:2006). This tool evaluates the environmental footprint of a product "from cradle to grave", considering a number of impact categories such as water footprint, energy use, global warming potential, ecological footprint and many others (Ruini et al., 2013). Fresh-cut manufacturers may thus use this tool to identify improvement options in relation to water and energy issues associated with the production of ready-to-eat vegetables.

Accordingly, consumers need to be able to identify and distinguish products which are more respectful with the environment from those which are not and producers are motivated to differentiate those environmental friendly products. To this regard, LCA method and its results can be used to create sustainability labels which give the consumers the opportunity to take into account environmental and ethical aspects when making food choice. For instance, data relevant to energy consumption and relative greenhouse gases emission allow the creation of carbon footprint label as well as water management data are translated into water footprint label. In conclusion, eco-labelling and environmental information can help creating a system of production and consumption that is more environmental friendly and that means creating a greener market (Peano et al., 2015).

Chapter 2 Materials and Methods

2.1 Sample preparation

Electrolyzed water solutions

Electrolyzed water solutions were generated using an Envirolyte Demonstration Unit (mod. Dem-30, Envirolyte Industries International Ltd., Tallin, Estonia) having two platinum–iridium coated titanium electrodes separated by an yttria-zirconium membrane. The EW solutions were produced at room temperature using a continuous supply of NaCl (Carlo Erba, Milano, Italy) aqueous solution at increasing concentration up to 3.4 g/L. Neutral electrolyzed water was collected after electrolysis without the use of the separation membrane. When membrane separation was performed, Acidic electrolyzed water and alkaline solutions were collected at the anode and cathode side, respectively. Electrolyzed water pH adjustment was carried out with 0.1 M HCl (Sigma Aldrich, Milano, Italy) and 0.1 M NaOH (Sigma Aldrich, Milano) for NEW and AEW respectively.

NaOCl aqueous solutions (Titolchimica, Rovigo, Italia) at increasing concentration of free chlorine and pH adjusted to 6.5 with HCl (0.1 M, Sigma Aldrich) were also prepared as control.

Salad and wastewater

Lamb's lettuce, provided by a local farm, was used. Salad was stored at 6 °C and processed within 1 day. Aliquots of 100 g of Lamb's lettuce were washed in tap water (0.3 mg/L free chlorine), electrolyzed water or NaOCl aqueous solutions at 8 °C for 2 min. The salad/water ratio during washing was 1:10 (w/v). Lamb's lettuce leaves were manually separated from wastewater and centrifuged (mod. ACX01, Moulinex, Ecully, France) for 1 min. Water drained from leaves by centrifugation was added to the previously collected wash water.

Wastewater was then treated with UV-C or pulsed light and reused for up to 5 washing cycles. In each cycle a new batch of lettuce was washed with UV-C or pulsed light treated water to simulate industrial recycling of wastewater during salad washing. Water and lettuce samples were immediately used for analysis.

Apple slices

Raw apples (*Malus domestica* Borkh., var Golden Delicious) were purchased at the local market and maintained at 6 °C until use. Apples were washed in potable water (0.3 mg L^{-1} residual chlorine), wiped, cored and manually cut into 1 cm thickness slices. During handling operations the hygienic conditions were strictly preserved. Apple slices were firstly submitted to pulsed light treatment and then introduced in sterile Petri dishes and stored in darkness at 6 °C for up to 7 days. At increasing times during storage, samples were removed from the thermostated cell and submitted to the analyses.

Polyphenoloxidase solution

Mushroom tyrosinase (polyphenoloxidase, PPO) (T3824 50KU, Sigma, St. Louis, MO, USA), from *Agaricus bisporus* was used. PPO solution was prepared diluting the enzyme in 0.1 M potassium phosphate buffer pH 7.0 (Carlo Erba, Milano, Italy). PPO initial activity on 1.5×10^{-3} M L-Dopa (Carlo Erba, Milano, Italy) was 0.004 Abs min^{-1}. PPO solution was immediately treated by HP-CO_2.

Apple juice

Raw apples (*Malus domestica* Borkh., var Golden Delicious) were purchased at the local market and maintained at 6 °C until use. Apples were washed, wiped, cored and centrifuged by using a domestic centrifuge (Moulinex, Groupe SEB, Ecully, France). Such operations were carried out at 4 °C. The juice was then clarified by centrifuging for 5 min at 5000 rpm at 4 °C (Beckman Avanti J-25 Beckman Instruments Inc., Palo Alto, CA, USA). Apple juice was immediately submitted to HP-CO_2 treatments.

Potato cubes

Raw potatoes (*Solanum tuberosum*, var. Rooster) were purchased at a local supermarket and stored in the dark at room temperature for a maximum of 7 days. Potatoes were manually peeled, washed in tap water, drained with paper and cut into 2×2×2 cm cubes (8.5 g). Potato cubes were immersed in tap water at 20 °C prior to PEF processing, which occurred within 20 min.

Fried potatoes

A commercial deep-fat fryer (Cookworks, Argos Ltd, Milton Keynes, United Kingdom) was used for the frying tests. Untreated, blanched and PEF treated potato cubes were par-fried in sunflower oil at 190 °C for 1 min (potato-oil ratio 1:40, w/w). The frying sieve was shaken to remove the excess oil and the potato cubes were drained for few seconds on paper. After cooling at room temperature for 10 min, potato cubes were frozen in an air blast freezer (Mod. FT36, Armfield, Hampshire, United Kingdom) at -30 °C for 30 min. Samples were then packed in plastic bags (Mod. 021ODC681, Webomatic, Bochum, Germany) and stored at -18 °C in a thermostated cell for 7 days. Potato cubes were then finish-fried for up to 4 min adopting the same procedure previously described.

2.2 Non-thermal technologies

UV-C light treatments

UV-C treatments of wastewater and lamb's lettuce were carried out at 6 °C into a thermostated cell (Climacell 222, MMM Medcenter, Einrichtungen GmbH, Graefelfing, Germany) equipped with up to six 15 W lamps (TUV G15 T8, Philips, Amsterdam, The Netherlands) with maximum emission at 253.7 nm. In particular, aliquots of 10 mL of wastewater were placed in 5.5 cm diameter sterile Petri dishes to get a 4 mm thick-sample. Petri dishes were placed at 5 cm distance from the lamp corresponding to 20 W/m^2 UV-C light irradiance on wastewater surface. Samples were exposed to UV-C light for increasing time up to 60 s. Relevant fluence on the samples was equal to 0.1, 0.2, 0.4, 0.6 and 1.2 kJ/m^2. In the case of lamb's lettuce, aliquots of 20 g were positioned on a UV-C light transparent support between six UV-C lamps and exposed to 20 W/m^2 for increasing times up to 300 s. Relevant fluence on the samples was equal to 0.2, 0.4, 0.9, 1.2 and 6.0 kJ/m^2. Differently processed lamb's lettuce samples were packed under air in 20x20 cm bags of commercial bioriented polypropylene (BOPP, 30 µm thickness) (Radici Film, S. Giorgio di Nogaro, Italy). According to the supplier the BOPP gas transmission rate were 1800 cm^3 O_2 m^{-1} day^{-1} bar^{-1}, 6 g H_2O m^{-1} day^{-1} (at 38 °C and 90% RH). Bags were sealed by a packaging machine (Easy Packer EP-400-C, AVC, Torino, Italy) and stored at 6 °C for up to

21 days in a thermostated cell. At increasing times during storage, samples were removed from the thermostated cell and used for analyses.

Pulsed light treatments

Pulsed light treatments of wastewater and apple slices were carried out by using a pulsed light mobile decontamination unit (Claranor, Rouaine, France) equipped with 4 xenon lamps (JA series, Verre et Quartz, Bussy Saint Georges, France) with maximum emission in the range 200–1000 nm (200–400 nm: 41%; 400–700 nm: 51%; 700–1000 nm: 8%). In the case of wastewater, aliquots of 10 mL of sample were placed in 5.5 cm diameter sterile Petri dishes to get a 4 mm thick-sample. Petri dishes were placed on a 5 mm thickness quartz plate at a distance of 10 mm from the lamps positioned above, below and at the two sides of the sample, and exposed at increasing light fluence up to 17.5 kJ/m^2, by modifying capacitor voltage (1000-3000 V). Each light pulse had a duration of 50 µs. After pulsed light treatment sample were used for analysis.

In the case of apple slices, samples were placed on the quartz plaque and exposed at increasing light fluence from 0 to 157.5 kJ/m^2. Increasing fluence was obtained by delivering to the sample increasing number of pulses, each having a fluence of 17.5 kJ/m^2.

High-pressure CO_2 treatments

Liquid carbon dioxide (purity 99.995%, Sapio, Monza, Italy) was cooled down to 4 °C using a F34-ED chiller (C; Julabo, Milano, Itlay) after been filtered with a 15 µm filter (Ham-Let, Milano, Italy). Subsequently, CO_2 was pressurised with a Orlita MhS 35/10 diaphragm pump (ProMinent Italiana S.r.l., Bolzano, Italy) into two identical stainless steel cylinders (internal volume 150 mL) with a screwed cap, previously filled with 10 mL of polyphenoloxidase aqueous solution or apple juice. The vessels connected in parallel, were immersed in a water bath connected to a CB 8-30e thermostatic bath (Heto, Allerød, Denmark) that ensured maintenance of constant temperature. Before starting the pressurisation, the temperature of the sample was allowed to reach the equilibrium. The time needed to reach the desired temperature (20, 35 or 45 °C) was lower than 3 min. Once the desired temperature was reached, less than 2 min were needed to reach the target pressure. As a consequence, the desired

conditions of temperature and pressure were reached in less than 5 min. After reaching the desired pressure (6, 12 and 18 MPa), the pump was switched off and valves connected to each vessel were tightly closed. In order to carefully control operating parameters such as pressure and temperature during experiments, two thermocouples connected to a digital data logger and a manometer were used. The mixing between samples and CO_2 inside the vessels was assured by two magnetic stirrers (Microstirrer, Velp Scientifica, Usmate, Italy) placed below each vessel. After increasing treatment time up to 30 min, vessels were depressurised. The valves were heated in a water bath connected to a thermostatic bath to prevent freezing during decompression. In order to assure an adequate heat exchange in both water baths, two small water pumps were used. In all experiments, depressurisation was completed within 3 min and the outlet flow was controlled using a PFM 750 digital flowmeter (SMC Italia S.p.A., Milano, Italy). Additional control samples were prepared by treating the enzyme solution or the apple juice in the vessels at atmospheric pressure (0.1 MPa) and thus at CO_2 partial pressure equal to 0.0039 MPa.

After HP-CO_2 treatments, aliquots of 1 mL of polyphenoloxidase solution were introduced in Eppendorf® vials of 1.5 mL capacity. In the case of apple juice, aliquots of 10 mL were introduced in vials of 10 mL capacity. Samples were stored for up to 7 days at 4 °C in a refrigerated cell. At increasing time during storage, samples were removed from the refrigerator, equilibrated at 22 °C and submitted to the analysis.

Pulsed electric field treatments

A lab scale pulsed electric fields (PEF) unit (ELCRACK HVP5, DIL, German Institute for Food Technologies, Quakenbrück, Germany) with a maximum output voltage of 25 kV was used. The system employed a bipolar rectangular-shaped pulse having pulse width of 20 μs and a repetition rate of 250 Hz. The system was connected to a digital oscilloscope (Model No. TDS 2012, Tektronix, Beaverton, OR, USA). The treatment module consisted of a batch chamber with 8 cm electrode gap and 37.5 cm^2 area for each electrode, resulting in a 300 cm^3 total volume, modified after Haughton et al. (2012). For each PEF treatment, 9 potato cubes (75 g) were placed on the teflon base of the chamber in a

3×3 configuration. Tap water (225 g) was added to guarantee uniform distribution of the electric field. Samples were processed at room temperature and were subjected to PEF treatments characterised by a total energy (*Q)* of 18.9 kJ/kg. The latter was calculated according to Zhang, Barbosa-Cánovas and Swanson (1995) based on:

$$Q = \frac{V^2 t}{R\, m} \quad (1)$$

where *V* is the voltage (kV), *t* is the treatment time (s), *R* is the resistance (ohm) and *m* is the sample mass (kg).

Two PEF treatments were performed: (i) 9000 pulses at 0.75 kV/cm electric field ("Low PEF") (ii) 810 pulses at 2.50 kV/cm electric field ("High PEF"). After the treatment, samples were taken from the PEF chamber, drained with paper and stored for further analysis.

Control samples were prepared by dipping just-cut potato cubes in tap water at 20 °C for 20 min (potato-water ratio 1:10, w/w). Additional control samples were prepared by blanching potato cubes in a 0.25% (w/v) sodium metabisulfite aqueous solution (Merck KGaA, Darmstadt, Germany) in a thermostated bath (Mod. JB5, Grant Instruments Ltd, Cambridge, UK) at 70 °C for 16 min (potato-water ratio 1:10, w/w) (Bingol et al., 2014). Potato cubes were then cooled in tap water at 20 °C for 2 min (potato-water ratio 1:20, w/w). After the treatments, control samples were drained with paper and used for further analysis.

The total energy (*Q)* of potato blanching was 209.3 kJ/kg. The latter was calculated based on:

$$Q = c_p \Delta T \ (2)$$

where c_p is the specific heat capacity of liquid water (kJ/ °C kg) and ΔT is the temperature increase from 20 to 70 °C.

Figure 2.1 summarizes the vegetable matrices studied and the respective treatments applied in this PhD thesis.

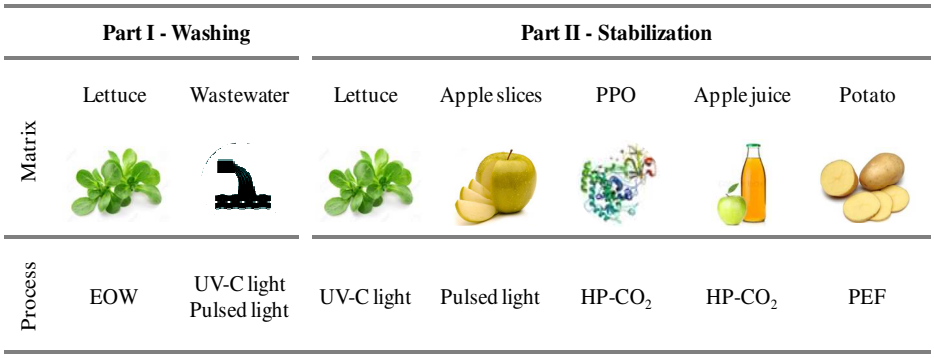

Figure 2.1 Matrices and technologies considered in the present PhD thesis.

2.3 Bacteria Strains and Growth Conditions

Pseudomonas fluorescens

Pseudomonas fluorescens L22 was obtained from the Department of Food Science Collection, University of Udine (Italy). The strain was maintained at −80 °C in Triptone Soya Broth (TSB, Oxoid) with 30% glycerol added as a cryogenic agent. The strain was kept in TSB at 37 °C for 48 h and subsequently spread on Tryptone Soya Agar (TSA, Oxoid) and incubated under the same conditions. The strain was cultured in 5 mL TSB at 37 °C for 24 h, collected by centrifugation at 13,000 rpm for 10 min at 4 °C (Beckman, Avanti TM J-25, Palo Alto, CA, USA) and washed three times with Maximum Recovery Diluent (MRD, Oxoid). Final pellets were suspended in MRD and used for *in vitro* tests. Pseudomonas Agar base (PSA, Oxoid) plates were used for enumeration of *Pseudomonas fluorescens.* Plates were incubated for 48 h at 30 °C.

Salmonella enterica, Listeria monocytogenes* and *Escherichia coli

Experiments were performed with pure cultures of *Salmonella enterica* subsp. *enterica* 9898 DSMZ, *Listeria monocytogenes* 20600 DSMZ and *Escherichia coli* obtained from the Department of Food Science Bacterial Culture Collection (University of Udine, Italy) and stored at -80 °C in Brain Hearth Infusion Broth (BHI, Oxoid) with 30% glycerol until needed. The stock cultures were maintained by regular subcultures in BHI Agar at 4 °C. A loopful of bacteria was transferred to 5 mL of BHI Broth,

incubated at 37 °C, collected by centrifugation at 13,000 rpm for 10 min at 4 °C (Beckman, Avanti TM J-25, Palo Alto, CA, USA) and washed with MRD (Oxoid) twice. Each final pellet was suspended in MRD and used to inoculate. Tap water samples were previously filtered with a sterile cellulose acetate filter (ALBET LabScience, Barcelona, Spain) 0.20 μm pore size and 25 mm diameter while wash water samples derived from the first washing cycle. The samples were inoculated with each strain separately in order to obtain a final concentration of 10^6-10^7 CFU/mL. The inoculated samples were incubated at 30 °C for 1 h and subsequently treated. Xylose Lysine Desoxycholate Agar (XLD, Oxoid), Palcam Agar (Oxoid) and Coli ID (BioMerieux, Mercy L'Etoile, France) were used for enumeration of *S. enterica*, *L. monocytogenes* and *E. coli* respectively. Plates were incubated at 37 °C for 24 h.

Lactobacillus brevis* and *Listeria monocytogenes

Lactobacillus brevis 20054 DSMZ and *Listeria monocytogenes* 20600 DSMZ were respectively used as alterative and pathogenic strains for inoculation of apple slices. The strains were maintained at -80 °C in Triptone Soya Broth (TSB, Oxoid, Milano, Italy) with 30% glycerol added as a cryogenic agent. The original strains were kept in TSB at 37 °C for 24 h and subsequently were spread on Tryptone Soya Agar (TSA, Oxoid, Milano, Italy) and incubated under the same conditions. Fresh cultures were prepared by inoculating one colony from the pure culture within 10 mL of TSB and incubated at 37 °C for 24 h to give an initial inoculum of 10^8-10^9 CFU/mL. Fresh-cut apple slices were inoculated with 100 μL of *L. brevis* or *L. monocytogenes* fresh cultures over the entire upper surface by spreading with a sterile micropipette to obtain an inoculum of about 10^5-10^6 CFU/cm^2. Man Rogosa Sharpe (MRS) Agar (Oxoid) with 0.025% Delvocid (DSM, Heerlen, the Netherlands) was used for enumeration of *L. brevis*. Plates were incubated for 48 h at 30 °C. Palcam Agar (Oxoid) was used for enumeration of *L. monocytogenes* and plates were incubated for 24 h at 37 °C. Results were expressed as log CFU/cm^2.

2.4 *In vitro* inactivation of *Pseudomonas fluorescens*

For *in vitro* inactivation of *Pseudomonas fluorescens* 1 mL of bacterial suspension was added to 9 mL of NEW, AEW or NaOCl solutions in a sterile test tube and vortexed (Fisher Scientific, Illkirk, France). The inactivation treatment was carried out for 2 min at room temperature. Inactivation experiments were stopped by transferring 1 mL of each treated sample to a sterile tube containing 9 mL of neutralizing 0.5% (w/v) sodium thiosulphate ($Na_2S_2O_3$, Sigma Aldrich) aqueous solution. After neutralization, samples were then used for microbial enumeration.

2.5 Microbiological analysis

Washing solutions, lamb's lettuce and apple slices were sampled for microbial plate counts. In particular, before plating, 10 g of lettuce and 25 g of apple sample were diluted 1:10 (w/v) and 1:5 (w/v) in Maximum Recovery Diluent (MRD, Oxoid, Basingstoke, UK), respectively and homogenized for 2 min in a Stomacher Lab-Blender 400 (PBI International, Milano, Italy). Serial dilutions of each suspension were made in MRD (Oxoid) and appropriate aliquots (0.1 mL or 1 mL) were spread on agar plates. Plate Count Agar (PCA, Oxoid) and Pseudomonas Agar base (Oxoid) added with Cetrimide, Fucidin, Cephaloridine supplement (Oxoid) were used for enumeration of total mesophilic bacteria and *Pseudomonas* spp. respectively; plates were incubated at 30 °C for 48 h. Violet Red Bile Glucose Agar (VRBG, Oxoid) and Coli ID (BioMerieux, Mercy L'Etoile, France) were used for enumeration of Enterobacteriaceae and total coliforms, respectively; plates were incubated at 37 °C for 24 h. Oxytracycline-Glucose-Yeast Extract (OGY) agar (Oxoid) for enumeration of yeasts and molds; plates were incubated for 72 h at 28 °C.

2.6 Temperature measurements

Temperature was measured by a thermocouple probe (Checktemp1, Hanna Instruments Inc., Woonsocket, RI, USA). In the case of apple slices, the temperature of the sample during pulsed light treatment was monitored using a T-type thermocouple placed 2 mm under the surface of

the fruit and connected to a portable data logger (mod. 502A1, Tersid, Milano, Italy).

2.7 pH and ORP measurements

pH and ORP were measured with a Crison 50 12T electrode (Hach Lange, Barcelona, Spain) and a InLab®Redox Combined electrode (Schwerzenbach, Switzerland) respectively, connected to a pHmeter (PH301, Hanna Instruments, Smithfield, RI, USA).

2.8 Chlorine determinations

Free chlorine was determined by a HI38020 kit based on the use of N,N-diethyl-p-phenyldiamine (DPD) (Hanna Instruments Inc., Salaj, Romania).
Hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) concentrations were assayed spectrophotometrically at 234 and 292 nm respectively by using a UV-Vis spectrophotometer (Shimadzu UV-2501PC, Shimadzu Corporation, Kyoto, Japan) and determined by Beer's law equation (Len *et al.* 2000).

2.9 Optical properties of wastewater

Absorbance
The absorption spectroscopy measurements from 250 to 700 nm were performed by a UV-Vis spectrophotometer (Varian Techtron Pty. LtD., Mulgrave, Australia) at 25 °C with a 1 cm path-length cuvette.

