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Decontamination treatments to prolong the shelf-life of minimally processed vegetables

MSc. Vicente Manuel Gómez López

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"When a man is riding through this desert by night and for some reason -falling asleep or anything else -he gets separated from his companions and wants to rejoin them, he hears spirit voices talking to him as if they were his companions, sometimes even calling him by name. Often these voices lure him away from the path and he never finds it again, and many travelers have got lost and died because of this. Sometimes in the night travelers hear a noise like the clatter of a great company of riders away from the road; if they believe that these are some of their own company and head for the noise, they find themselves in deep trouble when daylight comes and they realize their mistake.....Even by daylight men hear these spirit voices, and often you fancy you are listening to the strains of many instruments, especially drums, and the clash of arms. For this reason bands of travelers make a point of keeping very close together."

---- Marco Polo, *Travels*

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MSc. Vicente Manuel Gómez López

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NOMENCLATURE

Nomenclature

A	Amperes
AcEW	Acidic electrolysed oxidizing water
AlEW	Alkaline electrolysed oxidizing water
Anova	Analysis of variance
APC	Aerobic plate count
atm	Atmosphere
a_w	Activity of water
BA	Big amount of sample
CFU	Colony forming units
DNA	Deoxyribonucleic acid
DPD	N,N-diethyl-p-phenylenediamine
EMAP	Equilibrium modified atmosphere packaging
EOW	Electrolysed oxidizing water
FDA	Food and Drug Administration
F-test	Fisher test
GC	Gas chromatography
H	Hours
HPLC	High performance liquid chromatography
Hz	Hertz, pulses per second
ILP	Intense light pulses
J	Joules
kPa	Kilo Pascal
LAB	Lactic acid bacteria
LMG	Laboratorium Microbiologie, Universiteit Gent, Belgium
log	$\log_{10}$
M	Meters
MAP	Modified atmosphere packaging
min	Minutes
ml	Milliliters
mM	Milimolar
MP	Minimally processed
MPV	Minimally processed vegetales

N	Number of microorganisms after treatment
No	Number of microorganisms before treatment
n	Number of samples
NEW	Neutral electrolysed oxidizing water
OVQ	Overall visual quality
P	Probability
ppm	Parts per million
PPO	Polyphenol oxidase
pps	Pulses/second
PSS	Physiological saline solution
RH	Relative humidity
R^2	Coefficient of determination
s	Seconds
SA	Small amount of sample
S.D.	Standard deviation
t-test	Student t-test
UK	United Kingdom
USA	United States of America
UV	Part of the electromagnetic spectrum with wavelengths in the range 200-400 nm
UV-C	Part of the electromagnetic spectrum with wavelengths in the range 200-280 nm
Volts	Voltage
v/v	Volume/volume
W	Watts
w/v	Weight/volume
µg	Microgram

INTRODUCTION AND RESEARCH OBJECTIVES

Introduction and research objectives

Previous work on the application of decontamination methods to minimally processed vegetables (MPV) was mainly devoted to pathogen inactivation. Research usually ignored an aspect of foremost importance in food technology namely the effect of treatments on the sensorial quality of foods. No matter how efficient a decontamination treatment can be to inactivate foodborne pathogens, the technique is useless if MPV lose their fresh-like attributes. Moreover, MPV do have a short shelf-life. The same decontamination treatments tested for pathogen inactivation might be useful to prolong the shelf-life of MPV. Consequently, the main objective of this research was to test the feasibility of using some decontamination treatments to prolong the shelf-life of MPV stored under EMAP at refrigeration temperatures. The shelf-life was evaluated by using standard microbiological procedures and sensorial evaluation. The decontamination techniques were applied as such in order to evaluate their advantages and disadvantages; improvements were only attempted when drawbacks had already been reported in the scientific literature.

The methods tested in this thesis can be divided in physical (intense light pulses), and chemical (chlorine dioxide and neutral electrolysed oxidizing water (NEW)) methods. The body of this research can be divided in two sections: a small section on the basics of the microbial inactivation by intense light pulses (ILP), and the rest on decontamination and its consequences on the shelf-life of MPV.

The first specific research objective was to furnish a critical and comprehensive review of the protocol used to evaluate shelf-life extension of MPV, and reviewing the current knowledge on some decontamination methods encompassing: mechanism of action, effect on pathogens, spoilage microflora, sensory attributes and physiology, nutrients and phytochemicals; and toxicological issues. This objective is fulfilled in chapter 1.

The second specific research objective of this thesis was the study of some factors that influence the efficacy of microbial inactivation by ILP. This is described in chapter 2 and at the beginning of chapter 3.

The third specific research objective was the evaluation of the effect of some decontamination treatments on the shelf-life of MPV. ILP, gaseous and liquid ClO_2 and NEW were evaluated following a similar protocol. Firstly the efficacy of the methods to decontaminate MPV was measured, which corresponds to the results presented in chapter 3 for ILP, and in chapter 4 for the chemical methods. The set-up of the shelf-life studies was decided according to those results. MPV were intended to be stored under EMAP. In order to reach an EMAP, packaging configurations are designed taking the respiration rate of the commodity into account. The respiration rate of a MPV can be altered by the decontamination treatment. Therefore, the effect of the selected decontamination methods on the respiration rate of some MPV was measured. Subsequently, the main subject of this research was investigated, namely the effect of decontamination methods on the post-processing quality and shelf-life of MPV stored under EMAP at refrigeration temperatures. Classical microbiological methods and sensory evaluations were performed, together with monitoring pH changes and headspace oxygen and carbon dioxide concentrations during MPV storage. The fulfilments of the this part of the research can be found in chapter 3 for ILP, chapter 5 for gaseous ClO_2, and chapter 6 for NEW.

Moreover, results of chapter 4, as well as published one demonstrated that gaseous ClO_2 induces browning of MP lettuce and MP cabbage. Therefore, the inhibition of the browning occurring during the decontamination of these two commodities by action of ClO_2 was also tested. Results can be appreciated in chapter 5.