UV-C light transmittance of wastewater
UV-C light penetration depth in wastewater was determined photometrically using a portable luminometer (HD-2102.2 Delta Ohm, Padova, Italy) equipped with a UV-C (LP471 UVC, Delta Ohm, Padova, Italy) light probe according to Manzocco et al. (2013). A plastic beaker allowing 80% of the UV-C radiation to reach the sample (polycoupled Combiflex PA/PE 090, 20/70, Savonitti, Codroipo, Italy) was adapted to the luminometer sensor. Wastewater samples with increasing thickness were introduced in the beaker and exposed to 20 W/m^2 UV-C light. The

intensity of the light transmitted through samples was calculated according to the Beer-Lambert law:

$$\frac{I}{I_o} = e^{-\alpha x} \qquad (3)$$

where x is water depth and α is the experimental absorption coefficient. The reciprocal values of α were computed to estimate the penetration depth (δ), that can be defined as the depth at which the light irradiance inside the material (I) falls to $1/e$ (about 36%) of the irradiance of the incident light at its surface (I_o).

2.10 Colour

Colour of the apple slices and potato cubes was analysed using a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR-300 measuring head. The instrument was standardised against a white tile before measurements. Colour was expressed in L*, a* and b* Hunter scale parameters. Colour changes were measured by following the changes in L*, a* and b* parameters.

2.11 Histological preparation of apple slices

For light microscopy, apple samples were fixed in 4% (v/v) formaldehyde buffered solution for 7 days. After fixation, samples were processed by an automatic histoprocessor (TISBE tissue processor, Diapath, Martinengo, Italy) to embed the tissue in paraffin (ParaplastPlus, Diapath, Martinengo, Italy). Serial 5 μm sections were cut to obtain transversal specimen to the fibre direction by a programmable microtome (Reichert-Jung 2050, Nussloch, Germany). For histological evaluation the paraffin sections were stained with toluidine blue (Sigma Aldrich, Milano, Italy). The specimens were finally examined at 20x by light microscope (Leica DMRB, Leica Microsystems GmbH, Solms, Germany) and images acquired by a digital camera (Leica, ICC50, Solms, Germany) using a LAS-EZ (Leica, Solms, Germany) software.

2.12 Texture analyses

Firmness of fresh-cut salad

Firmness of the lamb's lettuce was measured using a ten-blade Kramer shear cell, attached to an Instron 4301 (Instron LTD, High Wycombe, UK) equipped with a 100 N load cell. The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, UK). On the test day, aliquots of 10 g of fresh-cut salad at 4 °C were compressed 50 mm. The test speed was 2.5 mm s^{-1}. Force-distance curves were recorded and firmness was taken as the maximum force (N) required to compress the salad.

Firmness of fresh-cut apples

Firmness was measured by a puncture test using an Instron 4301 (Instron LTD, High Wycombe, UK) equipped with a 100 N load cell. The instrumental settings and operations were accomplished using the software Automated Materials Testing System (version 5, Series IX, Instron LTD, High Wycombe, UK). On the test day, apple slices were punctured with a 1.5 mm cylindrical probe. Crosshead speed was set at 5 cm/min. Force-distance curves were obtained from the puncture tests and firmness was taken as the force (N) required to puncture the slices 0.5 cm.

Texture of potato samples

An Instron Universal Testing machine (Mod. 5544, Instron Corporation, High Wycombe, UK) equipped with a 500 N load cell was fitted with a Warner Bratzler flat blade (1.0 mm thick). The test was performed at a cross head speed of 250 mm/min for the first 2.5 mm followed by a 50 mm/min speed for a total length of 20 mm. Data were analysed by Bluehill 2 software package (Version 2.5, Instron Corporation, High Wycombe, UK). The parameters measured from the force deformation curve were the maximum peak force (N) and total energy calculated as the area below the curve (J).

2.13 Moisture, Weight loss and Drip loss determination

The moisture content of samples was determined according to oven drying method at 105 °C for 18 h (AACC, 2000).
Samples weight loss was calculated as the percentage weight difference between initial and final weight as a result of treatments or storage time.
For drip loss determination a potato cube was weighted, wrapped in cheesecloth and inserted into a 50 mL capacity plastic centrifuge tube half fitted with cotton wool. Following the centrifugation at 1,000 rpm for 10 min (Rotina 380, Hettigh Instruments LP, Tuttlingen, Germany), the sample was removed from the tube and reweighed. Drip loss was calculated as the percentage ratio between initial and final weight of the potato cube.

2.14 Oil content

Potato cubes were weighted and dried at 70 °C for 12 h in a convection oven (FD 115, Binder GmbH, Tuttlingen, Germany). The oil extraction was conducted with petroleum ether 40-60 DAB (AppliChem GmbH, Darmstadt, Germany) at 110 °C for 90 min in a Soxtec System apparatus (Mod. HT6, Foss Tecator, Höganäs, Sweden). Solvent residues were removed in a convection oven (FD 115, Binder GmbH, Tuttlingen, Germany) at 100 °C for 30 min. Recovered oil was then weighted and oil content was expressed as percentage ratio between oil and sample weight.

2.15 Polyphenoloxidase activity

The polyphenoloxidase activity was assayed spectrophotometrically (Shimadzu UV-2501PC, UV-Vis recording spectrophotometer, Shimadzu Corporation, Kyoto, Japan) at 25 °C according to the methodology of Kahn (1995). The reaction was started by the addition of 20 µL of polyphenoloxidase solution to 2 mL of 0.1 M potassium phosphate buffer pH 7 and 1.5 10^{-3} M L-Dopa (Carlo Erba, Milano, Italy). The absorbance at 420 nm was monitored each minute for 10 min. The changes in absorbance per min were calculated by linear regression, applying the pseudo zero order kinetic model. The eventual final stationary phase was excluded from regression data. One arbitrary unit of polyphenoloxidase

was defined as the amount of enzyme that produced an increase in absorbance at 420 nm of 0.001 Abs min^{-1} under the testing conditions. Polyphenoloxidase residual activity was calculated as the percentage ratio between enzymatic activity of the treated solution and that of the control treated at atmospheric pressure (0.1 MPa).

2.16 SDS-polyacrylamide gel electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970). Aliquots of polyphenoloxidase in the aqueous solution were added with sample buffer containing 0.2% bromophenol blue, 0.5 M Tris-HCl, pH 6.8, 10% (w/v) SDS, 50% (v/v) glycerol and 25% (v/v) β-mercaptoethanol. The samples were loaded on a 13% (w/v) polyacrylamide separating gel containing 40% (v/v) acrylamide and 2% (v/v) bis-acrylamide cross linker, mounted on a Mini 2-D (Bio-Rad, Richmond, CA, USA) vertical electrophoresis cell. The electrophoretic separation were obtained by running the samples at 15 mA with standard proteins of known molecular weight (Precision Plus Protein™ Dual Color Standards, Bio-Rad, Segrate, Italy) were used to determine the apparent molecular mass of the sample bands. An additional sample, obtained by thermal inactivation of PPO was also prepared. In particular, 10 mL enzyme solution were placed into 50 mL capacity glass tube and heated in a water bath (IKA-Werke, Staufen, Germany) at 70 °C for 5 min.
The gels were subsequently stained with NOVEX ® Colloidal Blue Staining Kit (Invitrogen, Carlsbad, CA, USA) for one night and then destained in bi-distilled water. Gels were scanned and analysed with the ImageQuant TL Image Analysis Software (Amersham Bioscience Inc., Piscataway, NJ, USA).

2.17 Images

Sample images were captured using a digital camera (Nikon D3, Nikon Corporation, Tokyo, Japan) mounted on an adjustable stand positioned 50 cm above a white cardboard base where the salad was placed. Light was provided by two 250 W frosted photographic floodlights (Bron Elektronik AG, Allschwill, Switzerland), in a position allowing minimum

shadow and glare. A white balancing was conducted on the white base and set at 4170 K. Other camera settings were: shutter time 1/160 s, F-Number F/13, focal length 70 mm. Images were saved in the *jpg* file format and resulted in pictures of 4256 × 2832 pixels, 300 × 300 dpi.

2.18 Image analysis

Digital images relevant to fresh-cut lamb's lettuce were analyzed for evolution of sliminess using Image-Pro Plus (ver. 6.3, Media Cybernetics,Inc., Bethesda, Md., U.S.A.). In particular, RGB (*Red Green Blue*) values corresponding to the slimy film on salad surface were identified: R(160-220), G(180-230), B(100-160). The percentage of sliminess was calculated as the percentage ratio between the sum of pixels of salad surface having RGB values in the selected color range and the overall sum of pixels relevant to sample surface.

2.19 Sensory analysis

Fresh-cut salad

Images relevant to fresh-cut salad stored for different lengths of time were evaluated by a panel of 12 Italian assessors, aged 30 to 55, with sensory evaluation experience in fresh-cut produce. The panel was trained in fresh-cut salad quality evaluation using images on a computer screen. Five images of fresh-cut salad, corresponding to increasing storage times, were presented to the panelists for visual examination. Assessors examined the visual characteristics of the product represented in the images and, after discussion, set up a 5-point overall visual quality scale with relevant lexical description of the fresh-cut salad appearance. 5 stood for excellent quality and 1 for poor quality. Afterwards, each assessor was asked to evaluate unknown images of fresh-cut salads labeled with a 3-digit code, by attributing them a quality index score using the previously set up scoring scale. The images were presented to the assessors in a random order, one after another. Reference images were available during the test to help the judges in anchoring their response.

Fresh-cut apple

A panel of 12 Italian assessors, equally distributed between males and females, of age between 30 and 55, was selected and trained. They all had a minimum of 2 years experience in discrimination and descriptive sensory analysis. The panel was allowed to decide through consensus which descriptor better discriminated samples exposed to pulsed light from the fresh one. To this purpose, a preliminary test was performed to identify the sensory changes most likely to appear as a result of pulsed light treatment. In particular, a treated sample (157.5 kJ/m^2) and a fresh one (untreated apple) were presented to the judges, who were asked to write down the descriptors which differentiated the samples. The samples were presented to the judges just after their preparation. The assessors agreed that browning, apple flavour and cooked flavour were the descriptors that best discriminated the treated sample from the fresh one. Assessors were trained by evaluating different samples using a continuous non-structured scale, with the left end corresponding to the lowest intensity (value -5) and the right end to the highest intensity (value +5). The scale was anchored with "reference" in correspondence of the middle of the scale (value 0). For sensory testing, differently treated apple slices (0, 17.5 and 157.5 kJ/m^2) were identified by a three-digit code and served in odourless plastic dishes at 6 °C. Water was used between samples.

2.20 Data analysis

Each experiment was performed in duplicate and measurements performed at least in triplicate. Results are reported as mean value ± SD.

In the case of HP-CO_2 treatments apparent inactivation rate constants of polyphenoloxidase were analysed by using a conventional first-order equation:

$$\frac{dA}{dt} = -k \cdot A \qquad (4)$$

Where A is the residual activity of polyphenoloxidase (%) at time t (min) and k is the inactivation rate constant (min^{-1}). The value of k was obtained by regressing the natural logarithm of A vs. t. The inactivation rate constant (k) was then derived from the slope of the obtained line.

The kinetic parameter D_P was obtained using procedures analogous to that employed in thermal death time studies. In particular D_P is the

decimal reduction time, i.e. the treatment time needed for 90% enzyme activity reduction at a given pressure and temperature. D_P was computed as:

$$D_p = \frac{2.303}{k} \qquad (5)$$

The pressure increase needed for a 90% reduction of the D_P value is computed as z_p (MPa). The value of z_p was obtained by regressing the decimal logarithm of D_P versus pressure (P):

$$logD_p = \frac{-P}{z_P} \qquad (6)$$

The z_P was then derived as the negative reciprocal slope of the regression line.

Goodness-of-fit was evaluated by means of the determination coefficients (R^2) and analyses of variance (ANOVA) was performed with significance level set to $p<0.05$ (Statistica for Windows, ver. 5.1, Statsoft Inc. Tulsa, OK, USA, 1997). The Tukey procedure was used to test for differences between means.

PART I Salad washing and wastewater decontamination

Salad entering the fresh-cut industry is generally characterised by a microbial load ranging from 5 to 9 Log units, depending on the type of salad, cultivation system, harvesting and handling procedures among other factors (Barth and Breidt, 2010; Ölmez and Kretzschmar, 2009). Washing is a common practice to enhance product quality and shelf-life and improve food safety through the removal of foreign materials, plant tissue exudates and the inactivation of both pathogens and spoilage microorganisms. During salad washing a significant contamination of wash water occurs, reaching microbial loads analogous to those of the unwashed salad (Selma et al., 2008). For this reason, washing of fresh-cut salad is generally performed with water containing antimicrobial sanitizer in order to prevent cross-contamination of pathogenic microorganism between contaminated leaves and uncontaminated product (FDA, 2009). Chlorine, especially in the form of sodium hypochlorite (NaOCl), is the most exploited water disinfectant. It is used at levels of 120-200 mg/L of free chlorine and pH values of 6.0-7.5 to ensure the presence of hypochlorous acid (HOCl) and minimize equipment corrosion (Parish et al., 2003). However, chlorine is known to react with organic matter to produce undesirable by-products, including trihalomethanes and haloacetic acids which are recognized as carcinogenic and/or mutagenic (Richardson and Ternes, 2005; Krasner et al., 2006).

For these reasons, increasing attention is being focused on sustainable technologies which are capable of efficaciously decontaminate wash water and, at the same time, allowing its reuse and recirculation for multiple washing cycles guaranteeing high hygienic quality of fresh-cut product.

The aim of the first part of this PhD research was thus to investigate the efficacy of decontamination methods other than highly chlorinated aqueous solutions in salad washing. In particular, to reduce chlorine concentration during washing, electrolyzed water solutions were considered as direct sanitizers of wash water and salad. In addition, to completely avoid the use of chemical compounds during salad washing, light technologies such as UV-C and pulsed light were used for decontamination of wastewater, allowing its reuse.

Chapter 3 Decontamination efficacy of neutral and acidic electrolyzed water in fresh-cut salad washing

The critical issues associated with the use of highly chlorinated aqueous solutions for fresh-cut salad washing, address the research towards novel disinfection strategies. Currently, electrolyzed water (EW) is gaining importance as a sanitizer in the food industry. It is usually generated by electrolysis of a NaCl aqueous solution in a chamber, within which the electrodes are separated or not by a semipermeable membrane. When membrane separation is performed, two types of water are produced. At the anode side, an acidic electrolyzed water (AEW) solution with pH 2.3-2.7, containing free chlorine (HOCl and OCl^-) and having high oxidation reduction potential (ORP, >1100 mV) is produced. On the contrary, an alkaline EW solution (pH 11-11.7) with low ORP (-800 to -900 mV) and absence of chlorine is generated at the cathode side (Hsu, 2005). In the case of electrolysis in the absence of a separating membrane, a neutral electrolyzed water (NEW) solution with pH in the range 8.0-8.5, ORP of 650-750 mV and free chlorine content is produced (Al-Haq et al., 2005).
Several studies attributed to AEW and NEW a high antimicrobial efficacy *in vitro* (Issa-Zacharia et al., 2010; Rahman et al., 2012). The enhanced bactericidal effect compared to highly concentrated chlorinated solutions obtained by addition of NaOCl, could be attributable to the presence in EW of reactive oxygen species (ROS) such as $\cdot OH^-$, O_3 and H_2O_2 (Jeong et al., 2006; Hao et al., 2012). Due to the intense antimicrobial effect, AEW and NEW have been proposed for decontamination of working surfaces and equipments (Park et al., 2002; Ayebah et al., 2006) as well as of food products including meat, seafood, fruit and vegetables (Park et al., 2009; Pangloli and Hung, 2011; Xie et al., 2012; Rahman et al., 2013). Particularly interesting seems the application of electrolysed solutions as washing agents of fresh-cut produce. To this regard, Forghani and Oh (2013) demonstrated that electrolysed water, even at low free chlorine concentration of 21-22 mg/L, allowed inactivation of microorganisms inoculated in leafy vegetables. In addition, Tomás-Callejas et al. (2011), not only proved that electrolyzed water was as effective as the conventional disinfection with NaOCl in salad decontamination, but also indicated that it did not affect the quality of the fresh-cut product.

Furthermore, some studies investigated the effect of electrolyzed water on microbial contamination of salad wash water. The latter was efficaciously decontaminated allowing to maintain the microbiological and physiochemical quality of wash water (López-Gálvez et al., 2012; Gómez-López et al., 2014).
However, available literature is limited to the study of the antimicrobial activity of electrolyzed solutions having a given free chlorine concentration with no clear indication on the optimal physicochemical properties (i.e. free chlorine concentration, pH and ORP) allowing to obtain the same bactericidal effect of concentrated NaOCl aqueous solution. In addition, there is a lack of information about the effect of electrolyzed water on both wash water and salad.

Aim of the study

The aim of this study was to determine which are the physicochemical properties that allow neutral electrolysed water (NEW) and acidic electrolysed water (AEW) to replace sodium hypochlorite as a disinfectant in fresh-cut salad washing. To this purpose, NEW and AEW at increasing concentrations of free chlorine and different pH and ORP values were prepared. The germicidal efficacy of electrolyzed solutions was firstly evaluated on pure culture of *Pseudomonas fluorescens* in order to identify the minimum concentration of free chlorine for bacteria complete inactivation. Subsequently, NEW and AEW were used for salad washing. The decontamination efficacy was tested in both wash water and salad.

Results and discussion

Aqueous solutions containing increasing concentration of sodium chloride were electrolysed to obtain neutral electrolysed water (NEW) solutions that were analysed for free chlorine concentration, pH and oxidising reduction potential (ORP) (Table 3.1). Free chlorine concentration progressively increased with the initial sodium chloride concentration. The latter slightly affected the pH of the electrolysed solutions, which ranged from 8.0 to 8.5. By contrast, ORP resulted quite high, independently on the initial NaCl concentration, in accordance with literature data (Abadias et al., 2008).

Table 3.1 Physicochemical properties of neutral electrolyzed water (NEW) obtained by electrolysis of NaCl solutions at increasing concentration.

NaCl (g/L)	Free chlorine (mg/L)	pH	ORP (mV)
1.00	16±1[g]	8.0±0.0[d]	689±13[a]
1.25	31±1[f]	8.2±0.0[c]	666±27[a]
1.60	43±4[e]	8.4±0.0[b]	656±20[a]
1.80	64±4[d]	8.4±0.0[b]	642±12[a]
2.00	81±5[c]	8.4±0.1[a,b]	637±35[a]
2.80	123±7[b]	8.5±0.1[a,b]	650±26[a]
3.30	152±5[a]	8.5±0.0[a]	646±14[a]

[a] Means with same letter in each column are not significantly different ($p \geq 0.05$).

Analogous electrolysed solutions were produced by separating the acidic and alkaline water during electrolysis by a separation membrane. The alkaline water presented high pH (11.7±0.3), low ORP (-882±23 mV) and absence of chlorine compounds. Based on these properties and not exerting antimicrobial effect, the alkaline water was not considered in this study and the attention was focused on the acidic electrolysed water (AEW) that contained high levels of antimicrobial chlorine compounds. Table 3.2 shows the physicochemical properties of AEW obtained by electrolysis of NaCl solutions at increasing concentration. At the same initial concentration of sodium chloride, AEW showed chlorine concentration (Table 3.2) not significantly different ($p \geq 0.05$) from that observed in the NEW electrolysed water (Table 3.1). However, the AEW was characterized by much lower pH and much higher ORP. As shown in table 3.2, even in this case, pH and ORP values were slightly affected by NaCl concentration.

Table 3.2 Physicochemical properties of acidic electrolyzed water (AEW) obtained by electrolysis of NaCl solutions at increasing concentration.

NaCl (g/L)	Free chlorine (mg/L)	pH	ORP (mV)
1.00	14±2[g]	2.4±0.0[a]	1177±3[c]
1.25	29±2[f]	2.4±0.0[a]	1181±2[c]
1.60	42±4[e]	2.3±0.0[b]	1183±3[c]
1.80	61±5[d]	2.3±0.0[b]	1183±5[b,c]
2.00	78±4[c]	2.3±0.0[b]	1185±2[b,c]
2.80	115±7[b]	2.3±0.0[b]	1188±1[b]
3.30	146±5[a]	2.3±0.0[b]	1193±3[a]

[a]Means with same letter in each column are not significantly different ($p \geq 0.05$).

Based on these properties, both NEW and AEW solutions could be used as washing solutions to avoid cross-contamination during fruit and vegetable washing in the fresh-cut industry. However, their different physicochemical properties could be associated with diverse antimicrobial activity. In order to evaluate the germicidal effect of electrolysed solutions, *in vitro* tests against *Pseudomonas fluorescens* were performed. The latter belongs to a *genus* particularly diffused in leafy vegetables and was chosen as a representative microorganism responsible for hygienic quality decay (Ongeng et al., 2006; Manzocco et al., 2010; Schwaiger et al., 2011). The survival of *P. fluorescens* at 9 Log concentration was tested in the presence of NEW and AEW solutions containing increasing concentration of free chlorine. Figure 3.1 reports the effect of the different solutions on the microbial load of *P. fluorescens.*

By increasing the concentration of free chlorine, NEW solutions (Table 3.1) exerted increasing bactericidal effect on *P. fluorescens*, reaching a microbial load reduction of *circa* 7 Log at 150 mg/L free chlorine. On the contrary, AEW solutions (Table 3.2) completely inactivated the microorganism at the lowest free chlorine concentration here tested (15 mg/L). This result can be attributed to the well known antimicrobial effect of acid pH on cell membranes as well as to the high ORP value (1177±3 mV, Table 3.2).

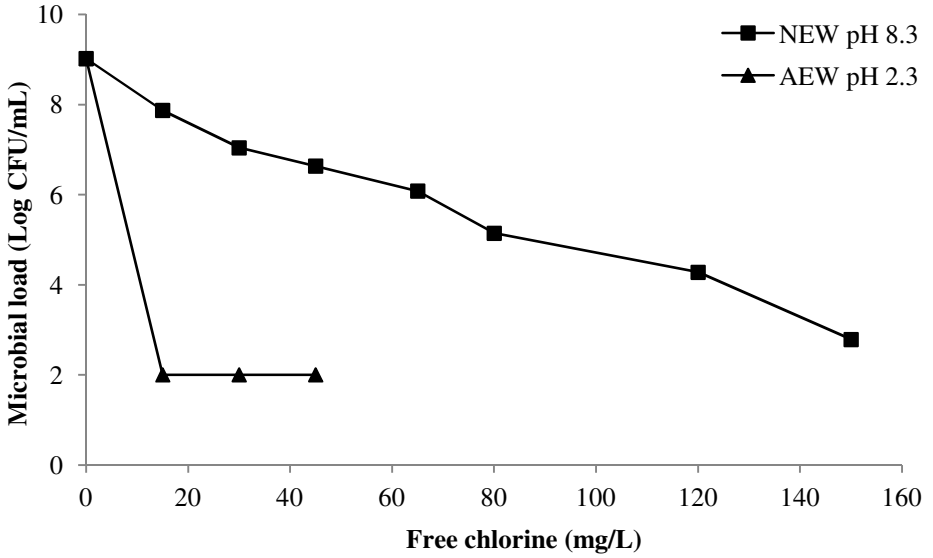

Figure 3.1 Microbial load of *Pseudomonas fluorescens* as a function of free chlorine concentration of neutral (NEW) and acidic (AEW) electrolyzed water. (Detection limit 2.0 Log CFU/mL)

Data shown in Figure 3.1 are in agreement with those reported by Issa-Zacharia et al. (2010) on microorganisms other than *P. fluorescens*. These authors observed almost 6 Log reductions of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp. in the presence of electrolyzed water with 1140 mV ORP, pH 2.6 and 25 mg/L free chlorine. By contrast, at the same free chlorine content, the increase in pH exerted a lower germicidal effect (Issa-Zacharia et al., 2010).

Literature data suggest that the germicidal efficacy of chlorinated water solutions is highly affected by pH adjustment (Len et al., 2000; Hricova et al., 2008). The original pH of aqueous solutions of sodium hypochlorite (NaOCl) intended for vegetable washing is about 8, depending on the concentration. The solution pH is generally adjusted to pH ranging from 4 to 6.5 to maximise the germicidal effect (Len et al., 2000; Park et al., 2004). Under these conditions, the equilibrium among the different chlorine species (gaseous chlorine, hypochlorous acid and hypochlorite ion) largely favours the prevalence of hypochlorous acid, which is the most active antimicrobial form (Kim et al., 2000; Abadias et al., 2008). A similar equilibrium is also reached in electrolysed solutions. To this regard, figure 3.2 shows the concentration of HOCl, OCl^- and their sum in

AEW obtained by electrolysis of 1.25 g/L NaCl aqueous solution (initial pH 2.4±0.0, Table 3.2) adjusted at different pH up to 9.0.

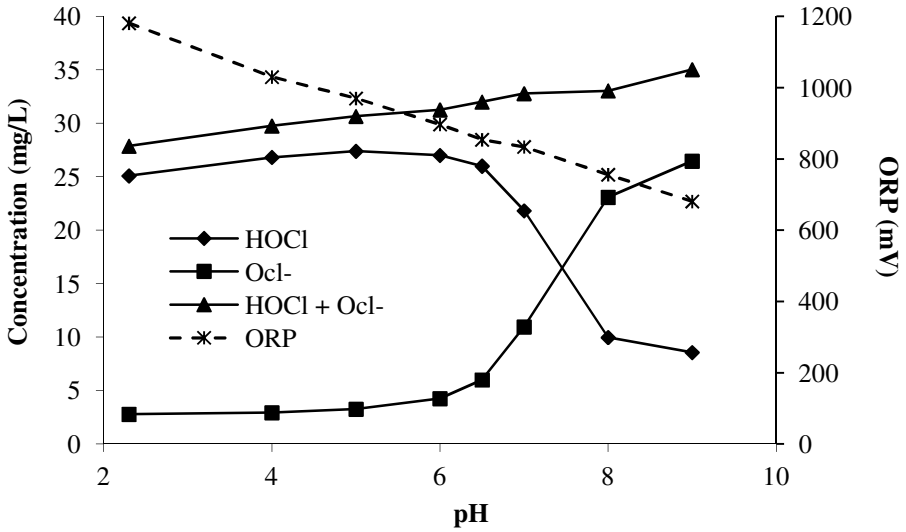

Figure 3.2 Concentration of HOCl, OCl^-, their sum and ORP in acidic electrolysed water (AEW) obtained by electrolysis of 1.25 g/L NaCl aqueous solution and adjusted to increasing pH.

At alkaline pH, the concentration of HOCl was lower than that of OCl^-, while opposite equilibrium of these species was observed at acid pH making the solution highly germicidal (Len et al., 2000; Hao et al., 2012). In fact, HOCl was demonstrated to be 20 times more effective against microorganisms than OCl^- (White, 1999). Below pH 4, HOCl is known to dissociate and form chlorine gas, which is highly volatile and toxic (Shigeto et al., 2000). Similar results were also achieved by adjusting the pH of NEW solutions (data not shown), reflecting the general behaviour of chlorinated aqueous solutions that depends on pH solely. Similarly, also the chemical equilibrium of electrolysed solutions only depends on pH and is not affected by the initial concentration of NaCl before electrolysis. However, the latter affects the overall amount of chlorine species in AEW and NEW solutions and, consequently, their germicidal efficacy. In addition, the modification of equilibrium among chlorine species upon pH adjustment strongly modified ORP value (Figure 3.2). In

particular, the increase in pH was associated with a decrease in ORP and thus potentially in the antimicrobial activity (Figure 3.2).
Based on these considerations, the pH of NEW and AEW solutions (Table 3.1 and 3.2) could be adjusted to slightly acid values to maximise the antimicrobial capacity. However, the physicochemical properties of electrolyzed solutions should not impair product quality. To this regard, optimal pH of washing solutions should approach that of the vegetable. Since salad is characterised by a pH of *circa* 6.5, its washing should be performed at such a value to maintain product quality (Rico et al., 2008; Tomás-Callejas et al., 2011). NEW and AEW solutions having increasing free chlorine concentration were thus adjusted to pH 6.5. Table 3.3 shows that the modification of the equilibrium among chlorine species allowed to obtain NEW and AEW with similar ORP. In particular, pH adjustment increased ORP values of NEW by *circa* 150 mV as compared to the non pH-adjusted NEW (Table 3.1). It can be hypothesised that the increase in NEW oxidative capacity could improve its germicidal activity. On the contrary, by increasing the pH of AEW a decrease in ORP of *circa* 300 mV was observed, probably leading to a decrease in the solution germicidal activity.

Table 3.3 ORP (mV) of neutral (NEW) and acidic (AEW) electrolyzed water containing increasing free chlorine concentration and adjusted to pH 6.5.

Free chlorine (mg/L)	**NEW**	**AEW**
15±2	812±11[b]	865±13[a]
30±2	805±9[b]	862±17[a]
42± 3	789±12[b]	879±6[a]
63±4	795±14[b]	881±9[a]
80±4	807±4[b]	878±11[a]
119±7	803±7[b]	886±8[a]
151±6	806±15[b]	889±12[a]

[a]Means with same letter are not significantly different ($p \geq 0.05$).

To verify these hypothesis, the effect of AEW and NEW solutions adjusted at pH 6.5 on the microbial load of *P. fluorescens* was tested (Figure 3.3). An aqueous solution containing increasing amount of free chlorine compounds but obtained by adding NaOCl was also considered

as control. As expected, by increasing the concentration of free chlorine, the control NaOCl solution showed a higher bactericidal effect on *P. fluorescens*. In particular, the microbial load was reduced below the detection limit when the free chlorine concentration of NaOCl solutions approached 150 mg/L. It is noteworthy that this concentration is conventionally used in industrial salad washing (Beuchat, 1998). In the case of the electrolysed solutions, the complete inactivation of *P. fluorescens* was achieved at 30 mg/L free chlorine. At the same concentration and pH, the higher germicidal activity of electrolysed solutions as compared to NaOCl solution could be attributed to their higher ORP. The higher bactericidal effect of electrolysed solutions could be also due to the well known presence of reactive oxygen species such as O_3 and $\cdot OH^-$ (Jeong et al, 2006).

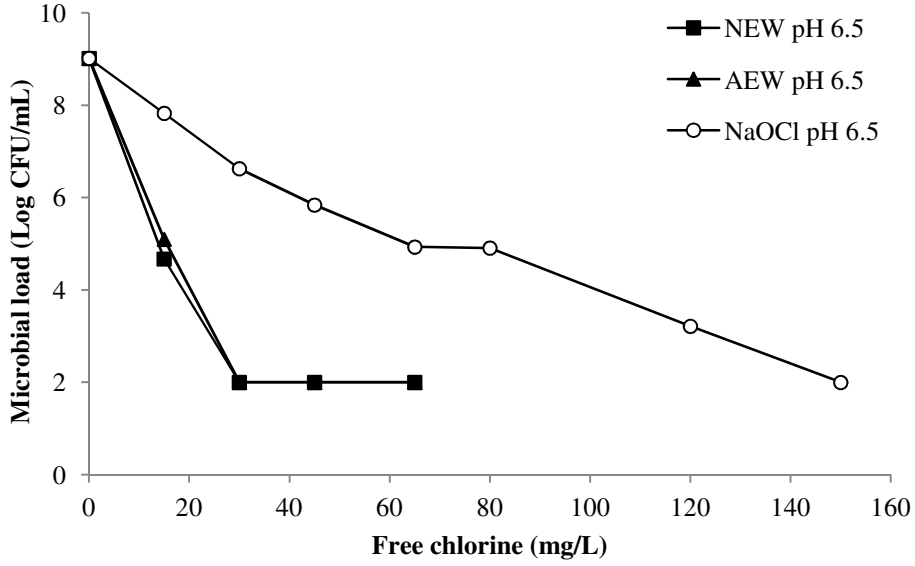

Figure 3.3 Microbial load of *Pseudomonas fluorescens* as a function of free chlorine concentration of neutral (NEW) and acidic (AEW) electrolyzed water solutions at pH adjusted to 6.5. (Detection limit 2.0 Log CFU/mL)

The complete inactivation of *P. fluorescens* by electrolyzed water was also reported by Ongeng et al. (2006) at a chlorine concentration of *circa* 4 mg/L. In this study, no indication on the pH and ORP of the solution was provided but the complete inactivation of *P. fluorescens* was

achieved upon 45 min of contact with the electrolyzed water solution. By contrast, in the present experiment, only 2 min of contact was allowed.
To verify the applicability of electrolyzed solutions in vegetable washing, NEW and AEW at pH 6.5 and containing the minimum free chlorine concentration able to guarantee the complete inactivation of *P. fluorescens* (30 mg/L, Figure 3.3) were used to perform lamb's lettuce washing. Washing was also carried out by using a NaOCl solution containing 150 mg/L free chlorine (pH 6.5±0.1 and ORP 724±43 mV). This concentration was chosen since allowed *P. fluorescens* to be reduced below the detection limit (Figure 3.3). The decontamination efficacy was also evaluated in the absence of chlorine compounds by washing salad with deionised water (pH 6.8±0.3 and ORP 375±32 mV).
Unwashed salad confirmed to be characterized by a microbial population greatly represented by *Pseudomonas* spp. (Table 3.4). The presence of total coliforms, which are typical inhabitants of fresh-cut plants deriving from soil and manure contamination, was also detected. Table 4 shows that, independently on the washing solution used and their free chlorine concentration, *circa* 1.5 Log reductions in total mesophilic count and *Pseudomonas* spp. were achieved. Similar results were obtained for total coliforms with *circa* 1 Log reduction.

Table 3.4 Native microflora of lamb's lettuce washed with neutral (NEW) or acidic (AEW) electrolyzed water. Samples washed with deionised water or NaOCl solution are shown as control.

Washing	Free chlorine concentration (mg/L)	Microbial load (Log CFU/g)		
		Total mesophilic count	*Pseudomonas* spp.	Total coliforms
None	-	7.6±0.1[a]	7.4±0.2[a]	3.3±0.1[a]
Deionised water	0	6.3±0.3[b]	5.9±0.2[b]	2.0±0.1[b]
NaOCl	150±4	6.1±0.4[b]	5.9±0.2[b]	1.9±0.3[b]
NEW	30±2	6.5±0.1[b]	6.4±0.3[b]	2.4±0.2[b]
AEW	30±2	6.5±0.2[b]	6.0±0.3[b]	2.2±0.3[b]

[a] Means with same letter in each column are not significantly different ($p \geq 0.05$).

This antimicrobial efficacy is in accordance with literature data on leafy vegetable washing and can be attributed to the ability of water turbulent flow to mechanically remove the contaminating bacteria at the product surface (Allende et al., 2008). Therefore, no germicidal effect on the internalised microorganism in vegetable tissue can be achieved upon washing (Hou et al., 2013). For this reason the overall antimicrobial effect of washing on salad rarely exceeds 1.5-2 Log reductions. However, it can be hypothesised that the decontamination efficacy of electrolyzed solutions could be mainly observed in wash water. Based on these considerations, microbial analyses were also performed on the wastewater recovered after lettuce washing with the solutions here considered (Table 3.5).

Table 3.5 Native microflora of wastewater recovered after lamb's lettuce washing with neutral (NEW) or acidic (AEW) electrolyzed water. Wastewater from washing with deionised water or NaOCl solution are shown as control.

Washing	Free chlorine concentration (mg/L)	Microbial load (Log CFU/mL)		
		Total mesophilic count	*Pseudomonas* spp.	Total coliforms
Deionised water	0	6.5±0.4	5.5±0.3	2.6±0.2
NaOCl	150±4	<DL	<DL	Nd
NEW	30±2	<DL	<DL	Nd
AEW	30±2	<DL	<DL	Nd

nd: not detectable; DL: detection limit 2.0 Log CFU/mL

As expected, wastewater deriving from salad washing in deionised water resulted highly contaminated with counts approaching those of unwashed salad (Table 4). Microbial load also resulted in the same magnitude range reported in the literature for wastewater from industrial washing of different vegetables (Selma et al., 2008; Barrera et al., 2012). NaOCl solution containing 150 mg/L free chlorine was able to reduce the microbial count of wastewater below the detection limits. Analogous results were also obtained with NEW and AEW solutions at pH 6.5. However, the latter were characterized by a free chlorine content of 30

mg/L, which is 5 times lower than that of the NaOCl solution. It can be inferred that electrolysed solutions with low free chlorine concentration could be efficiently employed to perform vegetable washing. Their application should necessarily guarantee that wash water maintains its germicidal activity during multiple washing cycles. This could be achieved by maintaining a constant 30 mg/L concentration of free chlorine compounds in wash water by continuous enrichment with newly generated electrolysed water.

Conclusion

Results demonstrate that electrolyzed water can be exploited as a sanitizing washing agent in the fresh-cut industry. The use of electrolysed solutions would allow to replace conventional chlorination of washing water with highly concentrated NaOCl, guaranteeing an analogous decontamination efficacy and decreasing the overall presence of potentially toxic chlorine compounds in wastewater. This would contribute to meet the global requirement of reducing the water footprint of industrial washing. In addition, the possibility to produce *in situ* germicidal washing water by electrolysis of NaCl aqueous solutions would avoid the presence of harmful NaOCl within the food industry, limiting the health risks for operators as well as for product contamination.

Chapter 4 Decontamination efficacy of UV-C light on wastewater deriving from fresh-cut salad washing

Wash water used for fresh-cut salad becomes highly contaminated if proper sanitizing agents are not used, as previously described in chapter 3. To avoid microbial cross-contamination and the presence of harmful chemical compounds water disinfection may be also accomplished by physical means, including microfiltration, ultrasound, pulsed electric field and light treatments (Selma et al., 2008; Poyatos et al., 2010; Bazyar Lakeh et al., 2013). Among the physical disinfection methods, ultraviolet (UV) radiation is one of the most successful in terms of disinfection efficacy. UV light occupies the non-ionizing region of the electromagnetic spectrum, between 200 and 400 nm. The UV spectrum can be divided in three regions: short-length (UV-C) from 200 to 280 nm; medium-length (UV-B) from 280 to 320 nm; long-length (UV-A) from 320 to 400 nm. The intensity of UV radiation is expressed as irradiance (W/m^2), while the dose which is a function of the intensity and time of exposure, is expressed as radiant exposure or fluence (J/ m^2) (Bintsis et al., 2000). The antimicrobial effect is mainly attributable to the UV-C component, with a maximum at 254 nm (Bintsis et al., 2000). Low-pressure mercury lamps are used to generate monochromatic radiation at 254 nm which has the ability to damage microbial DNA, causing cross-linking between neighbouring thyamine and cytosine in the same DNA strand (Rame et al., 1997). The resulting effect is that the DNA transcription and replication are blocked, compromising cellular functions and eventually leading to cell death (Sastry et al., 2000).

UV-C light processing is confirmed to be easy to use and characterized by favourable costs of equipments, energy and maintenance (Bintsis et al., 2000; Guerrero-Beltrán and Barbosa-Cánovas, 2004). It not leave residues and has been claimed as not forming known toxic or significant non-toxic by products (Keyser et al., 2008). To this regard, ultraviolet processing of food was approved in US, while does not require authorization according to EU novel food regulation and is successfully applied to disinfect water, air, working surfaces and packaging material (Bintsis et al., 2000; Koutchma et al., 2004).

Aim of the study

The aim of the present research was to investigate the possibility to exploit UV-C light processing in decontaminating wastewater deriving from washing of fresh-cut salad. To this aim, wastewater obtained by washing fresh-cut lamb's lettuce was exposed to increasing doses of UV-C light. The germicidal efficacy of the treatment was evaluated on native microbiota as well as on inoculated pathogenic bacteria. To this regard, *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* were considered as species associated with faecal contamination deriving from irrigation water or salad handling after harvesting. The possibility to reuse UV-C decontaminated wash water in multiple washing cycles up to 5 was studied considering both wash water and washed produce. Decontamination efficacy was related to UV-C light penetration depth in reused wash water.

Results and Discussion

The effect of washing on lamb's lettuce and water microflora is reported in table 4.1. Total viable count of lettuce resulted in the same magnitude range of literature data relevant to leafy vegetables cultivated on soil (Abadia et al., 2008; Selma et al., 2008). In particular, *Pseudomonas* spp. resulted being the prevalent genus which is in agreement with its diffusion in leafy vegetables and lamb's lettuce (Manzocco et al., 2010; Schwaiger et al., 2011). *Enterobacteriaceae* and total coliforms were also detected since they are typical inhabitants of salads deriving from soil and manure contamination.

Table 4.1 Effect of washing on microflora in lamb's lettuce (Log CFU/g) and water (Log CFU/mL).

	Lettuce	Washed lettuce	Wash water	Wastewater
Total viable count	7.50±0.01	6.55±0.19	2.07±0.11	5.85±0.07
Pseudomonas spp.	7.31±0.11	6.42±0.20	<DL	5.78±0.37
Enterobacteriaceae	3.14±0.34	1.48±0.17	nd	2.46±0.04
Total coliforms	1.91±0.13	1.70±0.07	nd	1.81±0.02

nd: not detectable; DL: Detection limit 0.95 Log CFU/mL

Following lettuce washing with tap water in the absence of chemical sanitizers, 1 Log reduction was achieved in total viable count, *Pseudomonas* spp. and *Enterobacteriaceae*. This result is in accordance with data reported by Allende et al. (2008) and can be attributed to the ability of wash water to mechanically remove contaminating bacteria at the product surface. By contrast, no significant decrease in total coliforms was achieved upon salad washing. This is well known to be attributable to the capacity of many coliforms to enter lettuce plant through the root system and migrate to the edible tissues (Hou et al., 2013). These internalised cells are not removed by typical surface washing operations. A high microbial load was observed in wastewater recovered after salad washing. This contamination level was analogous to that reported by washing different vegetables under industrial procedures (Selma et al., 2008), suggesting that the washing conditions adopted in this study actually mimic a large scale salad washing process.

Wastewater recovered from salad washing was exposed to increasing fluence of UV-C light to evaluate the potential germicidal effect of this technology (Figure 4.1). Following the exposure of wastewater to UV-C light, total viable count significantly decreased. About 3 Log reductions were achieved at the lowest fluence of UV-C light considered in the experiment (0.1 kJ/m^2). On further increase in fluence, a progressive inactivation was detected so that exposure to 0.6 kJ/m^2 UV-C light was associated to a microbial load below the detection limit (10 CFU/mL). In the case of the other microorganisms, not detectable counts were observed by exposure to a much lower fluence (0.1 kJ/m^2). Literature data relevant to decontamination of escarole wastewater by UV-C light report a microbial reduction not exceeding 4 Log CFU/mL (Selma et al., 2008). In that case, wastewater was recirculated in a closed decontamination unit for 60 min. Due to the different set up of the experiment as compared to the one reported in the present paper, data comparison is not possible. It is however likely that lower decontamination levels were probably obtained by these Authors due to the higher thickness of the wastewater layer exposed to UV-C light.