CHAPTER 1

Decontamination methods to prolong the shelf-life of minimally processed vegetables: state-of-the-art[1]

[1] *Redrafted from: Gómez-López, V. M., Ragaert, P., Debevere, J., Devlieghere, F. (Submitted November 2006)*

Chapter 1. Decontamination methods to prolong the shelf-life of minimally processed vegetables: state-of-the-art

1.1 Abstract

Minimally processed vegetables (MPV) are any fresh vegetable that has been physically altered from its original form, but remains in a fresh state. Microorganisms present in MPV can cause foodborne illnesses or spoilage, hence, decontamination of MPV can produce more stable products. The present review can be divided in three sections. The first part proposes five approaches that should be considered in the research of novel decontamination methods, namely: reducing the risk of foodborne infections and intoxications, decreasing microbial spoilage, preserving fresh attributes, preserving nutritional quality, and avoiding presence of unacceptable levels of toxic residues or formation of unacceptable levels of toxic by-products. The second part examines the difficulties to decontaminate and prolong the shelf-life of MPV, critically analysing the current way of data analysis and interpretation. It addresses the different aspects of the problem of the accessibility of sanitizers to microorganisms (irregularities of produce surface, injuries, internalisation, attachment, and biofilms). It also includes a critical exposition of the methodological problems to estimate the prolongation of shelf-life due to a decontamination method, namely: the variability among samples, the reproducibility of the results, the interpolation when lacking some crucial data, and especially the difficulties to keep microbial populations in treated samples at lower counts than in untreated samples during storage (the enhanced growth rate of microorganisms in decontaminated MPV, the patterns of microbial growth in non decontaminated and decontaminated MPV, and the role of temperature in keeping the decontamination effect). The third part describes the inactivation mechanism, advantages, disadvantages, effects on microorganisms, and toxicological issues of several decontamination treatments, together with their effects on sensory attributes, physiology, and nutritional and phytochemical composition of MPV. The explained methods are: continuous UV-C, intense light pulses, chlorine, electrolysed oxidizing water, aqueous and gaseous chlorine dioxide, and aqueous and gaseous ozone.

Keywords: decontamination, minimally processed, UV-C, chlorine dioxide, electrolysed oxidising water.

1.2 Introduction

Minimally processed vegetables (MPV) are any fresh vegetable that has been physically altered from its original form, but remains in a fresh state. Regardless of commodity, it has been trimmed, peeled, washed and cut into 100% usable product that is subsequently bagged or pre-packaged (IFPA, 2006). Eleven steps can be differentiated in the production chain of MPV: harvesting, refrigerated storage (or direct processing), preparation, washing, size reduction (trimming, peeling, cutting), washing/decontamination, removal of washing water, packaging, distribution, wholesaling/retailing, and finally holding by ultimate consumer (Wiley, 1994).

Going into more detail about the packaging step, the most studied packaging method for MPV is modified atmosphere packaging (MAP). The metabolism of MPV is reduced in MAP by low oxygen and/or high carbon dioxide concentrations in the headspace of the packages, which results in a lower rate of autolitic spoilage. The modified atmosphere can be created passively by produce respiration, or actively by direct injection into the packages. An equilibrium MAP (EMAP) is established inside the package when the permeability for oxygen of the packaging film is matched to the oxygen consumption rate of the packaged commodity (Kader et al., 1989).

Microorganisms present in MPV can cause foodborne illnesses or spoilage, hence, decontamination of MPV can produce safer and more stable products. The extension of the shelf-life of MPV is nowadays a challenge for the food industry, and for research institutions, where decontamination methods are currently evaluated. The structure of the research for testing decontamination methods to prolong the shelf-life of MPV should encompass five different approaches, namely:

1) Reducing the risk of foodborne infections and intoxications.
2) Decreasing microbial spoilage.
3) Preserving fresh attributes.
4) Preserving nutritional quality.
5) Avoiding presence of unacceptable levels of toxic residues or formation of unacceptable levels of toxic by-products.

Depending on their goals, available research articles have been focused in one or several, but not all these approaches. The integration of all of them will determine the adoption of a potentially successful new decontamination method by the food industry.

The biggest part of the literature deals with approach number 1, namely reducing the risk of foodborne infections and intoxications. Minimally processing can not ensure sterility of the product (Nguyen-the and Carlin, 1994), hence the risk of foodborne illnesses is high in comparison with that of other products, such as canned, frozen or dehydrated vegetables.

Approaches numbers 2 and 3 follow in the number of literature reports. Approach 2, decreasing spoilage microflora, is important to prolong the shelf-life. Approach 3, preserving fresh attributes, is essential for a decontamination method to be applicable, since decontaminated MPV should still have a fresh-look after the decontamination process. Most of the reports using approach 1 did not take into account this essential aspect of MPV preservation.

Approach 4 has been poorly studied. It is likely that some of the novel decontamination techniques decrease the nutritional quality of MPV. It is important to quantify the degree of nutrient losses if they can not be avoided, specially considering that MPV are frequently purchased by health conscious consumers (IFPA, 2006) that expect produce of nutritional quality equivalent to the fresh counterparts. Interestingly, there are evidences that minimally processing (Kang and Saltveit, 2002) and some decontamination methods (Cantos et al., 2001) can increase the content of phytochemicals.

Finally, approach 5, the toxicological aspects of the decontamination treatments for MPV, has been scarcely studied. It is possible that the mild nature of this kind of treatments, and definitively, the low potential of by-product formation of most of them, have accounted for the lack of interest in this area.

The efficacy of chemical methods used to reduce microbial populations is usually dependent upon the type of treatment, type and physiology of the target

microorganisms, characteristics of produce surface, exposure time and concentration of cleaner/sanitizer, pH, and temperature (Parish et al., 2003). Some of these aspects together with other ones are discussed below. It should be mentioned that literature on the use of some decontamination method is scarce. Therefore, it is difficult to draw definitive conclusions about methods whose efficacy would surely depend on treatment conditions and produce. Moreover, experiments on inactivation mechanisms give sometimes contradictory results. Consequently, conclusions given in this review will be confirmed or rejected by future experiments.

The present review does not disregard the importance of the presence of human pathogens in MPV. However, no emphasis was put on this subject because very comprehensive reviews on pathogen inactivation have area available (Beuchat, 1998; Parish et al., 2003). Consequently, this review is focused on the impact of decontamination techniques on the spoilage microflora of MPV, their sensorial and nutritional quality, and potential presence of toxic compounds. In this way, it attempts to provide summarized information regarding the production of MPV with extended shelf-life, high nutritional quality, and safety from the microbiological and chemical points of view. It starts with a discussion about general aspects of the use of decontamination treatments to prolong the shelf-life of MPV. Subsequently, some decontamination treatments will be explained, i.e.: continuous UV-C, intense light pulses, chlorine, electrolysed oxidizing water, chlorine dioxide, and ozone.

1.3 General aspects of the use of decontamination treatments to prolong the shelf-life of minimally processed vegetables

1.3.1 Accessibility of sanitizers to microorganisms

In order for a chemical decontaminant to kill microorganisms, contact has to occur between the microorganism and the sanitizer. Also when electromagnetic energy, such as UV-C, is used to decontaminate produce, contact has to occur between photons and microorganisms. Therefore, microorganisms will survive decontamination treatments when they are not accessible at the surface of the produce. The contact between sanitizer and microorganism can be difficult because microorganisms can be internalised in the produce, be present in surface irregularities, or form biofilms. Injuries caused during harvesting and transport can

offer protective places for microorganisms to survive and grow. Attached microorganisms are also more resistant to sanitizers although being exposed at the produce surface. The problem is worsened when organic matter present in the disinfectant solution or the same produce surface inactivates the disinfectant.