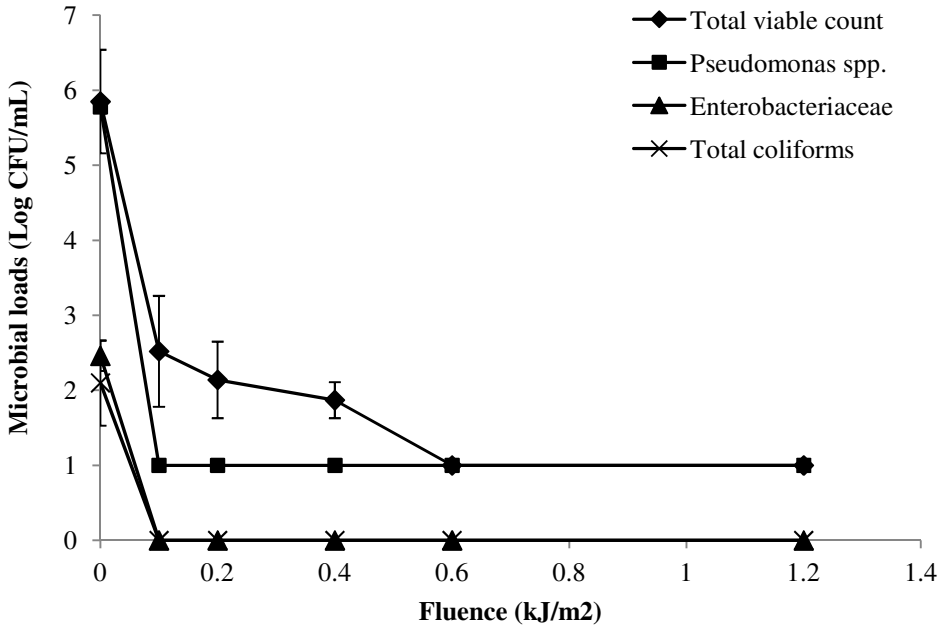

Figure 4.1 Native microflora in lamb's lettuce wastewater exposed to increasing fluence of UV-C light. (Detection limit: 10 CFU/mL for total viable count and *Pseudomonas* spp.; 1 CFU/mL for *Enterobacteriaceae* and total coliforms.)

To verify the inactivation efficacy of UV-C light on pathogens potentially contaminating wastewater, some representative microorganisms were selected. In particular, *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* were considered as species associated with faecal contamination deriving from irrigation water or salad handling after harvesting. These pathogenic microorganisms were inoculated in lamb's lettuce wastewater. The decontamination effectiveness of UV-C light at increasing fluence was then evaluated (Table 4.2).

Table 4.2 Microbial counts (Log CFU/mL) of *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* inoculated in wastewater and exposed to increasing fluence of UV-C light.

Microorganism	Fluence (kJ/m^2)				
	0	0.1	0.4	0.6	1.2
S. enterica	6.63±0.47	<DL	<DL	<DL	<DL
L.monocytogenes	7.02±0.04	<DL	<DL	<DL	<DL
E. coli	7.01±0.44	2.93±0.21	1.82±0.31	1.34±0.80	nd

nd: not detectable; DL: Detection limit 0.95 Log CFU/mL

Microbial counts reported in Table 4.2 show that *Salmonella enterica* was completely inactivated (> 5 Log reductions) in wastewater upon exposure to 0.1 kJ/m^2. Similar fluence was reported to efficaciously inactivate *Salmonella* in secondary effluents (Chang et al., 1985). Data reported in Table 4.2 also show that *Listeria monocytogenes* counts decreased below the detection limit (10 CFU/mL) by exposure to 0.1 kJ/m^2 UV-C light, in agreement with literature data (McKinney et al., 2009). By contrast, UV-C light doses equal to 0.4 kJ/m^2 were required to obtain more than 5 Log reductions in *Escherichia coli* inoculated in wastewater. This result suggests *Escherichia coli* to be more photoresistant than the other pathogenic microorganisms considered in this study. Actually, divergences in the UV susceptibility of most of vegetative bacteria have been observed within different strains (Sommer et al., 2000; Gayán et al., 2011).

A 5 Log reduction in pathogenic bacteria is generally considered to fulfil the requirement for safe water disinfection. Such a level of inactivation was reached for the most photoresistant bacteria (*Escherichia coli*) at a UV-C light dose corresponding to 0.4 kJ/m^2. Such a fluence is considerably lower than that generally required to decontaminate wastewater from other origin, such as municipal wastewaters effluents (Mounaouer and Abdennaceur, 2012). This can be related to the high presence of suspended particles which absorb light and decrease the fluence of the radiation actually penetrating wastewater. Suspended particles may provide protection to microbes and increase the light fluence required to kill the bacteria. This effect could be not negligible when wash water is reused in multiple washing cycles. Selma et al.

(2008) actually observed a significant increase in water turbidity after 2 h of vegetable washing in an industrial wash tank.

In order to evaluate the influence of suspended particles deriving from soil or leached from the leaves on the UV-C light treatment efficacy, the spectral properties of wastewater obtained upon multiple washing up to 5 recycling cycles were characterised. The increase in the number of recycling cycles was associated with an increase in wastewater absorbance at 670 nm (data not shown). This wavelength corresponds with the maximum of absorbance of the chlorophyllian pigments leached from salad leaves (Chappelle et al., 1992). Wastewater also showed an increase in absorbance at 254 nm, which accounts for dissolved organic matter, as well as at 680 nm (Figure 4.2). The latter was chosen as an indicator of suspended particles deriving from leaching of dirt particles from salad.

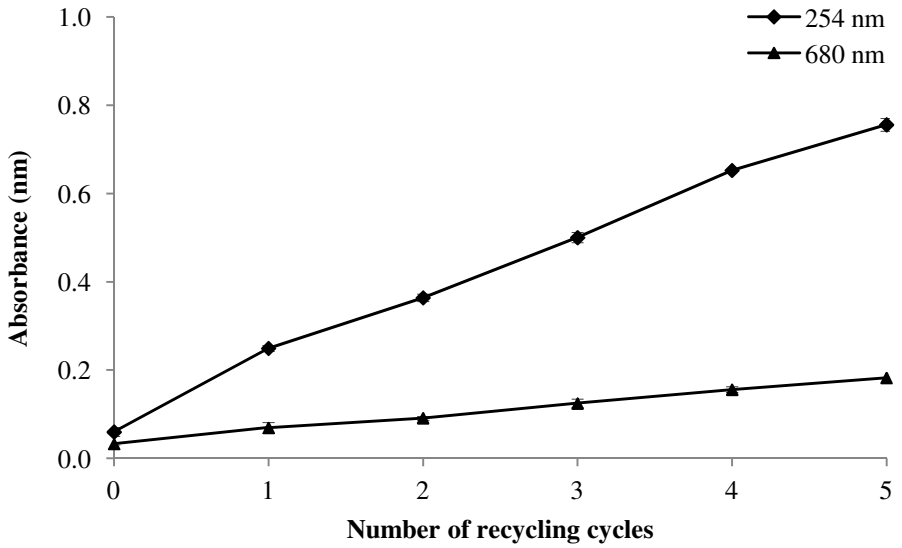

Figure 4.2 Absorbance at 254 and 680 nm of wastewater obtained by increasing washing cycles of fresh-cut lamb's lettuce.

It is noteworthy that 254 nm corresponds to the wavelength of maximum emission of the UV-C lamp used for wash water decontamination. Data shown in figure 4.2 confirm that recycling modifies the spectral properties of water, potentially reducing the transmittance of UV-C light through the

liquid and thus its germicidal efficacy. Further investigations were thus performed to estimate how deep UV-C light can penetrate into wastewater to inactivate microbial cells. To this aim, UV-C transmittance through wastewater was measured and penetration depth of the radiation calculated accordingly. In particular, wastewater samples of increasing thickness were exposed to a known irradiance of UV-C light and the irradiance of the light transmitted through wastewater was measured. Figure 4.3 shows transmittance of UV-C light at increasing depth in wastewater obtained by increasing washing cycles of fresh-cut lamb's lettuce.

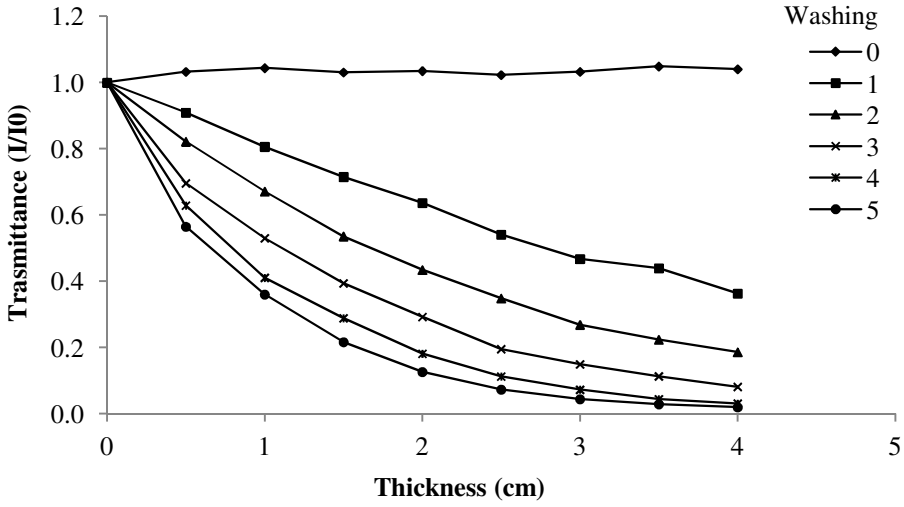

Figure 4.3 Transmittance of UV-C light at increasing depth in wastewater obtained by increasing washing cycles of fresh-cut lamb's lettuce.

As expected, transmittance decreased with water depth as well as with the increase in washing cycles. Data reported in figure 4.3 were well fitted (R^2>0.99) by the Beer-Lambert law (3). Furthermore, in the case of one washing cycle, at least 63% of the incident light at the surface is able to reach eventual microbial cells located at 4.20 cm water depth (Table 4.3). Penetration depth progressively decreased with the number of washing cycles. However, even in the case of 5 successive washing cycles, 63% of the light was able to reach any particle located at less than 0.95 cm water depth. These results indicate that, independently on the number of

recycling cycles, a considerable amount of UV-C light can efficaciously penetrate wastewater if its thickness is lower than *circa* 1 cm.

Table 4.3 Penetration depth of wastewater obtained by increasing washing cycles of fresh-cut lamb's lettuce.

Washing cycles	Penetration depth (cm)
1	4.20±0.58
2	2.37±0.44
3	1.58±0.32
4	1.15±0.12
5	0.95±0.16

On the basis of previous results, the possibility to exploit UV-C light in decontaminating wastewater and allow its reuse in further washing cycles was studied. To this aim, lamb's lettuce wastewater obtained upon multiple washing cycles up to 5 was exposed to 0.4 kJ/m^2 UV-C light. This fluence was chosen since it was found to be efficacious not only in reducing total viable count by about 4 Log but also to achieve more than 5 Log reductions in the pathogenic microorganisms possibly contaminating wastewater (Figure 4.1 and table 4.2). Sample thickness during the treatment was maintained equal to 0.4 cm since this value was well below the penetration depth of UV-C light in the wastewater exposed to 5 washing cycles (Table 4.3). Figure 4.4 shows that, independently on the number of washing cycles, about 3 Log reductions in total viable count and *Pseudomonas* spp. were achieved by exposure to UV-C light under the experimental conditions here selected. In addition, in all cases the UV-C light treatment allowed the counts of *Enterobacteriaceae* to be reduced by *circa* 2 Log, reaching the complete inactivation. Similar results were also obtained for total coliforms (data not shown). These results demonstrate that the germicidal efficacy of the UV-C light treatment is not impaired by treating wastewater from multiple washing cycles.

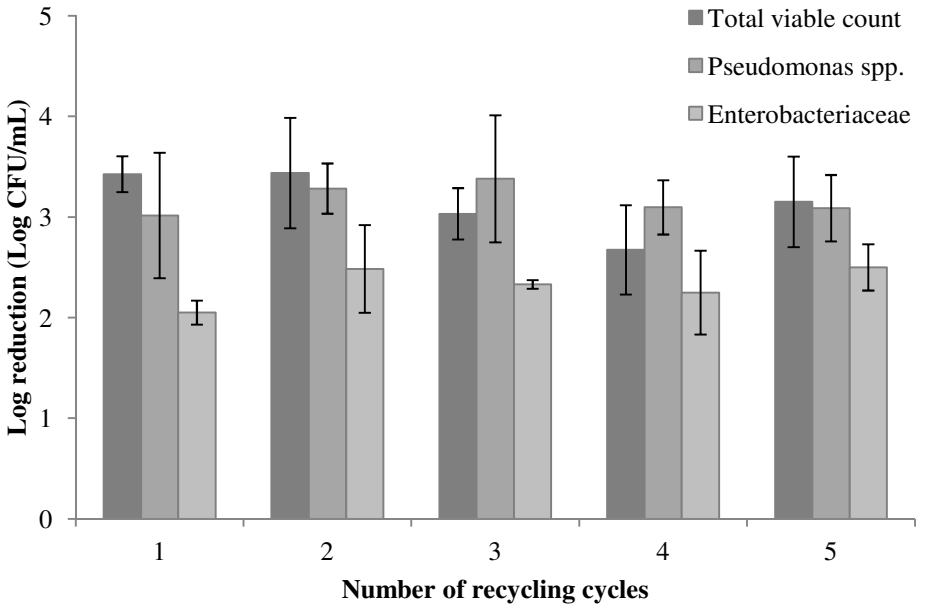

Figure 4.4 Microbial reduction of total viable count, *Pseudomonas* spp. and *Enterobacteriaceae* in wastewater obtained by increasing washing cycles of fresh-cut lamb's lettuce and exposed to 0.4 kJ/m^2 UV-C light.

Lettuce washing is well known to be associated with a reduction in natural microflora of *circa* 1 Log (Allende et al., 2008). In order to verify if UV-C decontaminated wastewater could be recycled without decreasing the hygienic quality of washed lamb's lettuce, salad was analysed for microbial counts before and after each of the 5 washing cycles (Figure 4.5). Independent of the number of wash water recycles, lamb's lettuce washing promoted a number of Log reductions in total viable count, *Pseudomonas* spp. and *Enterobacteriaceae* analogous to that performed with tap water during the first washing cycle.

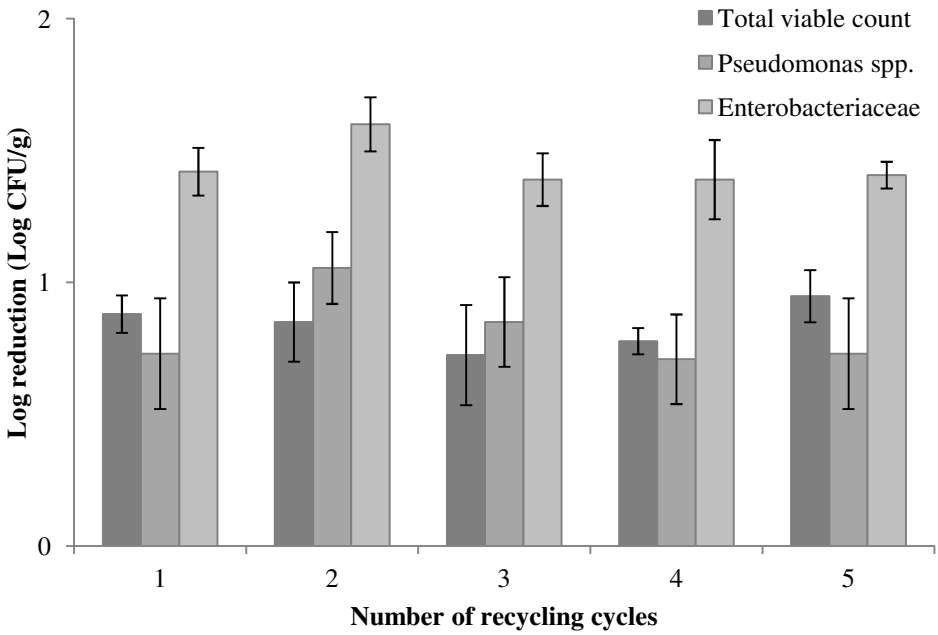

Figure 4.5 Microbial reduction of total viable count, *Pseudomonas* spp. and *Enterobacteriaceae* in lamb's lettuce washed by increasing washing cycles with water exposed to 0.4 kJ/m^2 UV-C light.

Conclusions

The results acquired in this work demonstrate that UV-C treatment represents an optimal tool for decontaminating wastewater deriving from salad washing. This can be attributed to the low amount of solid particles which scarcely decrease penetration depth of light through wastewater. Therefore, the application of this technology could decrease the water footprint of fresh-cut vegetables by minimising the overall requirement for water in industrial plants. In addition, it would decrease the risk for residuals of toxic chemicals in fresh cut vegetables by avoiding the use of sanitizers, such as chlorine, nowadays adopted to minimise cross-contaminations during washing operations.

Chapter 5 Decontamination efficacy of pulsed light on wastewater deriving from fresh-cut salad washing

Water disinfection may be efficaciously accomplished by physical treatments such as UV-C light as demonstrated in chapter 4. Although highly effective, water decontamination by UV-C light usually requires treatment times ranging from many seconds to more than 10 min, depending on processing conditions (e.g. light irradiance, plant geometry, water depth). This disadvantage could be overcome by using pulsed light, which can be considered an improved version of delivering ultraviolet radiation (Gómez-López et al., 2005a). Pulsed light is actually based on exposure to xenon lamp flashes, which typically last from μs to ms. Light flashes are characterised by an intense broad spectrum of wavelength (200–1000 nm) which includes not only ultraviolet (200-400 nm) but also visible (400-700 nm) and infrared (700-1000 nm) light. The latter are known to strengthen the antimicrobial effects of UV-C light by adding a local photothermal effect (Dunn et al., 1995; Fine and Gervais, 2004; Gómez-López et al., 2005a; Guerrero-Beltrán and Barbosa-Cánovas, 2004). The high energy flashes can also provide a photophysical disturbance leading to further structural changes to microorganisms (Krishnamurthy et al., 2008). When rapid disinfection is required, pulsed light may thus provide a practical advantage over ultraviolet technology, allowing multiple flashes to be delivered within few seconds of treatment. Other advantages of pulsed light treatment are the lack of residual compounds, as for UV-C light treatment, but, unlike the latter, xenon flash lamps are more environmental friendly than UV mercury lamps (Gómez-López et al., 2007).

Despite literature data confirming that pulsed light can reduce microbial load and inactivate pathogens in fruit juices and other liquids, no information is available about its bactericidal efficacy on wastewater deriving from the washing of vegetables (Ogihara et al., 2013; Palgan et al., 2011; Pataro et al., 2011).

Aim of the study

This study was designed to investigate the possibility of washing salad by recycling wastewater submitted to pulsed light decontamination. To this

purpose, wastewater obtained by washing lamb's lettuce was exposed to increasing doses of pulsed light. Similarly to the study performed with UV-C light, the germicidal efficacy of the pulsed light treatment was evaluated on native microbiota as well as on inoculated pathogens (*Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*). Also in the case of pulsed light, the possibility to reuse decontaminated wastewater in multiple washing cycles up to 5 was studied.

Results and discussion

Wastewater recovered from salad washing was exposed to increasing fluence of pulsed light to evaluate the potential germicidal effect of this technology (Figure 5.1). Water temperature before exposure to pulsed light was 8 °C and no changes in temperature were detected upon the treatment. Analogously to wastewater decontamination by UV-C light studied in chapter 4, a progressive inactivation of total viable count and *Pseudomonas* spp. was observed by increasing the fluence of pulsed light. Exposure to pulsed light fluence higher than 7.0 kJ/m^2 was associated with a microbial load below the detection limit (10 CFU/mL). Similar effects were also detected in the case of *Enterobacteriaceae* and total coliforms but in a different fluence range. In particular, due to their lower initial load, counts lower than the detection limit (1 CFU/mL) were observed when wastewater was exposed to fluence higher than 2.6 kJ/m^2.

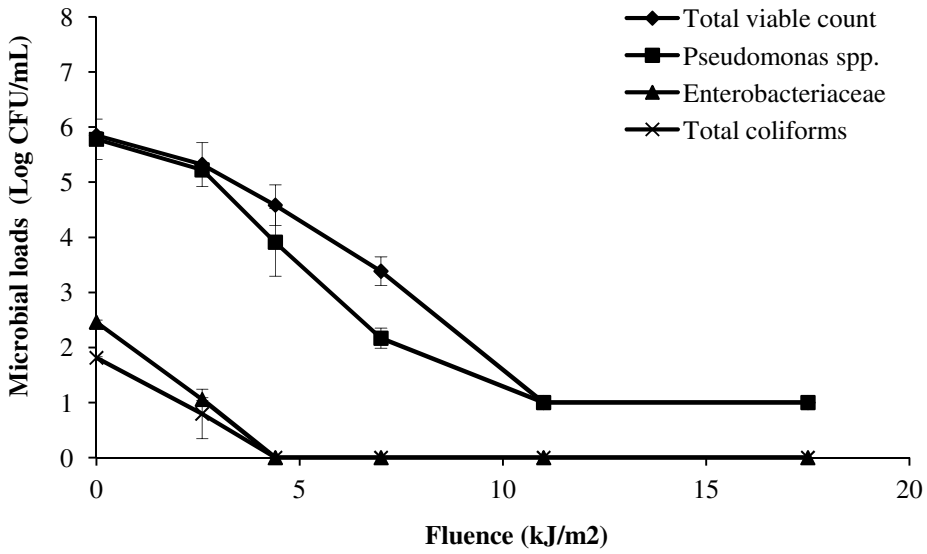

Figure 5.1 Total viable count, *Pseudomonas* spp., *Enterobacteriaceae* and total coliforms in wastewater recovered from lamb's lettuce washing and exposed to increasing fluence of pulsed light.

Pulsed light treatments needed much higher fluence than UV-C light exposure (Chapter 4, figure 4.1) to completely inactivate microorganisms in wastewater. This can be attributed to the different radiation emitted by the two light systems. While UV-C lamps mainly emit at 254 nm, which exert germicidal effect, xenon lamps used for pulsed light emit not only in the UV region but also in a broad spectrum, including visible and infrared (Bintsis et al., 2000). So that much more energy is required for pulsed light to obtain the same decontamination effect of UV-C.

Similarly to the application of UV-C for wastewater decontamination considered in chapter 4, to verify whether pulsed light is able to guarantee the requirement for safe water disinfection, by reducing pathogenic bacteria by at least 5 Log, the germicidal efficacy was tested on selected pathogens (*S. enterica*, *L. monocytogenes* and *E. coli*). Wastewater deriving from lamb's lettuce washing was inoculated with the pathogenic microorganisms selected and exposed to pulsed light with increasing fluence. Relevant microbial counts are shown in figure 5.2.