1.3.1.1. Irregularities of produce surface

The problem caused by the inaccessibility of sanitizers to microorganisms has been discussed by Adams et al. (1989). These authors observed by using scanning electron microscopy that surfaces of unwashed lettuce were liberally coated with bacteria. Washing with tap water removed many of them, although substantial numbers remained in hollows at the junction of epidermal cells and in folds of the epidermis. Since the microorganisms that survived in lettuce washed with hypochlorite were not intrinsically resistant, the authors suggested that they had not been in contact with the sanitizing solution, protected in groves or hollows on the surface as shown by microscopy. These features will be hydrophobic because of the waxy surface cuticle and thus serve as protective pockets where aqueous solutions can not penetrate and the bacteria can lie unmolested.

Entrance of *Escherichia coli* O157:H7 cells into stomata of cut lettuce and penetration into damaged tissue provide protection against chlorine inactivation (Takeuchi and Frank, 2000). Takeuchi and Frank (2001), also with *E. coli* O157:H7 inoculated onto lettuce pieces, showed by using confocal scanning microscopy that although cells on the leaf surface were mostly inactivated by chlorine, some viable cells were observed in cracks of cuticle and on the trichome, which demonstrates the importance of lettuce leaf structures in the protection of microorganisms from chlorine inactivation.

1.3.1.2 Injuries

Injuries at the surface of produce also protect microorganisms from the effect of sanitizers. Han et al. (2000a) demonstrated by using scanning electron microscopy that cells of *E. coli* O157:H7 preferentially attached to coarse and porous intact surfaces and injured surfaces. Injuries to the wax layer, the cuticle and underlying

tissues increased bacterial adhesion and resistance to washing and disinfecting treatment. Moreover, injuries can favour bacterial growth. *E. coli* O157:H7 grew in injured but not in uninjured green pepper surfaces. Injuries offer a hydrophilic surface where cells can attach, and provide nutrients allowing survival and multiplication.

Han et al. (2000b) hypothesized that because gas has greater penetration ability than liquid, gaseous disinfectants such as ClO_2 may be more effective for surface sanitation than aqueous compounds. Their results (Han et al., 2001b) demonstrated that ClO_2 gas was indeed more effective than aqueous ClO_2 in inactivating *Listeria monocytogenes* on injured green pepper surfaces, yet the inactivation was not complete. Furthermore, Han et al. (2000b) found that ClO_2 gas treatment showed significantly more log reductions (1.23-4.24 log) of *E. coli* O157:H7 on uninjured green bell pepper surfaces than on injured surfaces. The micrographs showed that bacteria preferentially attached to injured surfaces and those bacteria could be protected even from ClO_2 by the injuries. Therefore, although gaseous ClO_2 is indeed more effective than aqueous ClO_2, injuries also pose a problem for gaseous decontaminants.

1.3.1.3 Pre-harvest internalisation of microorganisms

Microorganisms can be naturally internalised in produce before harvesting or become internalised by or during process operations. Evidences of internalisation come from different sources: determination of microbial counts in samples taken from inner tissues, experiments where plants are grown in presence of marker microorganisms, or decontamination experiments. In the latter, differences between the levels of disinfection at the sample surface and in the whole sample indicate that part of the microorganisms are not accessible to disinfectants and must have been present in the inner tissue.

The inner part of sound produces is generally considered to be sterile. However, endophytic bacteria are known to reside in a wide range of plant tissues. Testing the effect of neutral electrolysed water on total microbial counts of several MPV, Izumi (1999) found that the decontamination was the greatest with spinach leaves, which had the maximum surface area/unit weight of tissue among the tested MPV. The

microbicidal effect was greater on the surface of the tissues than in macerate, which indicated that microbes inside of the tissue were more difficult to control. Moreover, Koseki et al. (2001) compared the effect of the decontamination of MP lettuce with acidic electrolysed water on microbial populations on the surface and in the whole MPV. After treatment, yeast and mould populations in the whole lettuce were higher than 2 log CFU/g but were not detectable on the surface. This indicates that the microorganisms on the surface of lettuce were easily disinfected (the detection limit was not reported). The residual microorganisms embedded in the whole lettuce were, according to the authors, either inside the vegetable tissue or making biofilm on the surface of lettuce. In fact, a biofilm was observed in this work on the surface of lettuce treated with acidic electrolysed water.

Several conclusions can be deduced from research and review articles published on internalisation of foodborne pathogens by plants. Microorganisms present in contaminated soil or hydroponic solution can penetrate plants through the root system. The mechanism of microbial internalisation into plant structures is a three-phase process: colonization of the root surface, infection of the vascular parenchyma, and invasion of the xylema. The production of plant cell wall-degrading enzymes is not an absolute requirement for invasion. Major points of entry appear to be wounds that naturally occur as a result of growth, through root hairs, at the root emergence zone, and at epidermal conjuctions (Guo et al., 2002). The process of internalisation seems to be a process that requires energy. Likewise, motility seems to be an important factor for infiltration of some microorganisms. The ability to move along the surface of the plant can assist in allowing microorganisms to find openings to enter the plant (Aruscavage et al., 2006). Once microorganisms are inside the root system, they can migrate throughout other parts of the plant, for instance, reaching the leaves of lettuce plants. However, it is also possible that microorganisms penetrate a root system without migration to other plant structures (Beuchat, 2006). Since once internalised, microorganisms can survive and will be more difficult to inactivate by decontamination treatments, it is the foremost importance to avoid pre-harvest contamination of vegetables.

1.3.1.4 Internalisation of microorganisms during washing of whole vegetables

Several authors have proved that microorganisms can be internalised into vegetable tissues when present in dump tanks given a negative temperature difference, i.e., when the water temperature is less than the produce temperature (Bartz and Showalter, 1981; Bartz, 1982; Zhuang et al., 1995). Microorganisms have been shown not only to infiltrate, but also to survive inside fruits and vegetables (FDA, 1999). This poses a problem for decontamination treatments. When cutting, a small fraction of the internalised microbial population gets exposed to the decontaminant agent, but the highest proportion stays out of reach, although that could depend on the size of the cut pieces and the relation area/surface of the intact vegetable. Furthermore, cutting exposes part of the produce inner tissue to the environment, which allows new contamination (King et al., 1991, Allende et al., 2004) and the net change will be a more contaminated produce.