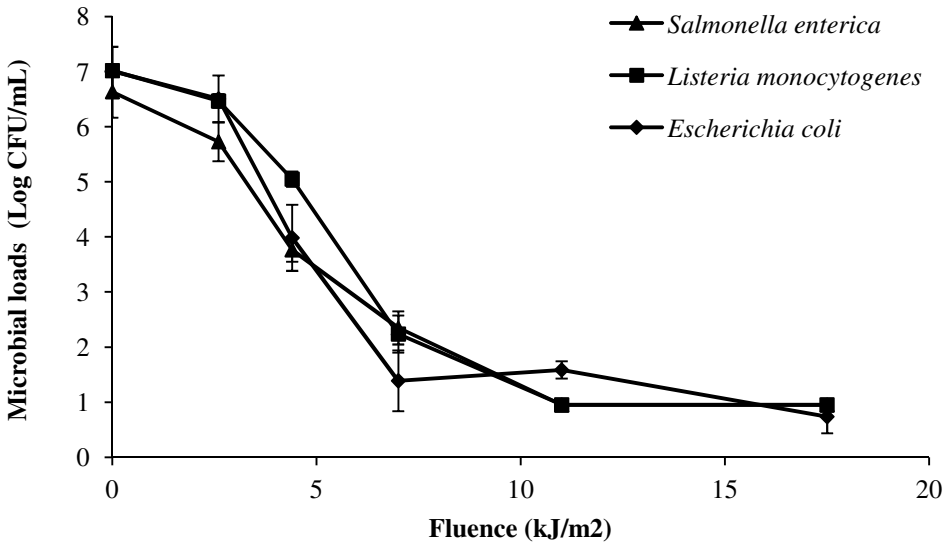

Figure 5.2 *Salmonella enterica, Listeria monocytogenes* and *Escherichia coli* inoculated in wastewater recovered from lamb's lettuce washing and exposed to increasing fluence of pulsed light.

Pathogenic bacteria inoculated in wastewater were highly sensitive to pulsed light, potentially allowing more than 6 Log reductions to be achieved. In particular, *S. enterica* and *L. monocytogenes* were completely inactivated (detection limit, 10 CFU/mL) upon exposure to fluence higher than 7.0 kJ/m^2 . Residual counts of *E. coli* were still detectable in wastewater exposed to the highest pulsed light fluence due to the lower detection limit of their assessment (1 CFU/mL). These data suggest the selected microorganisms to exert similar sensitivity to pulsed light, apparently contrasting with literature reports. The latter indicate possible divergences in the photosensitivity of different microorganisms inoculated in liquid and solid foods (Gómez-López et al., 2005b; Palgan et al., 2011). For instance, the thicker peptido-glycan cell wall of Gram-positive microorganisms would make them more resistant to light radiation. In addition, the conditions of inoculation and adaptation in the media could deeply affect microbial susceptibility. Most of the literature information is actually referred to the effect of pulsed light on microorganisms spread onto the surface of solid foods. In these cases, inoculation is followed by an adaptation period which may last hours. In the experimental conditions here tested, only 30 min of adaptation was

allowed before pulsed light treatment. This choice was performed to simulate the timing of a possible industrial decontamination of wastewater deriving from salad washing. It can be inferred that the limited adaptation time probably made negligible the effects of the different sensitivity of the microorganisms to pulsed light (Figure 5.2).
Based on the promising results obtained by analysing the germicidal effect of pulsed light in wastewater, the possibility to exploit this technology to decontaminate wastewater and allow its reuse in more than one washing cycle was studied. To this aim, lamb's lettuce wastewater obtained upon multiple washing cycles up to 5 was exposed to 11.0 kJ/m^2 pulsed light. This fluence was chosen since it allowed more than 5 and 6 Log reductions in native microflora and inoculated pathogens respectively (Figure 5.1 and 5.2).

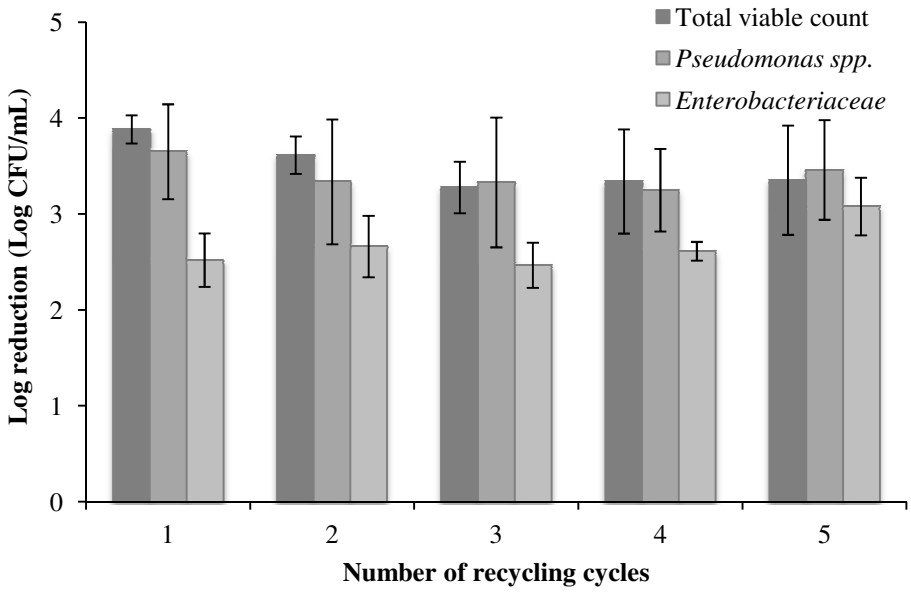

Figure 5.3. Microbial reduction of total viable count, *Pseudomonas* spp. and *Enterobacteriaceae* in wastewater obtained by increasing washing cycles of fresh-cut lamb's lettuce and exposed to 11.0 kJ/m^2 pulsed light.

Figure 5.3 shows that, independent on the number of washing cycles, more than 3 Log reductions in total viable count and *Pseudomonas* spp. were achieved by exposure to pulsed light under the experimental

conditions here selected. Also in the case of *Enterobacteriaceae*, the number of washing cycles did not affect the extent of inactivation. Similar results were also obtained for total coliforms (data not shown). These results demonstrate that the germicidal efficacy of pulsed light is not impaired when treating wastewater from multiple washing cycles, as also observed for UV-C light treatment (Chapter 4, Figure 4.4). The latter, however, required longer processing time than pulsed light for the same decontamination effect. In particular, wastewater samples were submitted to 20 s UV-C light (Chapter 4) while only 50 µs of exposure to pulsed light were needed.

In order to verify if the use of pulsed light treated wastewater could decrease the hygienic level of lamb's lettuce, salad was taken after each washing cycle and analysed for microbial counts. Independently of the number of wash water recycles, a 1 Log reduction in lamb's lettuce was observed in all cases, analogously to UV-C decontaminated wastewater recycle (Chapter 4, Figure 4.5). This can be attributed to the fact that salad decontamination promoted by washing only depends on the ability of the water flow to mechanically remove contaminating bacteria at the product surface. This physical property is obviously not affected by the eventual treatment of wastewater with pulsed light.

Conclusions

Analogously to UV-C light (Chapter 4), wastewater decontamination was efficaciously demonstrated for pulsed light treatment. Due to the wide emission spectrum of the xenon lamps, much more energy than UV-C light treatment was needed. However, pulsed light required significant lower processing time to obtain the same bactericidal effect achieved by UV-C. Results thus suggest that pulsed light technology could be exploited to perform wastewater decontamination, meeting the increasing industrial requirements for rapid and environmental friendly methods.

Conclusions part I

The first part of this PhD thesis investigated the possibility to adopt novel decontamination technologies in salad washing in order to avoid the use of highly chlorinated aqueous solutions and reduce the water amount required for this operation. In particular, electrolyzed water solutions characterized by adequate physicochemical properties, were demonstrated to be an alternative to conventional chlorination with NaOCl (Chapter 3). Data obtained indicate that high bactericidal effect in salad wash water can be obtained by using electrolyzed water solutions with a drastically reduction in the free chlorine concentration. The antimicrobial effect at lower free chlorine concentrations compared to NaOCl could be due to the synergistic effect of some physicochemical and chemical properties of washing solutions such as pH, ORP and the presence of oxidizing species other than chlorine compounds.

Furthermore, light technologies proved to be a successful decontamination method able to completely avoid the use of chemical compounds during salad washing (Chapters 4 and 5). In particular, UV-C and pulsed light allowed the re-use of wastewater for multiple washing cycles, without compromising the hygienic quality of fresh-cut salad. In addition, in an industrial perspective, pulsed light technology could be efficaciously applied since it demonstrated to be time-effective.

In conclusion, the technologies considered in this PhD thesis proved to be promising methods able to avoid cross-contamination during salad washing, reducing or completely eliminating the use of chemicals and positively affecting the water footprint of fresh-cut salad production.

PART II Application of novel non-thermal technologies for fruit and vegetables processing

Novel non-thermal technologies could be exploited in fruit and vegetables processing to overcome the safety, quality and environmental issues described in Chapter 1. In particular, emerging technologies could provide a killing step to enhance safety of fresh-cut produce as well as improve product quality acting on the physiological and biochemical aspects which influence product decay and shelf life. In addition, the adoption of novel technologies having minor environmental impact compared to the conventional food processing could be a step towards a greener food production. However, much research on the application of novel technologies is needed to optimize the processing conditions to guarantee safety while assuring appropriate quality of the final product.
The aim of the second part of this PhD research was thus to investigate the feasibility of selected emerging non-thermal technology for fruit and vegetable processing. In particular, the effect of UV-C light on lettuce and pulsed light on apple intended for fresh-cut produce were studied with reference to safety and quality issues. In addition, the possibility to exploit dense-phase CO_2 for quality enhancement of vegetable products was considered. Finally, the application of pulsed electric field on potato products was studied as an example of novel technology able to reduce energy and chemical compounds both of which have a positive environmental benefit.

Chapter 6 Effect of UV-C light on fresh-cut salad

UV-C light is a powerful non-thermal technology used in a broad range of antimicrobial applications including water decontamination, as previously investigated in chapter 4. In addition, ultraviolet radiation is exploited for disinfection of air, food preparation surfaces and packaging materials (Wang et al., 2005). More recently UV-C light has been proposed as a bactericidal technology for food treatment, being easy to use, with favourable costs of equipment, energy and maintenance (Bintsis et al., 2000). Although its poor penetration capacity, the application of UV-C

light is particularly suitable for surface treatment of products where microbial activity mainly occurs at the outer part. Furthermore, this technology was employed to decontaminate those food products which can not be subjected to heat treatment and/or need to retain their fresh-like appearance such as meat derivates, eggs and more recently fresh-cut fruit and vegetables (Sommers et al., 2010; Manzocco et al., 2011a,b; Bermúdez-Aguirre and Barbosa-Cánovas, 2013). The latter could also be treated with UV-C light in order to delay or inhibit the enzymatic activity that leads to quality loss during storage (Guerrero-Beltràn and Barbosa-Cánovas, 2006; Manzocco et al, 2009). Several Authors demonstrated UV-C light to be highly efficient against both microbial and enzymatic activity in fresh-cut fruit and vegetables. Gómez et al. (2010) promoted fresh-cut apple decontamination by using UV-C light treatment up to 14 kJ/m^2. However, the prolonged UV-C light exposure resulted in marked browning in apple surface during storage, probably due to cell damage and increased enzymatic activity. Bermúdez-Aguirre and Barbosa-Cánovas (2013) considered different vegetables as carrot, lettuce and tomato and concluded that product surface greatly affects light disinfection efficacy. In fact, data acquired in that study revealed that the more complex in terms of porosity and roughness is the vegetable surface, the less efficient is the UV-C treatment. Similar differences were observed by Birmpa et al. (2013) when comparing UV-C light antimicrobial efficacy in lettuce and strawberry which present different surface irregularities. In addition, as also observed by Gómez et al. (2010), high UV-C light fluence up to 72 kJ/m^2 allowed to increase the decontamination effect but had adverse consequences on product colour.
Thus, the efficacy of UV-C light treatment greatly depends on the food matrix characteristics as well as on the possibility to identify proper conditions able to impart the desired decontamination effect without impairing product fresh-likelihood.

Aim of the study

The aim of the present work was to evaluate the effect of UV-C light on microbial inactivation and overall quality of fresh-cut lamb's lettuce. The bactericidal effect of UV-C light was initially studied on native microflora such as *Pseudomonas* spp. The optimal dose was thus

identified by submitting lamb's lettuce to UV-C light in a wide range of fluence conditions. Following, samples exposed to the optimum dose were packed and stored at 4 °C for increasing time up to 21 days. During storage UV-C light treated samples were analysed for native microflora, weight loss, firmness, sliminess and sensory quality index.

Results and discussion

Lamb's lettuce washed in tap water to remove dirt deriving from soil was submitted to increasing fluence of continuous UV-C light up to 6.0 kJ/m^2. Initial microbial count of lettuce was mainly characterized by *Pseudomonas* spp. as previously shown in Part I (Table 3.4). To this regard, the effect of UV-C light was assessed on this microbial population (Table 6.1). Exposure to increasing fluence of UV-C light significantly reduced *Pseudomonas* spp., reaching *circa* 1 Log reduction at 0.9 kJ/m^2. However, further increase in light fluence appeared less effective and a maximum reduction of 1.3 Log was achieved at 6.0 kJ/m^2 corresponding to a total exposure time of 300 s, difficultly applicable in the fresh-cut industry. Literature reports similar results for other leafy vegetables exposed to UV-C light. In particular, less than 1 Log reduction in total aerobic mesophilic bacteria in Tatsoi baby leaves was achieved by exposure to 4.5 kJ/m^2 UV-C light (Tomás-Callejas et al., 2012), while psychrotrophic bacteria in baby spinach were reduced by *circa* 2 Log at much higher values exceeding 12 kJ/m^2 (Escalona et al., 2010). It could be thus inferred that UV-C light, which is a surface technology, is not able to completely inactivate the microorganisms that can internalize into leafy vegetables.

Table 6.1 *Pseudomonas* spp. in fresh-cut lamb's lettuce as a function of exposure time to 20 W/m^2 UV-C light.

Exposure time (s)	Fluence (kJ/m^2)	*Pseudomonas* spp. (Log CFU/g)
0	0	6.11±0.07^{a}
10	0.2	5.95±0.09^{a,b}
20	0.4	5.75±0.13^{b}
45	0.9	5.13±0.11^{c}
60	1.2	5.01±0.18^{c,d}
300	6.0	4.83±0.17^{d}

Means with same letter are not significantly different ($p \geq 0.05$).

Lamb's lettuce exposed to 0.9 kJ/m^2 was packed and stored for increasing times at 4 °C to simulate storage of fresh-cut salad along the refrigerated chain. Samples were then analysed for changes in *Pseudomonas* spp. (Figure 6.1). Despite the initial reduction of *circa* 1 Log in *Pseudomonas* spp. in the UV-C light treated sample, a significantly higher microbial growth compared to untreated lettuce was observed during storage. In fact, after 7 days of storage UV-C light treated lettuce presented higher *Pseudomonas* spp. count. Similar results were reported for other fresh-cut leafy vegetables where the initial microbial count was reduced by UV-C light treatment but higher bacterial growth was observed during refrigerated storage (Artés-Hernández et al., 2009; Escalona et al., 2010; Tomás-Callejas et al., 2012). Thus, it has been suggested that UV-C light could alter the cell permeability and may result in a greater leakage of nutrients such as aminoacids and carbohydrates which might stimulate microbial growth.

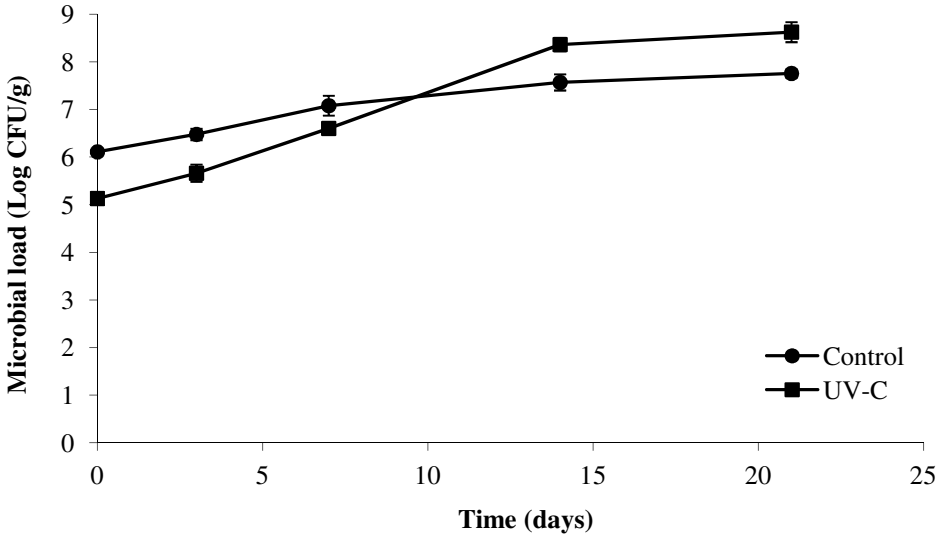

Figure 6.1 *Pseudomonas* spp. in lamb's lettuce exposed to 0 (Control) and 0.9 kJ/m^2 UV-C light as a function of storage time at 4 °C.

In order to evaluate the effect of UV-C light treatment on quality evolution of fresh-cut lettuce during storage, some quality attributes were considered. Weight loss of UV-C light treated lettuce significantly increased during refrigerated storage similarly to control sample (Table 6.2). This trend could be solely attributed to plant tissue respiration and surface dehydration (Martínez et al., 2008). Despite the significant increase in dehydration in both control and UV-C treated samples, a decrease in firmness was only observed in the irradiated lettuce at day 21 of storage (Table 6.2). It is noteworthy that this storage time is well above the shelf life expected for fresh-cut salad. It could be inferred that the lower firmness measured in the UV-C treated lettuce could be attributed to light induced tissue damage. To this regard, Allende et al. (2006) reported that 7.1 kJ/m^2 UV-C irradiation induced tissue softening in lettuce during storage. The loss of mechanical properties could be thus the result of the damaged tissue which enhance the activity of both enzymes and microorganisms. The latter could also affect the visual quality of the product. In fact, during storage a slimy film on the surface of lamb's lettuce was visually observed. In order to evaluate the variations in terms of visual quality, the extent of surface sliminess was calculated on both untreated and treated samples during storage. Table 6.2 shows the rapid

increase of sliminess at day 3 while during storage slightly differences can be observed. The presence of a slimy film can be mainly associated with the activity of psychrotrophic bacteria such as *Pseudomonas* spp. responsible for the depletion of the fresh-like appearance of the product (Ragaert et al., 2007). However, the sliminess on the surface of UV-C light treated lettuce resulted much higher than that on the untreated lettuce. As already discuss, UV-C radiation could induce tissue damage and increase microbial growth (Figure 6.1).

Table 6.2 Weight loss, firmness and sliminess of lamb's lettuce exposed to 0 (Control) and 0.9 kJ/m^2 UV-C light (UV-C) as a function of storage time at 4 °C.

Storage time (days)	Weight loss (%)		Firmness (N)		Sliminess (%)	
	Control	UV-C	Control	UV-C	Control	UV-C
0	-	-	7.8 ± 0.7^{aA}	7.7 ± 0.1^{aA}	-	-
3	2.1 ± 0.2^{dA}	1.4 ± 0.7^{cA}	8.6 ± 0.6^{aA}	7.8 ± 0.5^{aA}	3.1 ± 0.1^{bB}	8.4 ± 0.5^{aA}
7	4.1 ± 0.3^{cA}	3.2 ± 0.8^{bA}	8.7 ± 0.9^{aA}	8.2 ± 0.7^{aA}	3.2 ± 0.4^{bB}	8.5 ± 0.3^{aA}
14	4.8 ± 0.1^{bA}	5.6 ± 1.2^{aA}	7.5 ± 0.9^{aA}	6.7 ± 1.1^{aA}	3.4 ± 0.2^{bB}	9.7 ± 0.3^{bA}
21	6.2 ± 0.4^{aA}	5.4 ± 0.7^{aA}	8.9 ± 1.3^{aA}	4.1 ± 0.2^{bB}	4.4 ± 0.1^{aB}	9.8 ± 0.2^{bA}

For each property, means within column with same lowercase letter are not significantly different ($p \geq 0.05$).

For each property, means within row with same uppercase letter are not significantly different ($p \geq 0.05$).

Finally, a sensory evaluation of fresh-cut lettuce during storage was performed. In particular, images of lamb's lettuce exposed to 0.9 kJ/m^2 UV-C light and stored for increasing time were evaluate by a trained panel and results were expressed as quality index ranging from 5 to 1. Figure 6.2 shows the evolution of the quality index of the fresh-cut lettuce during refrigerated storage. The UV-C light treated lettuce was characterized by an initial lower quality index value than the control sample probably due to some damaged leaf as a consequence of the preparation procedures used. However, during storage no significant differences among sample were observed. The quality index of light treated lettuce drastically decreased after 3 weeks of storage, because of yellow, damaged and slimy leaves as partially described in table 6.2.

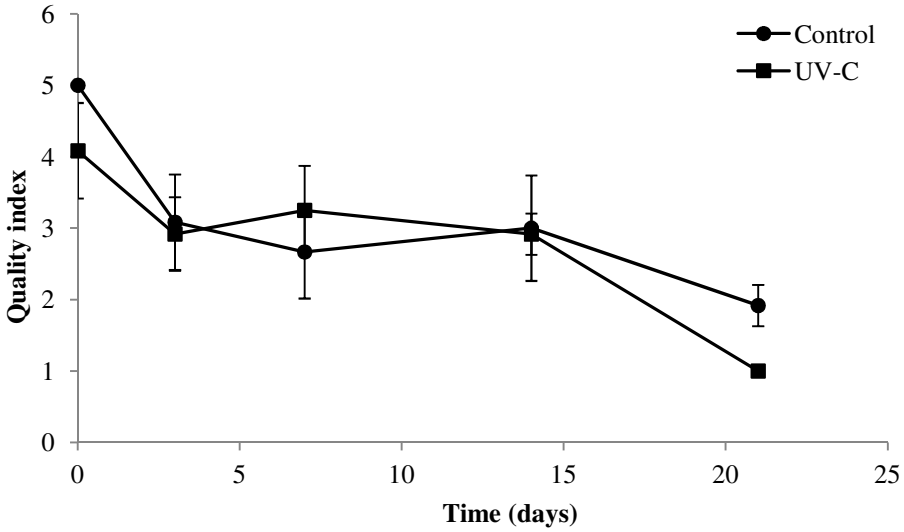

Figure 6.2 Quality index of lamb's lettuce exposed to 0 (Control) and 0.9 kJ/m^2 UV-C light (UV-C) as a function of storage time at 4 °C.

Conclusions

Results obtained in this chapter demonstrate that UV-C light could reduce initial microbial count of fresh-cut lettuce. However, in the experimental conditions here tested, light treatment promoted structural modification of vegetable tissue with consequent increase in microbial growth during refrigerated storage. In addition, the UV-C light treated lettuce was also characterized by a negative biofilm detectable by consumers.

Although highly efficient against microbial and enzymatic activity in several fresh-cut fruit and vegetables, UV-C radiation proved to be critical in the case of leafy vegetables. In fact, the latter are characterized by an extremely delicate structure and a microbial population internalized into the vegetable tissue. In addition, it could be hypothesized that even applying pulsed light, the decontamination efficacy can not be improved. For these reasons, further investigations were carried out on fresh-cut apple, chosen as representative of plant matrix having a more resistant and less delicate structure than leafy vegetables.

Chapter 7 Effect of pulsed light on fresh-cut apple

The possibility to exploit UV-C light to treat the surface of fresh-cut fruits such as apple has been already studied (Gómez et al., 2010; Manzocco et al., 2011;). However, the application of UV-C light requires long time as also demonstrated in Chapter 6. For this reason, pulsed light could overcome this disadvantage by exerting bactericidal effects in very short treatment time. In addition, pulsed light has been demonstrated to be highly capable of inactivating microorganisms in several liquids, as previously described in Chapter 5 for wastewaters, and on different surfaces due to its UV-C component. However, pulsed light efficacy is tightly influenced by the degree and nature of microbial contamination, process parameters and properties of the treated food matrix (Gómez-López et al., 2007). The latter influences the efficacy with regard to transparency or opacity, surface composition and morphology. In particular, in the case of solid foods, the target surface should be as smooth as possible, since vast irregularities and light-absorbing matter constitute a shelter for microbial contaminants and an obstacle for the incident light (Gómez-López et al., 2007; Sommers et al., 2010). In addition, most of the research carried out on the application of pulsed light to fresh-cut product involved the decontamination of vegetables having firm structure and texture, since more delicate products such as leafy vegetables have been already demonstrated to have some criticality when the sole UV-C light was applied (Chapter 6). Literature data has thus shown 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; Ramos-Villaroel et al., 2012a,b), as well as native microflora and inoculated bacteria in sliced apple (Gómez et al., 2012a). However, pulsed light required for decontamination (60–1200 kJ/m^2) can induce not only photochemical but also photothermal effects, as previously described in Chapter 5, impairing colour, structure and sensory characteristics of fresh-cut products (Oms-Oliu et al., 2010). Despite these findings, few data are available on pulsed light effects on quality and sensory properties of fresh-cut fruit and vegetables. For this reason, there is a need for

optimising treatment intensity to achieve specific decontamination levels without affecting product freshness.

Aim of the study

The aim of the present work was to evaluate the effect of increasing fluence of pulsed light on bacterial inactivation and overall quality of fresh-cut apple. To this regard, Golden Delicious apple slices were submitted to pulsed light in a wide range of fluence conditions. The bactericidal effect of pulsed light was initially studied on native microflora and inoculated bacteria (*Lactobacillus brevis* and *Listeria monocytogenes*). Samples were then stored at 4 °C for increasing times up to 7 days, and analysed for native microflora, colour, firmness, weight loss and sensory attributes. Temperature increase and structural modifications in sliced apple were also taken into account to hypothesise possible mechanisms of action of pulsed light.

Results and discussion

Apple slices equilibrated at 4 °C were submitted to pulsed light treatments with increasing fluence by delivering up to 9 pulses to the sample, each having a fluence of 17.5 kJ/m^2. Sample temperature was found to progressively increase with pulsed light fluence; each pulse producing an increase in sample temperature of about 2 °C. As a consequence, the temperature of apple slices exposed to the highest intensity treatment approached 26 °C (Table 7.1).

Table 7.1 Temperature of apple slices exposed to increasing fluence pulsed light.

Fluence (kJ/m^2)	Temperature (°C)
0	5.9±0.1
17.5	8.7±0.4
52.5	13.9±0.1
105.0	20.6±0.7
157.5	25.8±0.3

Initial microbial counts of apple slices were in the same range of fresh-cut apples reported in the literature (Rojas-Graü et al., 2007; Gómez et al.,

2012b). In particular, a low presence of mesophilic bacteria (2.26±0.12 log CFU/cm^2) was detected. Yeasts, moulds and *Enterobacteriaceae* counts were lower than the detection limit(50 CFU/cm^2). Following the exposure of apple slices to pulsed light, total viable count was not detectable. Considering that the temperature of apple slices exposed to pulsed light never exceeded 26 °C, the decrease in microbial counts can be attributed to the well known bactericidal effect of the UV-C component of pulsed light. The latter may actually perturb microbial cells, affecting the phase of adaptation to substrate and the ability to replicate (Bintsis et al., 2000; Giese and Darby, 2000). Table 7.2 shows the effect of pulsed light on *L. brevis* and *L.monocytogenes* inoculated on apple slices to verify the inactivation efficacy of pulsed light. These microorganisms were chosen as representative of spoilage and pathogenic microorganisms, potentially contaminating apple slices during preparation. Exposure to low intensity pulsed light (17.5 kJ/m^2) was found to significantly decrease *L. brevis* and *L. monocytogenes* by 3.0 and 2.7 Log reductions respectively. However, further increases in light fluence appeared less effective. Microbial inactivation data (Table 7.2) indicate that, independent of the intensity of the treatment and the bacterial type, microbial counts never decreased to values lower than circa 2 Log CFU/cm^2. This can be attributed to the fact that microorganisms will survive if they are shaded by any protective layers between them and the light source. Local surface roughness derived from cutting operations is expected to shade eventual microbial cells, thus impairing the germicidal effect. In addition, the inoculum procedure could stimulate the cells to physically locate into these microscopic surface irregularities. Finally, it is not excluded that, given the high microbial count, microbes could shield each other in protecting from light (Gómez-López et al., 2005a). It is noteworthy that Gómez et al. (2012a) observed 1.4 Log reductions in *Listeria innocua* inoculated on apple slices upon exposure to 1100 kJ/m^2.In the present work, much lower fluence was needed to obtained higher germicidal effects (Table 7.2).

Table 7.2 Microbial counts of inoculated microorganisms (*Lactobacillus brevis* and *Listeria monocytogenes*) in apple slices exposed to increasing fluence pulsed light.

Fluence (kJ/m^2)	*Lactobacillus brevis* 20054 DSMZ (log CFU/cm^2)	*Listeria monocytogenes* 20600 DSMZ (log CFU/cm^2)
0	5.88 ± 0.21^{a}	6.54 ± 0.44^{a}
17.5	2.90 ± 0.37^{b}	3.86 ± 0.12^{b}
52.5	$2.30\pm0.37^{b,c}$	3.69 ± 0.25^{b}
105.0	$2.28\pm0.28^{b,c}$	2.55 ± 0.22^{c}
157.5	2.15 ± 0.45^{c}	2.72 ± 0.22^{c}

Detection limit 1.70 log CFU/cm^2.

For each microorganism, means indicated by the same letter are not significantly different ($p\geq0.05$).

The different microbial response to pulsed light could be attributed to the different geometry of the lamps in the equipment. Apple slices submitted to increasing fluence of pulsed light up to 157.5 kJ/m^2 were stored for increasing times at 4 °C to simulate storage of fresh-cut apple along the refrigerated chain. Samples were then analysed for changes in native microflora. Total viable counts, yeast, moulds and *Enterobacteriaceae* were considered as indicators of the general hygienic condition of the products. Table 7.3 shows, as an example, the changes in total viable count and yeasts during storage of apple slices exposed to 17.5 kJ/m^2 pulsed light. Untreated apple slices (0 kJ/m^2) are also shown as controls. During refrigerated storage, the untreated control sample showed an increase in total viable count and yeasts, while no moulds or *Enterobacteriaceae* were detectable during storage (data not shown). By contrast, in the pulsed light treated apple slices, the counts of all microorganisms were lower than the limit of detection during the entire storage period. As expected, similar results were also obtained for samples exposed to higher fluence up to 157.5 kJ/m^2 (data not shown).These data not only confirm the decontamination efficiency of pulsed light, but also indicate its capability to promote higher microbial stability during storage, reasonably leading to a longer shelf life. To this regard, some European countries, such as Spain, France and Germany, have adopted specific microbiological criteria for minimally processed fruit and vegetables (Francis et al., 1999; BOE, 2001). Furthermore,

Ragaert et al. (2011) recently proposed specific microbiological guidelines, with 7 Log CFU/g as a maximum limit for total viable count of these products. In this study, the microbial count was within this limit in both control and pulsed samples during the entire storage period under study (7 days). It can be inferred that the product does not reach the end of shelf life because of excessive microbial count, but following the decrease of quality parameter due to physiological decay of plant tissue.

Table 7.3 Total viable count and yeast during storage at 4 °C of apple slices exposed to 17.5 kJ/m^2 pulsed light. Untreated apple slices (0 kJ/m^2) are shown as control.

Fluence (kJ/m^2)	Time (days)	Total viable count (log CFU/cm^2)	Yeasts (log CFU/cm^2)
0	0	2.62±0.15^c	<DL
	1	2.78±0.03^c	<DL
	3	3.57±0.12^b	2.04±0.15^a
	7	4.30±0.06^a	2.68±0.14^a
17.5	0	<DL	<DL
	1	<DL	<DL
	3	<DL	<DL
	7	<DL	<DL

DL: Detection limit 1.70 log CFU/cm^2.
For each microorganism, means indicated by the same letter are not significantly different (p≥0.05).

Apple slices exposed to increasing fluence pulsed light were analysed for firmness, weight loss and colour. No significant changes (p≥0.05) in firmness of the pulsed light treated samples were observed just after the treatment and during storage (data not shown). By contrast, a slight increase in weight loss was observed just after exposure to pulsed light (Table 7.4). Despite the moderate increase in temperature, it is not excluded that local temperature on apple surface could be higher than that actually measured, potentially favouring surface dehydration and sample weight loss (Table 7.4). The effect of pulsed light on weight loss was only observed in samples immediately after treatment. By contrast, a similar change in weight loss during storage was detected in all samples (data not shown). This can be attributed to the physiological dehydration of apple

tissues during storage, being much higher than the weight loss initially induced by the pulsed light treatment.

Table 7.4 Weight loss of apple slices just after exposure to increasing fluence pulsed light.

Fluence (kJ/m^2)	Weight loss (% w/w)
0	0.36 ± 0.04^{c}
17.5	0.37 ± 0.06^{c}
52.5	0.54 ± 0.05^{c}
105.0	0.87 ± 0.05^{b}
157.5	1.63 ± 0.15^{a}

Means indicated by the same letter are not significantly different ($p\geq0.05$).

The effect of increasing pulsed light fluence on apple slice colour during storage is shown in Table 7.5. Under the experimental conditions applied in this work, pulsed light did not affect the initial lightness and green to red point of apple slices. However, during storage, samples showed different changes in colour depending on the treatment intensity. As expected, in untreated apple slices (0 kJ/m^2), a decrease in lightness and an increase in a* values were detected. These typical changes are attributable to the development of enzymatic browning which rapidly occurs after cutting (Sapers and Douglas, 1987). Actually, slight colour changes were observed one day after sample preparation. Similar colour changes were also detected in apple slices exposed to 17.5 kJ/m^2 pulsed light. Higher intensity treatments significantly increased colour changes in apple slices so that after one week of storage at 4 °C, their overall browning level was higher than that of the untreated and 17.5 kJ/m^2 treated samples. Significant browning was also observed in the case of apple slices exposed to intense fluence of UV-C light (Gómez et al., 2010; Manzocco et al., 2011a). This effect could be attributed to the breakage of cellular membranes which would lead to a loss of cell compartmentalisation. The latter would increase the contact between enzyme and substrate favouring browning reactions.

Table 7.5 Lightness (L*) and colorimetric parameter a* (green to red) during storage at 4 °C of apple slices exposed to increasing fluence of pulsed light.

Parameter	Fluence (kJ/m^2)	Storage time (days)			
		0	1	3	7
L*	0	78.3 ± 1.1^{aA}	75.7 ± 1.5^{aA}	76.2 ± 1.5^{aA}	78.0 ± 1.3^{aA}
	17.5	77.6 ± 1.3^{aA}	$74.8\pm1.0^{a,bB}$	77.6 ± 0.7^{aA}	77.1 ± 0.8^{aA}
	52.5	78.4 ± 1.4^{aA}	$74.1\pm0.6^{a,bB}$	72.7 ± 0.6^{bC}	$73.1\pm1.0^{bB,C}$
	105.0	77.4 ± 0.7^{aA}	$73.4\pm0.7^{b,cB}$	$72.6\pm0.7^{bB,C}$	$71.9\pm0.4^{b,C}$
	157.5	78.7 ± 1.0^{aA}	$72.6\pm0.9^{b,cB}$	72.0 ± 0.6^{bB}	69.7 ± 0.8^{cC}
a*	0	-4.2 ± 0.6^{aB}	-1.5 ± 1.0^{aA}	-1.3 ± 0.3^{cA}	-1.2 ± 0.2^{cA}
	17.5	-4.6 ± 0.8^{aB}	-1.5 ± 0.4^{aA}	-1.6 ± 0.2^{cA}	-1.7 ± 0.2^{cA}
	52.5	-4.6 ± 0.3^{aB}	-0.7 ± 0.6^{aA}	0.1 ± 0.2^{bA}	0.3 ± 0.6^{bA}
	105.0	-4.2 ± 0.2^{aD}	-1.1 ± 0.2^{aC}	$0.3\pm0.3^{a,bB}$	1.8 ± 0.3^{aA}
	157.5	-4.5 ± 0.6^{aC}	0.4 ± 0.6^{bB}	0.9 ± 0.5^{aB}	2.7 ± 0.9^{aA}

For each parameter, means within column with same lowercase letter are not significantly different ($p \geq 0.05$).

For each parameter, means within row with same uppercase letter are not significantly different ($p \geq 0.05$).

The hypothesis of the loss of cell integrity upon pulsed light exposure was also supported by optical microscopy observations performed to assess the structure changes produced, at a cellular level, by the different treatments. The photomicrographs (Figure 7.1) show that in fresh tissue, cells are regular in shape and appear turgid with an apparent consistent cell wall structure. The large amount of cell volume was occupied by the central vacuole, and the protoplasm, bounded by the plasmalemma and tonoplast, was present as a thin layer lining the cell surface (Figure 7.1A). The same type of structure was also observed for the sample exposed to 17.5 kJ/m^2 (Figure 7.1B). However, the treatment with the highest fluence (157.5 kJ/m^2) caused the rupture of the membranes, a decrease in intracellular volume and the loss of turgidity of the cells (Figure 7.1C). These structural changes could be the reason for the effect of pulsed light on both weight loss (Table 7.4) and browning (Table 7.5).

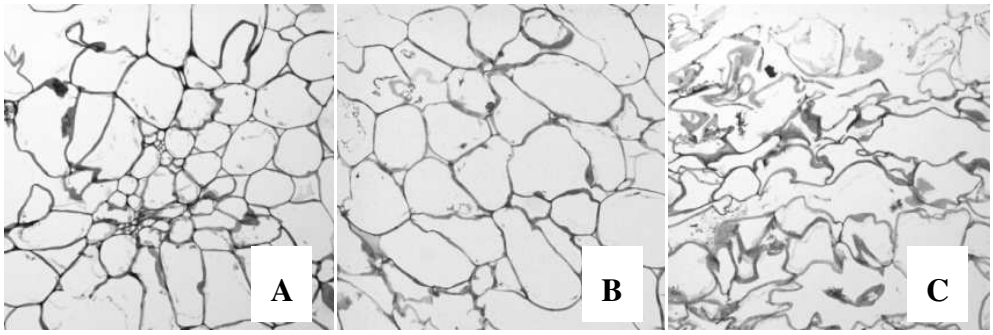

Figure 7.1 Microscopy images of fresh apple slice exposed to 0 (A), 17.5 (B) and 157.5 kJ/m^2 (C).

Additional analyses were also performed to investigate the impact of pulsed light on sensory properties of fresh-cut apple (Table 7.6). During storage, apple slices exposed to 157.5 kJ/m^2 were judged as more brown than untreated and 17.5 kJ/m^2 treated slices. The intensity of apple flavour significantly decreased upon expo-sure to intense pulsed light (157.5 kJ/m^2) with no further changes during storage. Slighter flavour changes were detected in apple slices exposed to 17.5 kJ/m^2. It is noteworthy that these changes were similar to those detected during storage of untreated apple slices. Flavour modifications in apple derivates are known to be attributable to changes in composition of volatiles, including esters, aldehydes and alcohols (Valappil et al., 2009). Pulsed light treatment was also associated with the development of an anomalous flavour, which was described as “cooked flavour”. This defect was only associated with apple slices submitted to 157.5 kJ/m^2 pulsed light and was detected starting from the first day of storage. In addition, even if not quantified during storage, the presence of anomalous fruity flavours (“banana”, “vanilla”) were perceived by 6 out of 13 judges testing the apple sample just after its exposure to 157.5 kJ/m^2. These anomalous flavours quickly disappeared so that judges were not able to detected them during the following sensory tests. It can be inferred that pulsed light affects the relative abundance of volatile compounds depending on their specific photosensitivity. This is probably related to photo-induced reactions of esters and other compounds contributing to the overall profile. In this regard, Lamikanra et al. (2002) demonstrated that UV-C light reduces ester concentrations in melons.

Table 7.6 Browning, apple flavour and cooked flavour during storage at 4 °C of apple slices exposed to increasing fluence pulsed light.

Fluence (kJ/m^2)	Time (days)	Browning	Apple flavour	Cooked flavour
0	0	0.0 ± 0.0^{e}	0.0 ± 0.0^{a}	0.0 ± 0.0^{c}
	1	$0.7\pm0.4^{d,e}$	-0.1 ± 0.2^{a}	0.0 ± 0.0^{c}
	3	$0.8\pm0.5^{c,e}$	$-0.6\pm0.6^{a,d}$	0.0 ± 0.0^{c}
	7	2.2 ± 0.7^{b}	$-0.9\pm0.5^{b,c,d}$	0.2 ± 0.2^{c}
17.5	0	0.0 ± 0.1^{e}	-0.1 ± 0.3^{a}	0.0 ± 0.1^{c}
	1	$0.9\pm0.5^{c,d}$	$-0.5\pm0.5^{a,b}$	0.1 ± 0.2^{c}
	3	$1.5\pm0.6^{b,c}$	$-1.4\pm0.4^{d,e}$	0.2 ± 0.2^{c}
	7	$1.5\pm0.6^{b,c}$	$-0.7\pm0.7^{a,d}$	0.2 ± 0.3^{c}
157.5	0	0.1 ± 0.1^{e}	$-1.4\pm0.6^{d,e}$	0.2 ± 0.3^{c}
	1	1.7 ± 0.5^{b}	$-1.2\pm0.5^{d,e}$	$0.7\pm0.7^{a,b}$
	3	3.2 ± 0.7^{a}	$-1.6\pm0.3^{c,e}$	1.2 ± 0.4^{a}
	7	3.6 ± 0.9^{a}	-1.7 ± 0.7^{e}	$1.0\pm0.3^{a,b}$

For each quality attribute, means indicated by the same letter are not significantly different ($p\geq0.05$).

Conclusions

Data from this work demonstrate that pulsed light may allow non-thermal decontamination of the surface of fresh-cut apple slices with negligible changes in fresh-like appearance of the product. Contrarily to the application of UV-C light to lamb's lettuce (Chapter 6), higher bactericidal efficacy was achieved, due to microbial contamination which was present at the product surface and not internalized in the vegetable tissue. However, the possibility of exploiting this technology to increase product safety and quality strictly depends on the application of pulsed light at proper fluences. Exposure to excessive pulsed light fluence should be avoided, since this is associated with critical cell damage favouring browning reactions. Moreover, it should be considered that the general issue concerning enzymatic inactivation in fresh-cut products can not be completely solved by applying surface treatments such as light technologies. In fact, in order to inhibit enzymes which negatively affect colour, flavour or texture in fresh-cut products, non-thermal technologies which are effective on the entire mass of the product (e.g. dense-phase carbon dioxide, pulsed electric fields) should be considered.