1.3.1.5 Internalisation of microorganisms during production of MPV

Cutting can also permit the internalisation of microorganisms. Takeuchi et al. (2000) showed that some species of microorganisms attach preferentially to cut edges while others to intact surface of cut lettuce leaves. More interestingly, some microorganisms at the cut edge penetrated the lettuce tissues, indicating that penetration occurred from the cut edge surface. When studying decontamination of cut lettuce from *E. coli* O157:H7 by using chlorine, Takeuchi and Frank (2000) found greater penetration of cells when the temperature of the inoculum was held at 4℃ compared with 7, 25, or 37℃. This finding is important from the practical point of view, because a cold temperature of the washing water during the preparation of MPV will promote microorganism internalisation. A more specific study (Takeuchi and Frank, 2001), also with *E. coli* O157:H7 inoculated onto lettuce pieces, showed that the deeper the penetration of cells the higher the proportion of survivors after chlorine disinfection. Moreover, Rodgers et al. (2004) showed that longer exposure times to ClO_2 and other sanitizers were necessary to produce 1 log reduction of *L. monocytogenes* and *E. coli* O157:H7 when lettuce was shredded compared to the whole lettuce.

1.3.1.6 Attachment and biofilms

There is increasing scientific evidence showing that microorganisms attached to surfaces are more resistant to sanitizers than their planktonic counterparts. As example, Peta et al. (2003) showed that attached cells of *Bacillus* spp. were more resistant to ClO_2 than planktonic cells grown in the presence of surfaces, the latter were also more resistant than simple planktonic cells. Moreover, attachment can be followed by the development of biofilms.

Biofilms pose a big problem for decontamination of MPV. Biofilms have been observed in the surface of different produce such as lettuce (Koseki et al., 2001), tomato, carrots (Rayner et al., 2004), and alfalfa, clover and mung bean sprouts (Fett and Cooke, 2005). Although biofilms adhering to peels will be removed during peeling, they will persist in non-peelable produces such as leafy vegetables. Biofilms were observed by Morris et al. (1997) on leaves of all sampled species (spinach, lettuce, Chinese cabbage, celery, leeks, basil, parsley, and broad-leaved endive). Morris et al. (1998) calculated that as much as 38 and 17% of bacteria present respectively in their samples of endive and parsley leaves were forming biofilms.

Chlorine concentrations measured in *Pseudomonas aeruginosa-Klebsiella pneumoniae* biofilms were typically only 20% or less of the concentration in the bulk liquid. The mass of the biofilm offered resistance to diffusion of chlorine together with chlorine degradation (de Beer et al., 1994). Bacteria of the biofilm were 150 to more than 3,000 times more resistant to HOCl than were unattached cells (LeChevallier et al., 1988). Norwood and Gilmour (2000) showed that *L. monocytogenes* is more resistant to sodium hypochlorite when it is in a multiespecies biofilm compared to when it is in a mono-culture biofilm or in the planktonic state. The possible causes for increased resistance to antimicrobial compounds of bacteria in biofilms have been revised by Kumar and Anand (1998). This resistance is attributed to the varied properties associated with the biofilm including: reduced diffusion, physiological changes and production of enzymes degrading antimicrobial substances. A characteristic feature of microbial biofilms is the presence of an exopolysaccharide matrix embedded with the microorganisms. This matrix may act to various degrees

as a diffusion barrier, molecular sieve and absorbent. Bacteria within the biofilm exhibit a varied physiological pattern, receiving less oxygen and fewer nutrients than those cells at the biofilm surface. Thick biofilms may include many metabolically dormant and/or dead cells. These cells may have an altered growth rate and physiology, resulting in increasing resistance to antimicrobial agents. Microorganisms can produce enzymes degrading antimicrobial substances. Such enzymes degrade antimicrobials as they permeate through the cell envelope to their target sites, but also the enzymes may become trapped and concentrated within the biofilm matrix, which will exhibit enhanced protective properties.

1.3.2 Initial contamination level

The efficacy of a decontamination treatment in terms of log reductions is influenced by the amounts of initial microbial populations, which might also determine the magnitude of the prolongation of the shelf-life. This fact can be illustrated by using an example from pathogen inactivation, where initial populations can be easily controlled inoculating desired amounts of microorganisms. Results reported by Singh et al. (2002a) on the decontamination of MP lettuce showed that the lower the levels of *E. coli* O157:H7 the higher were the log reductions due to treatment with aqueous ClO_2, ozonated water or thyme oil. The authors proposed that at high inoculum populations of *E. coli* O157:H7 on lettuce, a large number of bacteria may be entrapped in injured sites and thus may be minimally affected by sanitizer treatments. Contrastingly, when Beuchat et al. (2004) studied the inactivation of *L. monocytogenes* inoculated onto Iceberg lettuce pieces, shredded Iceberg lettuce, and Romaine lettuce pieces, reductions were generally higher as the inoculum level increased.

1.3.3 Criterion to establish a decontamination goal

Decontamination of MPV to prolong their shelf-life is based on the belief that it is necessary to decrease counts of the initial microbial populations of MPV in which the shelf-life is limited by microbial proliferation. But, there is no rule for determining how low the microbial microflora should be decreased to attain a certain shelf-life extension. It would even not be always necessary to decrease the microbial population, although results published by Allende et al. (2006) are uncommon. Those

authors found that after treating lettuce with UV-C, no significant decrease in total aerobic bacteria counts was observed. During storage, however, counts of treated lettuce were lower than those of untreated controls; a shelf-life extension of at least two days was estimated based on microbial counts.

The prolongation of the shelf-life of produce poses two possible safety problems. First, a shelf-life extension increases the likelihood that a produce with dangerous levels of pathogenic bacteria can be consumed, as it was warned by Brackett (1992). Second, decontamination eliminates spoilage population that could otherwise compete with pathogenic microorganisms for space and nutrients, thereby providing growth potential for pathogenic contaminants (Parish et al., 2003). One should ask the question, how low the spoilage flora can be decreased without causing safety problems?

The first problem can be illustrated with two examples. Berrang et al. (1989a) observed that controlled atmosphere storage extends the shelf-life of asparagus, broccoli, and cauliflower without appreciably hampering the growth of *Aeromonas hydrophila*. For example, broccoli stored under controlled atmosphere at 4°C is likely to remain acceptable for consumption for 21 days while broccoli stored under air would remain only 14 days acceptable. But after 14 days, *A. hydrophila* counts already reached their maximum. This 7-day extension increases the likelihood that broccoli with high counts of *A. hydrophila* would be purchased and consumed. Controlled atmospheres also extended the shelf-life of asparagus without abating *L. monocytogenes* growth. The extended shelf-life afforded the organism more time to grow before the product became unusable. Thus, the asparagus with extended shelf-life accumulated higher pathogen populations than did conventionally stored asparagus (Berrang et al., 1989b).

The second problem is more directly related to decontamination and decontamination levels. As examples, Bennik et al. (1996) showed that the natural microflora of MP chicory endive inhibits the growth of *L. monocytogenes*. They also demonstrated that when the pathogen was inoculated after decontamination, it grows faster in the disinfected produce than in the water rinsed one. The use of mild heat treatment, with the aim to prolong the shelf-life of lettuce, enhanced the growth of *E. coli* O157:H7 (Li

et al., 2001a) and *L. monocytogenes* (Li et al., 2002) during subsequent storage at 15°C when pathogens were inoculated before, and also after decontamination. Similar results were published by Francis and O´Beirne (2002) for *E. coli* and *Listeria innocua* on MP Irish lettuce inoculated before chlorination and stored at 8°C.

Studies on decontamination treatments have been mainly focused on pathogen inactivation. Moreover, studies performed to prolong the shelf-life frequently exclude pathogens from the research. Likely, future studies would include both approaches, although progress has already been done, such as the works by Sy et al. (2005ab). That would enable to determine how much the risk of outbreaks is influenced as a function of the achieved decontamination levels. The answer to the next two questions should be integrated in the future. Is it possible to estimate how much decontamination should be achieved to get certain shelf-life prolongation? and how much is the risk of outbreaks increased due to an extended shelf-life of a decontaminated MPV? The response to the last question will limit that to the first one.

1.3.4 Methodological problems to estimate the prolongation of shelf-life due to a decontamination method

1.3.4.1 The variability

The extension of the shelf-life of a MPV due to a decontamination treatment is frequently evaluated using mean values. It is considered that the MPV reaches the end of the shelf-life when counts of one of the groups of microorganisms reach a pre-established maximal count or when one of the sensory attributes reaches an unacceptable score. The first of these events that takes place will determine the shelf-life. Therefore, the shelf-life prolongation is evaluated subtracting the number of days that the treated MPV is acceptable minus that of the untreated MPV.

This approach does not take into account sample variability. Even though a count of a specific population or the score of a specific sensory attribute could be lower than the respective critical value, the 95% interval can surpass the limit, yet the MPV is still considered acceptable (Figure 1.1). The same happens with the difference in shelf-life of untreated and decontaminated MPV. One batch could be regarded as

unacceptable and the other still acceptable even when no statistical difference exists between both (Figure 1.2).

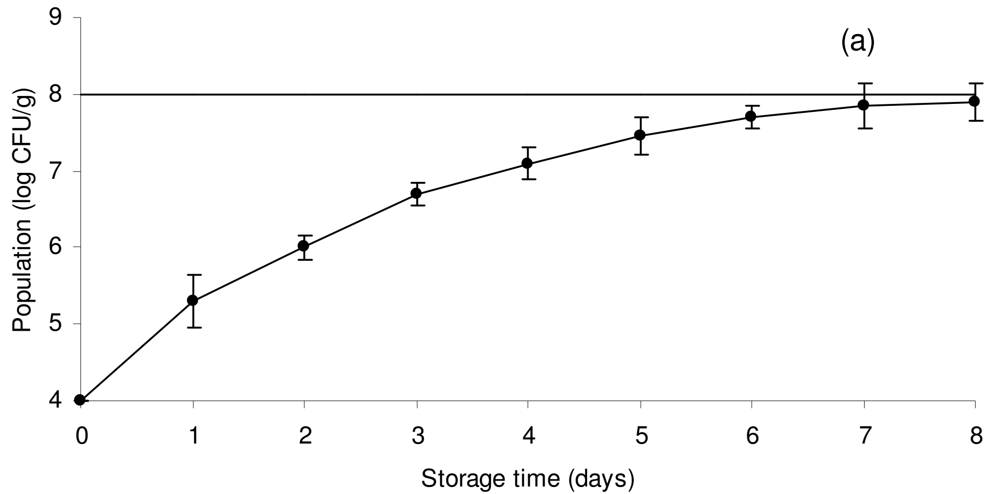

Figure. 1.1. The effect of sample variability in the decision of the end of the shelf-life. Product is considered acceptable although the 95% confidence interval surpasses the microbial limit. Horizontal line indicates the shelf-life limiting count. Error bars mean 95% confidence interval.

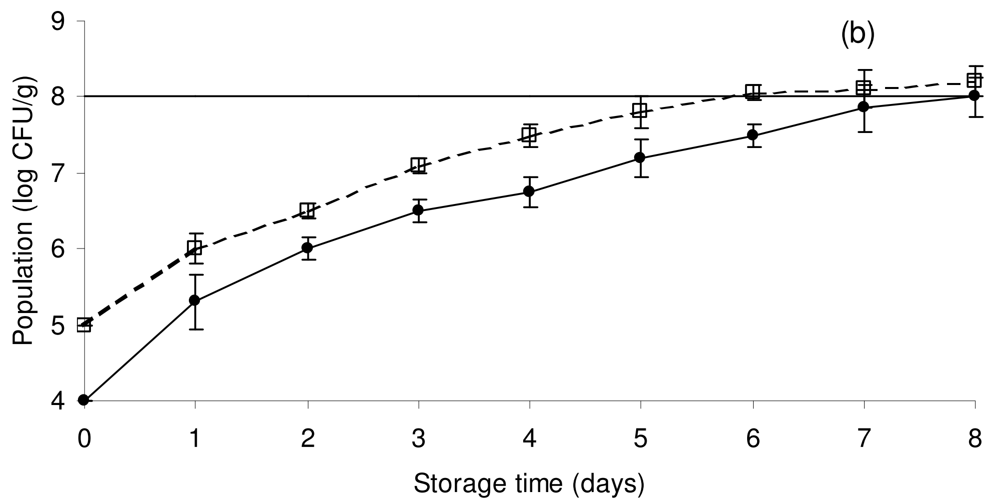

Figure 1.2. The effect of sample variability in the decision of the end of the shelf-life. One product is acceptable and the other does not, although the 95% confidence intervals overlap each other (see day 7). Horizontal line indicates the shelf-life limiting count. Error bars mean 95% confidence interval.

A novel approach for calculation shelf-life of MPV has been given by Corbo et al. (2006). The Gompertz equation modified by Zwietering has been used to calculate the shelf-life of MPV. The method generally adopted consists in estimating the Gompertz parameters (maximum cell increase attained at the stationary phase, maximal growth rate, and duration of the lag phase) by fitting the equation to the growth curve of a microbial population present in a MPV. Then, by fixing a maximum acceptable contamination value to the end of the shelf-life of the MPV (generally established by a legal regulation), the equation allows to calculate the respective shelf-life. The equation also permits the estimation of the confidence interval of each of the Gompertz' parameters, but it is not possible to estimate the confidence interval of the shelf-life since it does not appear explicity in the equation. The solution proposed by the authours is to rearrange the equation in such a way that the shelf-life appears directly as a parameter of the equation relating the microbial count to storage time. Being the shelf-life a parameter, its confidence interval can be estimated. The superposition of confidence intervals indicates when there is no significant difference in the estimated shelf-life of two batches of product, which can consist in control and treated MPV.