Chapter 8 Effect of dense-phase carbon dioxide on polyphenoloxidase in model systems and apple juice

Quality decay associated with enzymatic browning caused by polyphenoloxidase (PPO) activity in fresh-cut produce is of great concern for the food industry. PPO activity in fruit and vegetables is usually controlled by thermal processing, refrigeration, pH reduction and the use of antioxidants and/or enzyme inhibitors (Buckow et al., 2009). However, the efficacy of these methods to delay or avoid enzymatic browning results critical. Thus, in recent years non-thermal technologies have been proposed as alternatives to conventional methods for enzyme inactivation and browning control. As previously described in chapter 7, pulsed light can be effective in reducing browning in fresh-cut apple. In addition, UV-C light (Manzocco et al., 2009; 2011a,b), pulsed electric fields (Meneses et al., 2013) and cold plasma (Tappi et al., 2014) have been demonstrated to inactivate PPO and delay browning in vegetables. Moreover, high pressure carbon dioxide (HP-CO_2), also known as dense phase carbon dioxide (DP-CO_2) has been proposed as a promising non-thermal method for enzymatic inactivation. During the treatment, food is in contact with pressurised CO_2 at temperature/pressure conditions that may be below or above the critical point (31.1 °C, 7.38 MPa). Typical CO_2 pressure is generally within 4 and 30 MPa, rarely exceeding 50 MPa, while temperature is between 20 and 50 °C. PPO from different fruit juices and seafood has been demonstrated to be HP-CO_2 sensitive (Del Pozo-Insfran et al., 2007; Liu et al., 2010; Xu et al., 2011; Zhang et al., 2011). The extent of inactivation depends on the nature of the enzyme and is strongly affected by CO_2 pressure, temperature and treatment time. Besides processing parameters, the composition of the matrix surrounding the enzyme and its compartmentalisation could also affect sensitivity to HP-CO_2. For instance, as compared to enzymes within plant and animal tissues, higher inactivation was generally observed when the crude extracts were treated (Spilimbergo et al., 2013; Zhou et al., 2009; Zhang et al., 2011; Ferrentino et al., 2013).

The mechanism of enzyme inactivation by HP-CO_2 is hypothesised to be the result of local pH lowering (Balaban et al., 1991). According to other authors, pressurised CO_2 could cause changes in the conformation of the

secondary structure of the enzyme (Chen et al., 1992). To this regard, HP-CO_2 inactivation of different enzymes was observed in concomitance with a decrease in the residual α-helix content of the proteins, analogously to thermal induced inactivation (Gui et al., 2006; Ishikawa et al., 1996). Despite these findings, the effect of HP-CO_2 on the activity and structure of food enzymes is still under study.

Aim of the study

The present study was addressed to investigate the possibility to inactivate enzymes responsible for quality depletion in fresh-cut products by using HP-CO_2. To this aim, the attention was focused on PPO, taken as a representative enzyme responsible for product browning. In particular, aqueous solution containing mushroom polyphenoxidase and apple juice were exposed at 20, 35 and 45 °C for up to 30 min to CO_2 at increasing pressures up to 18 MPa. Samples were analysed for residual enzymatic activity. Inactivation rate constants were used to estimate decimal reduction parameters (D_P and z_P). In addition, to compare changes induced by pressurised CO_2 and conventional thermal treatment on PPO, SDS-PAGE separation patterns were also obtained. Finally, pH, turbidity and colour of HP-CO_2 treated juice were evaluated during storage at 4 °C for up to 6 days.

Results and discussion

Figure 8.1 shows the residual PPO activity of an aqueous solution containing 4 U of mushroom PPO as a function of treatment time at different CO_2 pressure at 20 and 35 °C. HP-CO_2 treatments promoted a significant decrease in the activity of the enzyme. In particular, while the PPO control sample, treated at 20 °C under environmental pressure (0.1 MPa), did not show significant changes in activity, exposure for increasing time to pressurised CO_2 strongly decreased this parameter (Figure 8.1 A). In particular, PPO was completely inactivated at room temperature within few minutes of treatment by selecting the proper pressure conditions. Independently on CO_2 pressure, samples were always subjected to CO_2 under subcritical conditions. When PPO was exposed to HP-CO_2 at 35 °C, inactivation was observed in even shorter times (Figure 8.1 B). Samples were always under supercritical conditions of CO_2 with

the only exception of those exposed to 6 MPa. It can be noted that no abrupt change in enzyme inactivation seemed to be associated with the transition from subcritical to supercritical condition. The effect of temperature on enzyme inactivation was further confirmed by additional trials carried out at 45 °C (data not shown). The exposure to the minimum pressure tested (6 MPa, subcritical conditions) for just 30 s was sufficient to completely inactivate the enzyme. It is noteworthy that the control sample, treated at the same conditions of temperature and time but at atmospheric pressure (0.1 MPa), presented a residual PPO activity approaching 90%. These data demonstrate the existence of a negative relation between PPO activity and the increase in both CO_2 pressure and temperature at least under the experimental conditions here tested.

Enzyme inactivation by pressurised CO_2 has already been reported for PPO from different origins (Gui et al., 2006; Zhang et al., 2011; Chen et al., 1992). However, in those cases, much more intense treatments were required. For instance, the complete inactivation of shrimp PPO was only achieved in the 4-25 MPa pressure range by carrying out prolonged treatments at 37 °C (> 30 min) (Zhang et al., 2011). The higher susceptibility to pressurised CO_2, as compared to that reported from the same enzymes extracted or included in food matrices other than mushroom tissue, is likely to be attributable to the enzyme origin. The latter is well known to affect the sensitivity of enzymes to different inactivation treatments, including those based on high pressure (Ortuño et al., 2013). In addition, the sensitivity of the enzyme could also be affected by its environmental conditions during the treatment. To this regard, Zhou et al. (2009) observed that the inactivation of pectin methylesterase in a buffer was enhanced as compared to the original enzyme in carrot and peach juices.

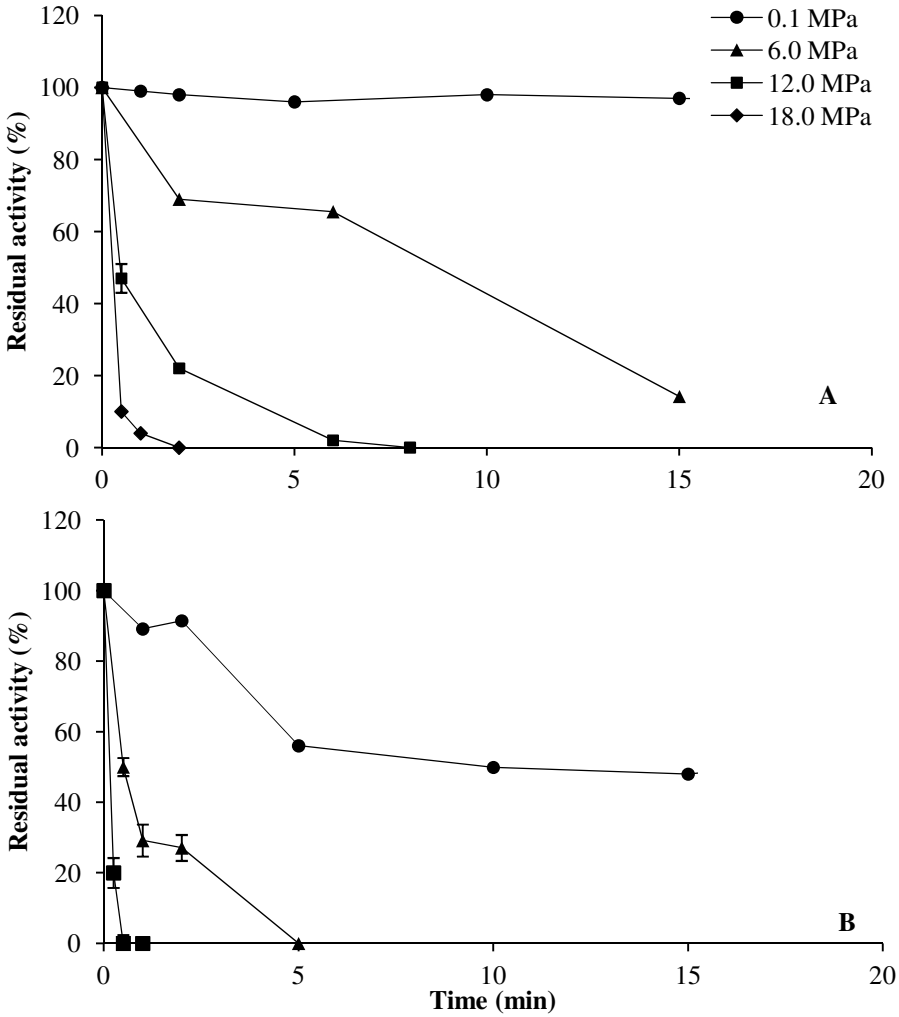

Figure 8.1 Residual activity of polyphenoloxidase activity in an aqueous solution as a function of exposure time to increasing CO_2 pressure at 20 °C (A) and 35 °C (B). Control was treated at environmental pressure (0.1 MPa).

Based on data shown in Figure 8.1, the apparent inactivation rate constants of PPO were computed (Table 8.1). The residual activity of PPO, expressed in natural logarithmic values, had good relationships (R^2>0.77, p<0.05) with the treatment time. D_P values were thus derived from the rate constants, using procedures analogous to that employed in thermal death time studies (Table 8.1). In particular, D_P was defined as the treatment time needed for 90% enzyme activity reduction at a given pressure. For instance, the treatment at 6 MPa and 20 °C leaded to a

tenfold decrease of activity in *circa* 18 min. At the same temperature, this goal was achieved at 12 MPa in *circa* 3 min. On the other hand, keeping constant CO_2 pressure at 6 MPa, inactivation was achieved at 35 or 45 °C in 3.7 or less than 0.2 min, respectively. It must be noted that, when the enzyme resulted completely inactivated following the shortest treatment considered in the experimental plan (0.2 min), the rate constant was not determinable. However, in such cases, the D_P value can be assumed as shorter than 0.2 min. These low D_P values are in agreement with the higher susceptibility of mushroom PPO to HP-CO_2 treatments, as compared to that from other origin. To this regard, Gui et al. (2006) reported PPO in cloudy apple juice to be inactivated in *circa* 220 min upon exposure at 35 °C to 30 MPa. Similarly, Zhang et al. (2011) reported Pacific white shrimp PPO to exert a D_p of *circa* 30 min at 15 MPa and 37 °C.

Table 8.1 Apparent inactivation rate constants (k), relevant determination coefficient and D_P values of polyphenoloxidase in aqueous solutions upon HP-CO_2 treatments carried out at increasing pressure at 20, 35 and 45 °C.

Temperature (°C)	Pressure (MPa)	k (min^{-1})	R^2	D_P (min)
20	0.1	0.0014	0.90	1645.1
	6.0	0.1264	0.95	18.2
	12.0	0.7272	0.96	3.1
	18.0	4.0829	0.99	0.6
35	0.1	0.0518	0.83	44.5
	6.0	0.6235	0.77	3.7
	12.0	6.6378	0.89	0.3
	18.0	*		<0.2
45	0.1	0.0723	0.80	31.8
	6.0	*		<0.2
	12.0	*		<0.2
	18.0	*		<0.2

* The enzyme resulted completely inactivated after the shortest treatment time (0.2 min).

D_p values relevant to experiments carried out at 20 and 35 °C (Table 1) were used to calculate the parameter z_P, describing the sensitivity of PPO to pressurised CO_2. The decimal logarithmic values of D_P resulted well correlated (R^2>0.91, p<0.05) with pressure for treatments carried out at 20

and 35 °C. The z_P value, which represents the pressure range within which the D_P changes tenfold, was calculated as the negative reciprocal slope of the regression line. The pressure sensitivity parameter z_P resulted *circa* 5.5 MPa, independently on whether the treatments were carried out at 20 or 35 °C. This indicates that an increase in pressure of 5.5 MPa is necessary to get a 90 % decrease in D_P at both temperatures.

PPO samples showing no residual activity after the treatments were also analysed for activity after storage for 7 days at 4 °C. The enzyme resulted unable to recover its activity upon storage. This result is in agreement with literature evidence showing the capability of pressurised CO_2 in irreversibly decomposing the α-helix of the protein structure (Chen et al., 1992).

To better understand the nature of the PPO modification induced by pressurised CO_2, the attention was focused on samples that underwent enzyme inactivation by exposure for 2 min at 20 °C at 6, 12 and 18 MPa. SDS-PAGE was performed on PPO samples treated at increasing pressure (Figure 8.3). Exposure of PPO to pressurised CO_2 (lane A-C), despite decreasing its residual activity, did not promote changes in the electrophoretic pattern as compared to that of the unpressurised enzyme (0.1 MPa). In fact, control and PPO treated samples showed one clear band of molecular weight of about 64 kDa (Duckworth and Coleman, 1970; Wickers et al., 2003; Wu et al., 2010). In other words, the treatments did not induce evident fragmentation and/or aggregation of the PPO subunits. This result is consistent with data obtained by HPLC gel permeation analysis of PPO submitted to pressurised CO_2 at 22 °C (Manzocco et al., 2014) and with indications relevant to the effect of this physical treatment on other enzymes, such as lipoxygenase (Liao et al., 2009). To verify whether the mechanism of PPO inactivation was different from that associated with thermal inactivation, an additional sample, with no residual activity, was prepared by maintaining the PPO solution at 70 °C for 5 min (Figure 8.3, lane D). Thermal inactivation of PPO was associated with the smearing of the band at the top of the lane, suggesting the presence of high molecular weight protein aggregates. Heating would thus promote enzyme inactivation by mechanisms other than those promoted by HP-CO_2 treatments. In particular, the exposure to HP-CO_2 could promote local pH lowering, possibly resulting in enzyme

inactivation (Damar et al., 2006; Balaban et al., 1991). In the present study, the pH of the treated sample resulted similar to that of the untreated one (7.0). However, due to plant constraints, sample pH was only analysed after depressurisation, not allowing to appreciate pH modifications during CO_2 solubilisation in the treatment vessel. It was also hypothesised that HP-CO_2 exposure could involve minor conformational modifications which would be however able to reduce enzyme functionality (Chen et al., 1992).

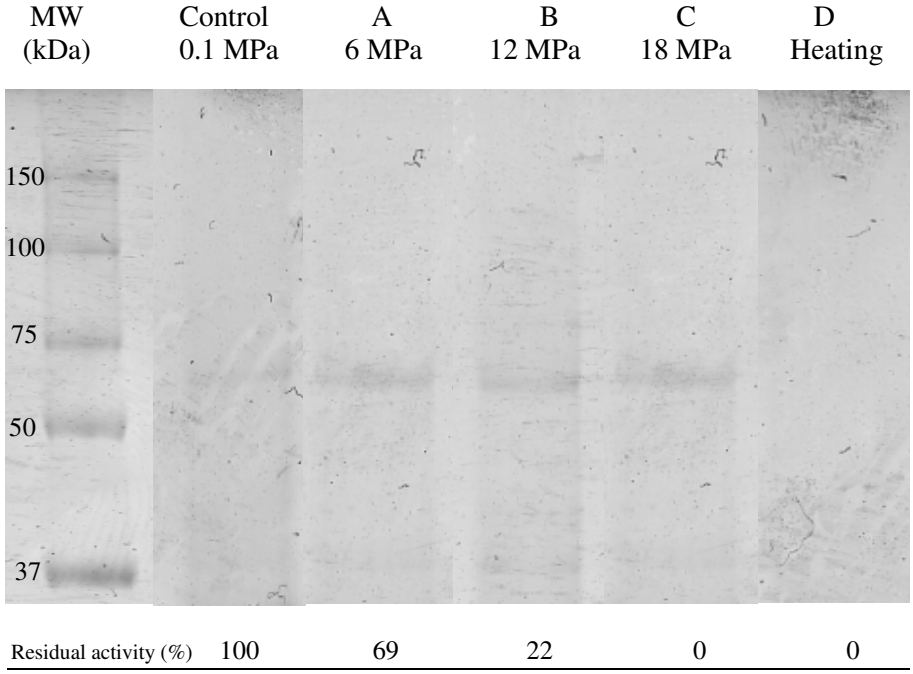

Figure 8.3 SDS-PAGE separation patterns of: control PPO (Control); PPO exposed for 2 min at 20 °C to HP-CO_2 treatments carried out at 6 (A), 12 (B), 18 (C) MPa; PPO heat treated at 70 °C for 5 min (D). Residual activity of each sample is also shown at the bottom of each lane.

As previously observed for PPO in the aqueous solution, also in the case of apple juice PPO, a significant inactivation was detected upon exposure to HP-CO_2. Figure 8.3 shows that, at each temperature, the increase in treatment pressure and time was associated to a progressive loss in PPO activity. In addition, the higher the temperature, the lower the residual activity of the enzyme. However, differently from the model system, the

complete inactivation of the enzyme was not achieved in apple juice even prolonging treatment time up to 30 min. For instance, in the case of the apple juice treated at the highest pressure (18 MPa) for 30 min, the minimum residual activity was equal to 25, 10 and 4% at 20, 35 and 45 °C, respectively.

Residual activity of PPO in apple juice upon exposure to HP-CO_2 is in accordance with literature data. In fact, treatments carried out at 35 °C at 8 MPa for up to 30 min were not able to decrease the activity of apple PPO (Xu et al., 2011; Soysal, 2008). This goal was only reached when apple PPO was submitted to longer treatments and/or higher temperatures (Xu et al., 2011).

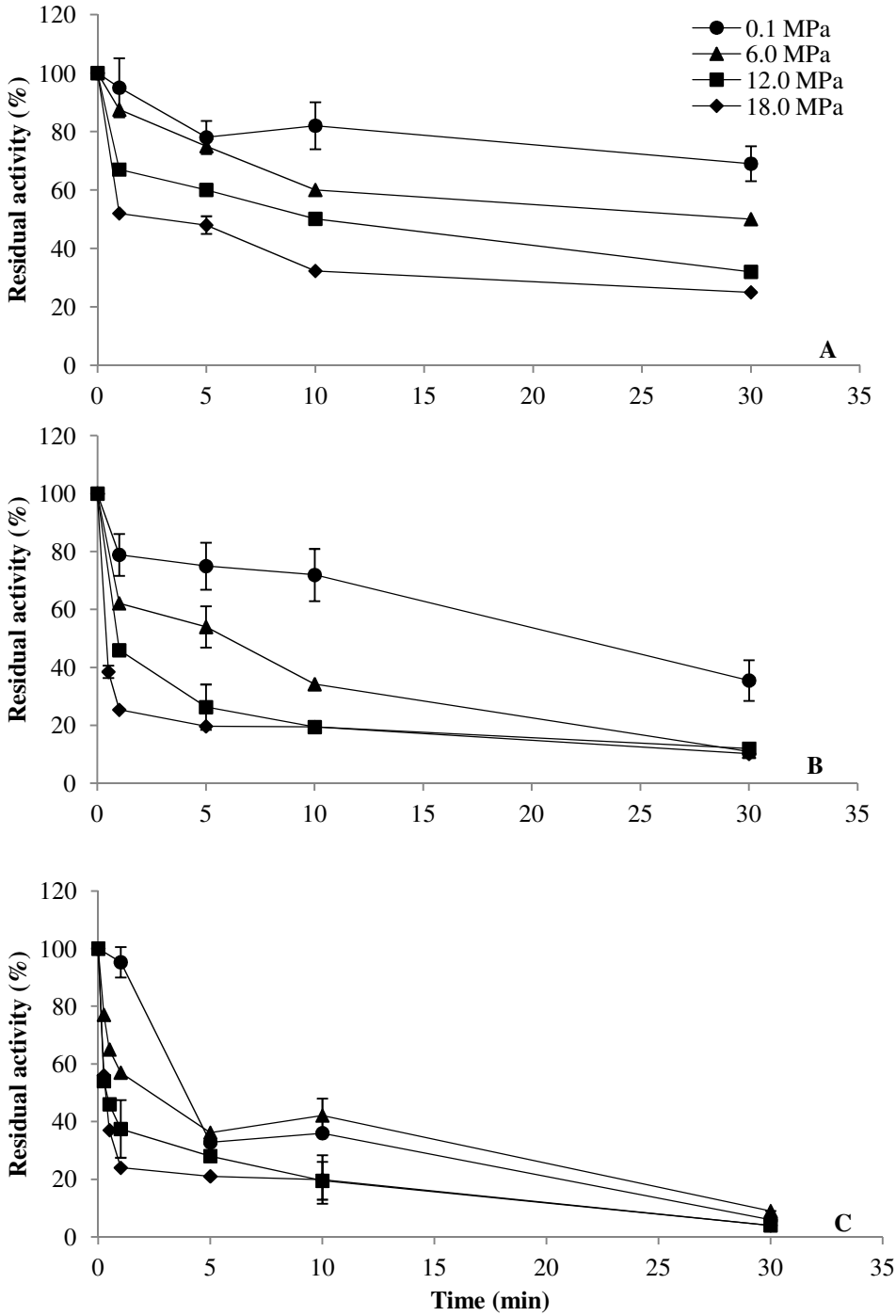

Figure 8.3 Residual activity of polyphenoloxidase activity in apple juice as a function of exposure time to increasing CO_2 pressure at 20 °C (A), 35 °C (B) and 45 °C. Control was treated at environmental pressure (0.1 MPa).

Data shown in figure 8.3 were used to estimate the kinetic parameters using procedures analogous to that described for PPO in the model system (Table 8.1). The eventual final stationary phase was excluded from regression data. As expected, the time required for a ten-fold decrease of PPO activity in apple juice resulted much higher than those needed for PPO activity in aqueous solution (Table 8.1).

Data reported in Table 8.2 were used to calculate the HP-CO_2 sensitivity parameter, z_P, of PPO in apple juice ($R^2 > 0.96$). The z_P parameter resulted 21.5, 11.7 and 10.4 MPa at 20, 35 and 45 °C, respectively. The higher values of D_p and z_p for PPO in apple juice as compared to those observed in the model system, indicate that enzyme inactivation by HP-CO_2 treatments is more difficult in apple juice. The higher resistance of PPO in apple juice can be reasonably attributed not only to its molecular specificity but also to the protective effect of the matrix complexity. The latter has been shown to be particularly critical in determining enzyme sensitivity to different physical stresses such as UV-C light (Manzocco et al., 2009) and ultrasound (Cheng et al., 2013; Sulaiman et al., 2015). In the case of HP-CO_2, higher inactivation rate were obtained when the crude extracts were treated as compared to enzymes within plant and animal tissues (Spilimbergo et al., 2013; Zhou et al., 2009; Zhang et al., 2011; Ferrentino et al., 2013).