In order to estimate the shelf-life from the sensorial point of view, Piagentini et al. (2005) proposed a kinetic approach to model changes in individual sensory attributes for individual and mixed fresh-cut leafy vegetables at different storage temperatures. However, the model does not estimate the variability.

1.3.4.2 The reproducibility

One of the most important features of a scientific research is that results have to be reproducible. The reproducibility of results is tested when the experiment is replicated, i.e. the same experiment is repeated several times. However, it is not possible to expect perfectly reproducible results in uncontrolled biological systems. There is a biological variability (for example of raw material or contaminating microorganisms), process variability and variability after processing in real scenarios

(for example storage time, storage temperature), at the distribution and consumer level (Zwietering, 2002). Nonetheless, it is important to quantify the reproducibility of the shelf-life extension before scaling-up decontamination processes to industrial level.

However, experiments on shelf-life are usually performed once. They are composed of two batches of MPV, untreated and treated. Data from the growth of microbial populations of each one of the batches during the storage experiment will produce a growth curve. The error bars presented in those curves are calculated from the results obtained by taking samples from different bags during the storage study. But all those bags come from a single batch. Therefore, the error bars represent the variability (see section 1.3.4.1) within one batch, but do not estimate the reproducibility of the growth curve. The same idea is valid for the change of any other quality parameter. The study would produce three outcomes: the shelf-life of the untreated and treated MPV, and the estimation of the shelf-life extension. The latter is the difference in days between the shelf-life of treated and untreated MPV.

But, given a positive result in shelf-life extension in one experiment, what would happen if the experiment were replicated? One of the few studies replicating experiments was performed by Allende and Artés (2003a) on treatment of MP Red Oak Leaf lettuce with UV-C (table 1.1). Based only on psychrotroph counts, and comparing the difference in time (days) when population reached the maximum allowed psychrotroph counts, a shelf-life extension of at least 4 days could have been concluded from the first experiment. However, in the second replication and for the same population, starting from the same initial counts and decontamination levels, only 3 extra days were gained. That could have two important implications. Firstly, in the first replication the estimated shelf-life of the decontaminated produce was 4 days longer than in the second. Therefore, what could be the recommended expiration date? Secondly, there is one day difference in the shelf-life prolongation between both replications, how long is the actual shelf-life extension? What would happen with a third replication?

Table 1.1. Time when psychrotroph counts reached unacceptable levels in two MP lettuces, showing the differences between replication of shelf-life experiments.

Variety	Replicate	Control	Treated	Reference
Red Oak Leaf	1	6 days	>10 days	Allende and Artés (2003a)
	2	3 days	6 days	
Lollo Rosso	1	6 days	8 days	Allende and Artés (2003b)
	2	5 days	7 days	

Another article reporting replicated experiments is Allende and Artés (2003b), on the application of UV-C to MP Lollo Rosso lettuce (table 1.1). Based only in psychrotroph counts again, a shelf-life extension of 2 days could have been concluded from results of both replicates, although the expiration dates differ by one day. Moreover, the same article (Allende and Artés, 2003b) reported coliform growth in two experiments. In the first one, a lag phase 5-day long can be observed for all the treatments and the control. However, in the second experiment, no lag phase was apparent after 3 days of storage.

Therefore it is important to remark that reports of successful prolongation of shelf-life of MPV only sign the potential of the decontamination method to do it. But the multiple replication of experiments is necessary to have a better assessment of the efficacy of the method to prolong the shelf-life.

1.3.4.3 Interpolation

Due to the intensity of the experiments for the determination of the shel-life of MPV, microbial counts and/or sensory evaluation are not often performed every day thorough the storage time. Therefore interpolation is a very common situation when evaluating shelf-life of MPV. It is important that actual determinations are performed before and after the produce reaches the expiration day, otherwise the accuracy of the shelf-life span will be compromised. Another alternative is modelling the quality evolution in order to estimate when the shelf-life limiting parameter reaches the cut-off.

1.3.5 Keeping microbial loads low

One of the purposes of treatments to decontaminate MPV is to reduce the initial microbial load of the product. Assuming that the microbial populations in decontaminated samples will grow at the same or at slower rate than in non decontaminated samples, the shelf-life of the product will be prolonged, at least from the microbiological point of view. However, different studies have demonstrated that decontamination treatments can enhance the growth rate of microorganisms. Consequently, after some days in storage, microbial populations in decontaminated produce could be higher than in their non decontaminated counterparts, and the benefit of the decontamination is lost.

1.3.5.1 Enhanced growth rate of microorganisms in decontaminated MPV

Bennik et al. (1996) decontaminated MP chicory endive leaves with hydrogen peroxide, and subsequently compared the changes in total plate counts in not disinfected and disinfected samples during storage at 10°C. Disinfection decreased counts by 1.6 log CFU/g, but they became equal at day 4. Li et al. (2001b) studied the changes in natural microflora on MP lettuce decontaminated with warm, chlorinated water, which were then stored at refrigeration temperatures. Psychrotroph counts were higher in treated than in untreated samples after 2 days of storage at 15°C, and after 4 days at 5°C. Similar patterns were observed for mesophilic aerobes, yeasts and moulds. In another work, Koseki and Itoh (2001, 2002) decontaminated MP lettuce and MP cabbage with acidic electrolysed water, then stored them at 5 and 10°C. Almost all bacteria treated with electrolysed water showed a higher growth rate and shorter lag time than did those treated with tap water. It is possible that some decontamination treatments cause some kind of damage to the tissue integrity of the produce that provides easier access to nutrients, allowing in consequence faster growth.

This fact has very important consequences. In such cases, decontamination is not only useless to prolong the shelf-life, but also counterproductive for the safety and

stability of the produce. Therefore, it is not only necessary to investigate techniques to decontaminate MPV, but also to explore ways to keep the microbial populations in decontaminated samples at lower levels than in non decontaminated samples throughout the shelf-life.

Some useful ideas can be taken from Cooksey (2005) and Aguayo et al. (2006). In the latest work, tomato slices were stored at 5°C up to 15 days under air or cyclic exposure to ozone gas. Growth of mesophilic and psychrotrophic bacteria, yeasts and moulds was retarded in samples exposed to ozone in comparison with controls. Similarly, Cooksey (2005) placed ClO_2 releasing sachets inside bags containing chicken breasts. Total plate counts during storage were 1.0-1.5 log CFU/piece lower in treated than in control breasts. Another possibility could be using packaging films that release those gases. However, the practical implementation of gaseous treatment during storage of MPV in retail bags can be a problem, since gas has to diffuse to all surfaces of the vegetables, and ozone and chlorine dioxide can be degraded by the food before reaching microorganisms.

1.3.5.2 Patterns of microbial growth in non decontaminated and decontaminated MPV

From the microbiological point of view, an extension of shelf-life means that microbial counts of untreated samples should reach the specified maximum before those of treated produce. Different situations can occur after decontamination, all but the last example result in shelf-life extension (see Figures 1.3 and 1.4):

1) No decontamination occurred, but

1.1) Growth rate of microorganisms in treated samples is slower than that of the untreated samples (Figure 1.3a). Example: Allende et al. (2006).

1.2) Growth curve of microorganisms in treated samples have a longer lag phase than that of the untreated samples (Figure 1.3b). Example: Beltrán et al. (2005a).

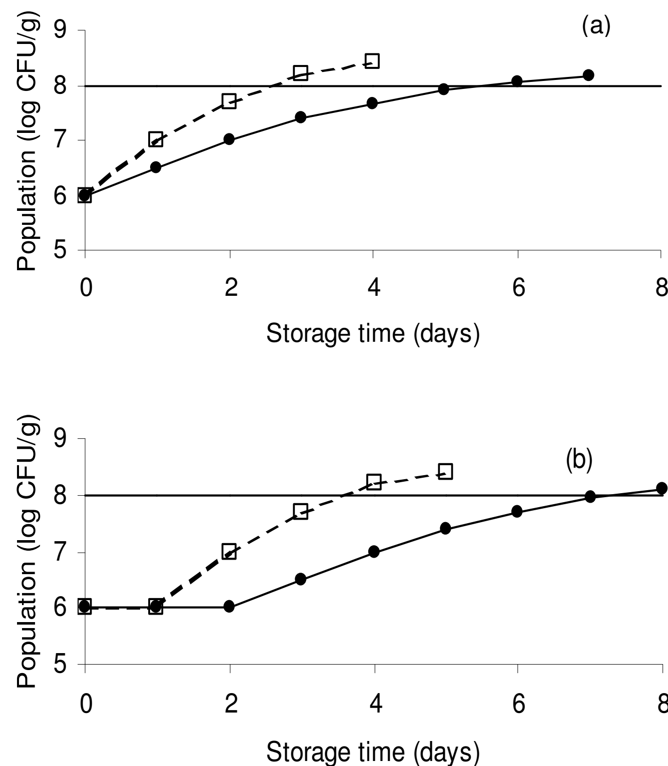

Figure 1.3. Schematic representation of two cases that can occur during the storage of MPV when no decontamination has been achieved. Untreated MPV (--□--), treated (—●—) MPV. See section 1.3.5.2 for explanation.

2) Decontamination occurred, and

2.1) Growth rate of microorganisms in treated samples is slower (or counts decrease) than that of the untreated samples (Figure 1.4a). Example: Chaudry et al. (2004).

2.2) Growth rate of microorganisms in treated samples is equal than that of the untreated samples (Figure 1.4b). Example: Beltrán et al. (2005b).

2.3) Microbial populations in treated samples do not grow or exhibited lag phase. (Figure 1.4c). Example: Ramamurthy et al. (2004).

2.4) Growth rate of microorganisms in treated samples is faster than that of the untreated samples, and growth curves of both kind of samples intercept each other before reaching the microbial limit (Figure 1.4d). Examples: Delaquis et al. (1999), Li et al. (2001b), and some others.

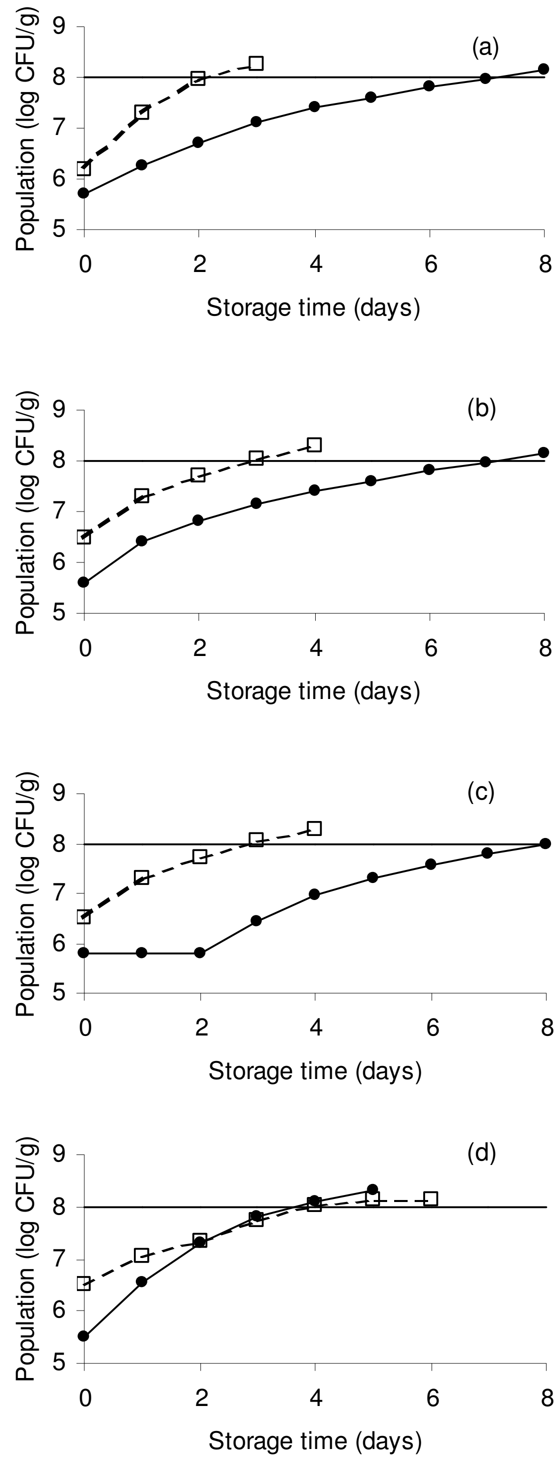

Figure 1.4. Schematic representation of four cases that can occur during the storage of MPV when decontamination has been achieved. Untreated MPV (--□--), treated MPV (—●—). See section 1.3.5.2 for explanation.

The specifications proposed by Debevere (1996) were used to determine the end of the shelf-life in these examples. Those are: 8 log CFU/g for psychrotrophs, 5 log CFU/g for yeasts, and 7 log CFU/g in combination with sensory analysis for lactic acid bacteria.

1.3.5.3 Role of temperature in keeping the decontamination effect

Authors attribute the success of a decontamination treatment in the prolongation of the shelf-life to the disinfection method itself, and sometimes to the influence of the used modified atmosphere. In various cases it is likely that some treatments have a residual effect on microorganisms, for example, sublethal damage, or have caused changes on the proportion of species of microorganisms present on the product. Moreover, treatment can cause physiological or structural changes to the produce, which could affect its role as substrate. But it is also possible that the storage conditions such as temperature usually play an important role in the success of a decontamination treatment.