Table 8.2 Apparent inactivation rate constants (k), relevant determination coefficient and D_P values of PPO in apple juice upon HP-CO_2 treatment carried out at increasing pressure at 20, 35 and 45 °C.

Temperature (°C)	Pressure (MPa)	k (min^{-1})	R^2	D_P (min)
20	0.1	0.0116	0.74	86.2
	6.0	0.0208	0.83	48.1
	12.0	0.0313	0.85	31.0
	18.0	0.0775	0.73	12.9
35	0.1	0.0313	0.96	31.9
	6.0	0.0669	0.96	14.9
	12.0	0.2352	0.84	4.3
	18.0	1.3704	0.95	0.7
45	0.1	0.0999	0.95	10.0
	6.0	0.1660	0.83	6.0
	12.0	1.5531	0.90	0.6
	18.0	1.9885	0.99	0.5

In order to evaluate whether HP-CO_2, despite promoting enzyme inactivation, may also contribute to modify some overall quality parameters, the HP-CO_2 treated juice was also analysed for pH, turbidity and colour. The analyses were performed on the sample obtained by the HP-CO_2 treatment leading to the lowest PPO residual activity within 10 min of treatment. As shown in Figure 8.3 the latter corresponded to the treatments performed at 12 MPa and 35 °C. The initial pH of apple juice (4.1±0.2) did not change following the HP-CO_2 treatment and remained constant on further juice storage at 4 °C. This result is in agreement with literature data and can be stated that CO_2 is highly removed during depressurization (Niu et al., 2010; Xu et al., 2011). In addition, the selected treatment did not significantly modified apple juice turbidity. In fact, the initial absorbance at 680 nm resulted 0.11±0.02 Abs in the just prepared apple juice and remained constant in the apple juice exposed to 12 MPa of HP-CO_2 at 35 °C for 10 min. The sample was thus stored at 4 °C for increasing time up to 6 days to evaluate colour changes under simulated refrigerated storage. Browning index of the HP-CO_2 treated apple juice is reported in Figure 8.4. The figure also shows the evolution of browning of untreated apple juice. As expected, the latter presented a rapid browning just after one day of storage, attributable to the

development of enzymatic activity which rapidly occurs after cell disruption (Sapers and Douglas, 1987). On the contrary, the HP-CO_2 treated apple juice retained its colour during storage. The HP-CO_2 treatment promoted a decrease of *circa* 80% in PPO residual activity (Figure 8.4B) which was sufficient to ensure colour stability during refrigerated storage.

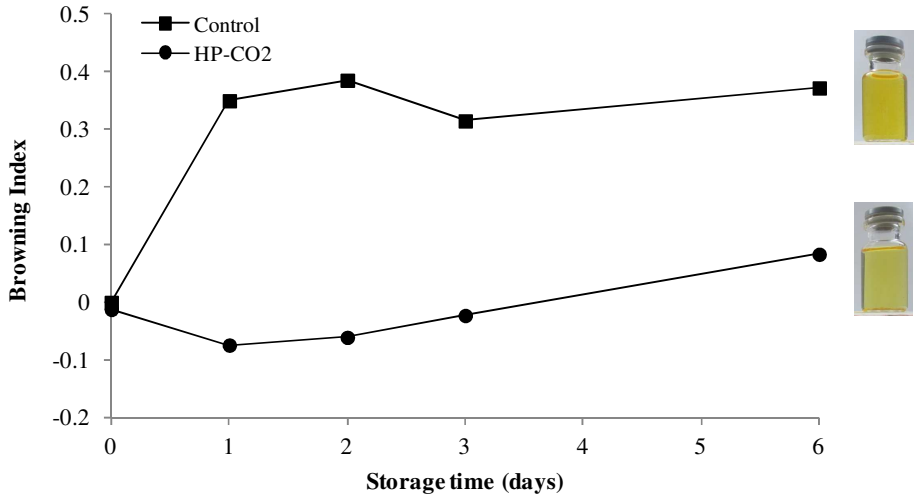

Figure 8.4 Browning index of apple juice exposed to HP-CO_2 at 12 MPa and 35 °C for 10 min. Control was treated at environmental pressure (0.1 MPa).

Conclusions

HP-CO_2 treatments allowed quick non-thermal inactivation of PPO in aqueous model system as well as in apple juice. In model system, the complete inactivation of the enzyme was achieved within minutes of treatment even at room temperature by selecting the proper CO_2 pressure. Kinetic analysis of the pressure and temperature induced inactivation demonstrated that the increase in temperature reduced inactivation time but did not affect enzyme sensitivity to the pressure variation. PPO inactivation by HP-CO_2 was not associated with changes in electrophoretic pattern and occurred following mechanisms other than those of thermal treatment. In the case of apple juice, due to the higher complexity of a real food matrix, PPO activity required longer treatment times to be reduced by HP-CO_2. However, no changes in the quality

parameters of apple juice after the treatment and during refrigerated storage were observed.

CO_2 is relatively inert, cheap, non-toxic and recyclable and the results acquired provide evidence of HP-CO_2 potential as an effective antimicrobial and sustainable non-thermal processing technology. HP-CO_2 treatment could be thus exploited in fresh-cut vegetable production to maintain fresh-likelihood of the product without impairing quality attributes such as colour.

Chapter 9 Effect of pulsed electric field on potato

Several vegetables can not be eaten raw and require treatments such as thermal blanching before further processing in order to guarantee their quality, for example in terms of colour and texture. This is the case of potatoes which are usually blanched before frying at industrial level. Blanching is generally performed in water at 55-75 °C for at least 10 min to get a uniform colour of the product by inactivating polyphenoloxidase and to soften the tissues by the activity of pectin methyl esterase. The latter also promotes strengthening of potato cell walls by increasing the amount of free carboxyl groups of pectin, thus allowing networking by Ca^{2+} bridges (Bartolome and Hoff, 1972; Andersson and Oste, 1994; Tajner-Czopek and Carbonell-Barrachina, 2008). Blanching has the drawback of being time-intensive and requiring high amounts of water. In addition, it has low energy efficiency, which further decreases when blanching water becomes saturated with sugars leached from the potato strips (Bingol et al., 2014). Potato iron and chlorogenic acid may form a colorless complex upon blanching that, upon oxidation, creates ferridichlorogenic acid providing an undesirable dark colour of the blanched tissue (Wang-Pruski and Nowak, 2004). In order to prevent this defect, the blanching aqueous solution is generally added with chemicals as sulphites. However, the latter may leave a residue in the product, causing an allergic reaction in sensitive consumers (Peroni and Boner, 1995).

The minimization of chemical additives as well as the excessive use of water and energy are thus major challenges for par-fried potato producers. In this context, pulsed electric fields (PEF) are recognised as an appropriate technology for different applications in food processing with rather low power consumption (Boussetta et al., 2013). PEF involves the application of short duration pulses (µs to ms) of high voltage electric fields to a sample located between two electrodes. The generation of transmembrane potential may cause temporary or permanent permeabilization of cell membranes through a process known as electroporation (Zimmermann, 1986). Besides its well-known use to decontaminate food, PEF is also exploited to modify plant tissue structure. A review of the literature shows that PEF promotes softening

of plant tissue such as carrots, apples and potatoes (Lebovka et al., 2004; Boussetta et al, 2013). PEF have also been applied to enhance mass transfer processes such as extraction, drying and frying in different fruits and vegetables (Górgora-Nieto et al., 2002; Amami et al., 2008; López et al., 2009; Donsì et al., 2010; Janositz et al., 2011a; Palgan et al., 2012). However, little data is available on the possibility for PEF treatments to improve the quality of fresh potatoes intended for deep-fat frying.

Aim of the study

The present study aims to investigate the potential of pulsed electric fields to replace thermal blanching, in order to modify raw potato structure and to influence the final quality of deep-fat fried potatoes. In particular, potato cubes were submitted to low electric field strength (0.75 and 2.50 kV/cm). The tissue modification were studied in comparison with water-dipped and blanched potato cubes considering the changes in drip loss, colour and texture. Following PEF pre-treatments, samples were par-fried, frozen, stored at -20 °C for 7 days to simulate industrial production of par-fried potatoes. Potato cubes were then finish-fried and analyzed for texture, colour and oil content.

Results and discussion

The effect of PEF on raw potato cubes was initially studied considering the role of the number of pulses and the intensity of the electric field applied during processing. To this aim, potato cubes were submitted to treatments characterized by the same energy (18.9 kJ/kg) delivered according to different conditions of pulsed electric field and number of pulses. Following the PEF treatments, the temperature of potato cubes increased by 3.9±0.2 °C and no significant temperature difference was observed depending on the PEF conditions applied. Table 9.1 reports moisture, weight loss and drip loss of potato cubes submitted to the different PEF treatments. The table also shows data relevant to a control sample (Dipped) obtained by dipping potato cubes in water at 20 °C for the same time required to allow PEF treatments (20 min). An additional control sample was also prepared by blanching potato cubes at 70 °C in sodium metabisulfite aqueous solution (Blanched). These conditions were chosen to simulate conventional blanching operations applied at industrial

level. It is noteworthy that the total energy required for potato blanching, although underestimated since not accounting for the energy of water evaporation, resulted more than ten-times higher than that required for potato PEF treatments.

Table 9.1 Moisture, weight loss and drip loss of potato cubes submitted to PEF treatments. Dipped and blanched potato cubes are shown as control.

	Moisture (% w/w)	Weight loss (%)	Drip loss (%)
Dipped	80.4±0.3[b]	-1.1±0.2[c]	2.3±0.6[b]
Blanched	81.3±0.4[a]	2.5±0.5[a]	2.9±0.4[b]
Low PEF[*]	81.8±0.3[a]	1.2±0.2[b]	9.9±1.4[a]
High PEF[*]	81.9±0.6[a]	1.5±0.4[b]	10.4±2.0[a]

Means with same letter in each column are not significantly different ($p \geq 0.05$).
[*] Low PEF: 9000 pulses at 0.75 kV/cm electric field; High PEF: 810 pulses at 2.50 kV/cm electric field.

Potato cubes submitted to PEF presented higher moisture than raw potato (78.7 ± 0.9 %) and no differences were detected depending on the number of pulses and electric field applied. However, the moisture of PEF treated samples was comparable to that observed in samples blanched at 70 °C but significantly higher than that detected in the control samples dipped in water. This evidence suggests that moisture increase upon PEF treatments cannot be solely attributed to the effect of the contact with water but possibly to a specific structural modification induced by the electric fields. This hypothesis was confirmed by the significant weight loss observed in potato cubes after PEF treatments as compared to that measured in the dipped samples. The latter actually showed a negative weight loss, indicating, as expected, that water absorption largely prevailed over leaching phenomena during dipping. It is noteworthy that an increase in turbidity of the aqueous phase used for both blanching and PEF treatments was visually observed, indicating the occurrence of starch leaching. While starch leaching from the bleached potato cubes derives from the modification of cell membrane permeability upon prolonged heating, PEF treatments cause starch release following tissue electroporation. The latter could account for the dramatic increase in drip loss as compared to the dipped and blanched control samples (Table 9.1).

It is known that plant material exposure to PEF leads to an enhanced mass transfer rate via permeabilization of the cell membrane (Lebovka and Vorobiev, 2007). Cell wall biopolymers are not only decreased but also degraded due to the effective break of intermolecular and intramolecular bonds, leading to cell membrane disintegration and cytoplasm release (Janositz et al., 2011b).

Table 9.2 Peak force, total energy, lightness, colour parameter a* and images of potato cubes submitted to PEF treatments. Dipped and blanched potato cubes are shown as control.

	Peak force (N)	Total energy (J)	Lightness (L*)	Green to red parameter (a*)	
Dipped	55.7±2.8[a]	0.857±0.043[a]	70.00±2.87[a]	-4.28±0.43[b]	
Blanched	44.9±5.7[b]	0.427±0.034[c]	58.16±2.55[c]	-6.47±0.31[c]	
Low PEF[*]	46.9±3.0[b]	0.570±0.063[b]	64.18±2.72[b]	-3.95±0.52[a,b]	
High PEF[*]	47.6±4.7[b]	0.536±0.066[b]	66.40±2.60[b]	-3.74±0.29[a]	

Means with same letter in each column are not significantly different ($p \geq 0.05$).
[*] Low PEF: 9000 pulses at 0.75 kV/cm electric field; High PEF: 810 pulses at 2.50 kV/cm electric field.

Potato cubes were also analyzed for their textural properties (Table 9.2). Potato cubes submitted either of the two PEF treatments and also blanched potato cubes presented lower peak force in comparison to the raw and dipped control samples. In addition, when considering the total energy needed to cut the samples, about 35% less energy was required to slice PEF treated samples while blanching leaded to nearly 50% energy reduction. This result can be attributed to PEF-induced textural changes of the potato tissue. Electroporation may actually cause partial loss in

turgor pressure, impairing plant tissue firmness (Lebovka et al., 2004; Pereira and Dejmek, 2009).

Table 9.2 also shows lightness and green-to-red colour parameter as well as images of the treated potato cubes. While no significant differences in b* parameter were observed (data not shown), PEF treated potato cubes presented lower lightness and higher a* parameter as compared to the dipped sample. These modification appeared moderate and not affecting the fresh-like appearance of the product, as clearly observed in their images. On the contrary, blanching significantly decreased both lightness and a* parameter of potato cubes, which also showed a more homogeneous colour, especially at the cube edges.

Potato samples submitted to PEF treatments were par-fried for 1 min. Table 9.3 shows moisture, weight loss and oil content following par-frying.

Table 9.3 Moisture, weight loss and oil content of potato cubes submitted to PEF treatments and par-fried for 1 min. Dipped and blanched potato cubes are shown as control.

	Moisture (% w/w)	Weight loss (%)	Oil content (% $w/w_{w.b.}$)
Dipped	74.4 ± 0.4^{a}	14.6 ± 0.5^{a}	2.83 ± 0.13^{a}
Blanched	75.8 ± 2.0^{a}	12.7 ± 0.0^{c}	2.33 ± 0.21^{b}
Low PEF*	74.2 ± 1.5^{a}	13.1 ± 0.4^{b}	2.06 ± 0.03^{b}
High PEF*	73.6 ± 2.2^{a}	13.3 ± 0.1^{b}	2.27 ± 0.05^{b}

Means with same letter in each column are not significantly different ($p \geq 0.05$).

* Low PEF: 9000 pulses at 0.75 kV/cm electric field; High PEF: 810 pulses at 2.50 kV/cm electric field.

Although no significant differences in sample moisture were detected, samples presented different weight loss. In particular, both PEF treated samples showed weight loss lower than that of the dipped potato cubes but higher than that of the blanched ones. It is known that during the frying process, moisture evaporates at the product surface due to the partial vapour pressure difference between the product and the frying oil whilst oil migrates into the product (Moreira and Barrufet, 1999). In the case of the PEF treated potato cubes, cell liquid diffusion from the core to the surface could be enhanced following cell membrane permeabilisation. This would result in higher water vapour pressure and thus thicker water

vapour layer at the surface, reducing weight loss and oil uptake (Janositz et al., 2011a). On the contrary, blanched potato cubes presented lower weight loss as a consequence of the structural changes induced by blanching. The latter promotes starch gelatinisation and maintains the integrity of the native pectin network through inactivation of pectolytic enzymes, probably leading to a lower water diffusion from the core to the surface (Pedreschi and Moyano, 2005). These phenomena could also account for the lower oil absorption in the blanched samples (Table 9.3).
Par fried potato samples previously submitted to PEF treatments were frozen, packed and stored at -20 °C as occurs during industrial production of frozen par-fried potatoes. After 1 week of storage, they were submitted to final frying to simulate preparation of deep-fat fried potatoes for consumption. In order to evaluate the effect of PEF on oil absorption during frying, samples were submitted to frying for 1, 2 or 4 min.

Table 9.4 Oil content of potato cubes submitted to PEF treatments and par-fried for 1 min as a function of frying time. Dipped and blanched potato cubes are shown as control.

	Oil content (% w/w $_{w.b.}$)		
	Frying time (min)		
	1	2	4
Dipped	3.61±0.13^{a}	4.56±0.16^{b}	5.20±0.38^{a}
Blanched	3.80±0.17^{a}	5.02±0.27^{a}	5.80±0.57^{a}
Low PEF*	2.54±0.08^{b}	2.62±0.29^{c}	3.98±0.47^{b}
High PEF*	2.38±0.04^{b}	3.03±0.37^{c}	4.07±0.33^{b}

Means with same letter in each column are not significantly different ($p \geq 0.05$).
* Low PEF: 9000 pulses at 0.75 kV/cm electric field; High PEF: 810 pulses at 2.50 kV/cm electric field.

As expected, in all samples oil uptake was found to increase with frying time (Table 9.4). At each frying time, dipped and blanched samples were characterized by having a similar oil content, which was significantly (p<0.05) higher than that measured in the potato cubes submitted to the PEF treatments. In this case, PEF-induced electroporation probably lead to the transfer of the intracellular cytoplasm solution to the extracellular environment so that more water is located outside the cells creating a barrier and leading to a reduced oil uptake during frying.

Independent of the treatment they were submitted to, samples fried for 4 min showed no significant difference in moisture (66.2±2.3% w/w) and weight loss (21.4±1.0%). It is likely that the freezing process could mask the structural effects induced by PEF and blanching. In other words, tissue damage deriving from the formation of ice crystals in potato tissue is reasonably expected to be much higher than that induced by PEF treatments or blanching, leading to the same water transfer during frying.
Texture and colour of potato cubes fried for 4 min are reported in table 9.5, which also shows the images of the samples. Independent of the treatments, samples show similar texture parameters. As regards colour and visual quality, PEF and blanching treatments leaded to fried potato cubes with uniform and bright colour. On the contrary, dipped samples resulted uneven darker, with brown edges. As known, the colour of fried potatoes is influenced by sugar content. The latter has been demonstrated by various authors (Jaeger and Knorr, 2010; Janositz et al., 2011a) to be efficaciously reduced by PEF process. This result confirms the possibility to exploit PEF as an alternative pre-treatment to blanching, allowing not only to control oil uptake but also the formation of brown melanoidins in fried potatoes.

Table 9.5 Peak force, total energy, lightness, colour parameter a* and images of potato cubes submitted to PEF treatments, par-fried for 1 min and finish-fried for 4 min. Dipped and blanched potato cubes are shown as control.

	Peak force (N)	Total energy (J)	Lightness (L*)	Green to red parameter (a*)	
Dipped	11.3±2.3[a]	0.131±0.035[a]	69.22±3.07[b]	-1.34±1.40[a]	
Blanched	11.8±3.7[a]	0.134±0.044[a]	74.13±2.66[a]	-6.11±0.56[c]	
Low PEF[*]	10.9±2.3[a]	0.125±0.022[a]	73.78±1.89[a]	-5.04±0.85[b]	
High PEF[*]	11.4±3.8[a]	0.127±0.025[a]	73.05±1.89[a]	-5.23±1.13[b,c]	

Means with same letter in each column are not significantly different ($p \geq 0.05$).
[*] Low PEF: 9000 pulses at 0.75 kV/cm electric field; High PEF: 810 pulses at 2.50 kV/cm electric field.

Conclusions

Data acquired in this work demonstrate that PEF treatments applied at different electric fields and pulse number conditions, could be a feasible alternative to the conventional thermal blanching of potatoes intended for frying. As a matter of fact, PEF enhanced colour and texture of fresh potatoes before further processing. In addition, the application of PEF technology could avoid the need for chemicals and reduce not only process time but also water and energy consumption. In addition, PEF could allow mass transfer to be controlled during frying, improving product colour and reducing oil uptake.

Conclusions part II

The second part of this PhD thesis investigated the possibility to exploit emerging non-thermal technologies to enhance safety, quality and sustainability of vegetables products. In particular, light technologies such as UV-C and pulsed light were proved to be powerful tools for surface decontamination of fresh-cut products. However, food matrix characteristics and processing parameters must be carefully evaluated in order to obtain the desired bactericidal effect as well as to maintain the fresh-likelihood of the product. In the case of UV-C light, its application on lamb's lettuce proved to be critical. Despite the initial decontamination efficacy, UV-C light accelerated microbial growth and induced tissue damage during storage, due to the highly delicate structure of the leaves. On the contrary, by selecting proper condition of pulsed light, it was possible to decontaminate apple slices whilst maintaining the fresh-like appearance of the product during storage. Pulsed light could be thus a fast antimicrobial treatment allowing shelf life extension for selected vegetable matrixes.

Light technologies are surface treatments and do not affect the entire mass of the product. To this regard, HP-CO_2 and PEF were studied as potential technologies able to positively affect the quality of the whole product, in terms of colour and texture. In particular, HP-CO_2 proved to be an efficacious and sustainable non-thermal processing technology able to inhibit enzymes responsible for browning in apple products. Furthermore, PEF processing could replace conventional thermal treatments, highly energy and water consuming, as a pre-treatment of fresh potatoes. In fact, the application of PEF allowed colour and texture enhancement in this vegetable matrix.

In conclusion, the emerging technologies considered in this PhD thesis were demonstrated to be feasible strategies allowing to enhance safety of fresh-cut produce as well as to improve product quality. In addition, these technologies could be promising sustainable strategies being able to avoid the use of chemical compounds, leaving no residues on the product and requiring low energy consumption.

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Manzocco, L., Ignat, A., Maifreni, M., Nicoli, M.C. *Effetto della luce UV-C continua e pulsata sulle caratteristiche igienico-sanitarie e sulla qualità di vegetali della IV gamma.* Convegno PostRaccolta, May 22-23, 2014, Barletta, Italy.

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