For example, Francis and O´Beirne (2002) dipped inoculated MP Irish lettuce in chlorinated water or in distilled water (control), and stored them. At 3℃, counts of *L. innocua* in treated and control samples equalled at day 14, while at 8℃ did it at day 7. Counts for *E. coli* were lower in treated samples stored at 3℃ during the 14 days of the study, but equalized at 8℃ at day 7.

An evaluation of the literature on shelf-life extension was performed for this review taken into account some arbitrary criteria in order to simplify and exemplify the proposal. Thirty nine cases were analysed. Shelf-life was estimated from published data (figures or tables) based on psychrotrophic counts. When they were not available, mesophilic counts were used. Only mean counts were taken into account. When figures were used, the intersection of the growth curves was taken into account. Only studies sampling at least at 5-day intervals were considered. The main point considered was whether the microbial population of treated samples was lower than that of controls during 7 days of storage.

From Figure 1.5, it is obvious that at storage temperatures between 1 and 4℃ the considered bacterial populations in decontaminated samples kept counts lower than in controls regardless the treatment in 100% of the cases. At storage temperatures between 5 and 8℃, some cases appeared where the counts in treated samples were higher than in controls, and the referred proportion fall to 67. Between 9 and 16℃, only 33% of the cases were successful in keeping lower counts in treated samples. Therefore, it can be suggested that, in general, lower temperatures are an important factor to avoid that microbial populations in treated samples reach higher counts than in controls during the storage of MPV.

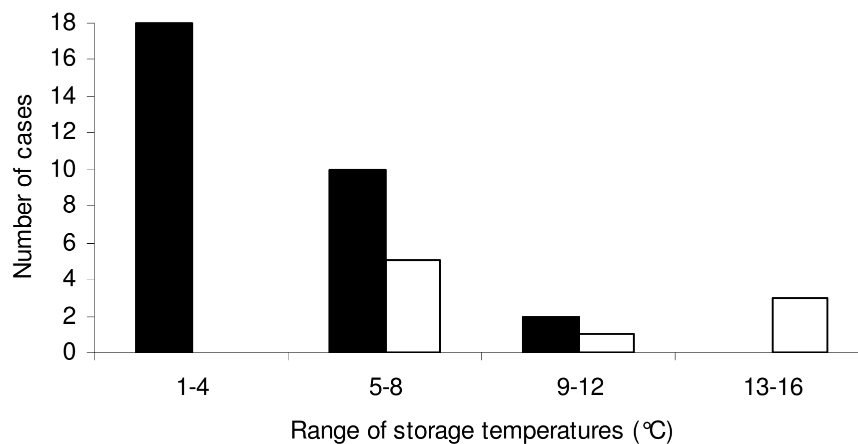

Figure 1.5. Number of successful (■) and unsuccessful (□) attempts to prolong the shelf-life of MPV as function of storage temperature.
References: Ahn et al. (2005), Allende and Artés (2003ab), Allende et al. (2006), Baur et al. (2005), Beltrán et al. (2005b), Bennik et al. (1996), Chaudry et al. (2004), Clyffe-Byrnes and O´Beirne (2005), Delaquis et al. (1999), Fan et al. (2003), Foley et al. (2002), Francis and O´Beirne (2002), Gaertner et al. (1997), Ihl et al. (2003), Kamat et al. (2005), Klaiber et al. (2005), Koseki and Itoh (2001, 2002), Li et al. (2001b, 2002), Lu et al. (2005), Nicholl et al. (2004), Orsat et al. (2001), Ramamurthy et al. (2004), Rodgers et al. (2004), Simon et al. (2004), Zhang et al. (2005, 2006).

Gamma irradiation seems to be a method that can really control by itself the microbial growth in treated samples regardless the storage temperature, since microbial populations in irradiated samples decrease during storage or remain long time at very low levels. Examples on this regard can be observed from the results by Ramamurthy et al. (2004), Chaudry et al. (2004), Ahn et al. (2005), and Kamat et al. (2005); although not in Foley et al. (2002), and Zhang et al. (2006).

1.4 UV-C

1.4.1 Definition

UV-C is the portion of the electromagnetic spectrum corresponding to the band between 200 and 280 nm. The knowledge of UV-C illumination as a technique to preserve foods was discovered in the 1930s (Artés and Allende, 2005). Inactivation of microorganisms with UV systems is frequently achieved by using low pressure mercury lamps designed to produce energy at 254 nm (monochromatic light), called germicidal light (Bintsis et al., 2000). More recently, medium pressure UV lamps have been used because of their much higher germicidal UV power per unit length. Medium pressure UV lamps emit a polychromatic output, including germicidal wavelengths from 200 to 300 nm (Bolton and Linden, 2003). In the food related industry, applications include disinfection of water supplies, food contact surfaces, air in a food preparation area (Bintsis et al., 2000), or food packaging materials (Mimouni, 2001).

1.4.2 Units

The following units are necessary to characterize an UV-C treatment.

- Exposure time: length in time (seconds) of the UV-C illumination process.
- Irradiance: Watt/meter^2 (W/m^2) is the flux incident from all upward directions on an infinitesimal element of surface containing the point under consideration divided by the area of the element surface.
- Fluence rate: Watt/meter^2 (W/m^2) is the flux incident from all directions on a small transparent imaginary spherical volume element containing the point under consideration divided by the cross-sectional area of that sphere. For a parallel and perpendicularly incident beam, not scattered or reflected, irradiance and fluence rate become identical.
- Fluence: Joule/meter^2 (J/m^2) is the product of the fluence rate and the exposure time. The term dose is sometimes used as synonym of fluence.
- Energy density: Joule/meter^2 (J/m^2) is the energy per unit area incident on one side of a small plane surface, the product of the irradiance (in watts/m^2) and

the exposure time in sec. For a parallel and perpendicularly incident beam, not scattered or reflected, fluence and energy density become identical.

(IUPAC, 1996)

1.4.3 Inactivation mechanism

The germicidal effect of UV light on bacteria is primarily due to the formation of pyrimidine dimmers, mainly thymine dimmers (cyclobutane dimmers) (Mitchell et al., 1992, Giese and Darby, 2000). The dimmer inhibits the formation of new DNA chains in the process of cell replication thus resulting in the inactivation (inability to replicate) of affected microorganisms by UV, called clonogenic death (Bolton and Linden, 2003). On bacterial spores, UV-C treatment results mainly in formation of the “spore photoproduct” 5-thyminyl-5,6-dihydrothymine, single-strand breaks, double-strand breaks and cyclobutane pyrimidine dimmers (Slieman and Nicholson, 2000). The shape of the inactivation curve for microbial inactivation by UV light is sigmoid. The initial plateau is due to an injury phase. Once the maximum amount of injury has been surpassed, minimal additional UV exposure would be lethal for microorganisms and survivor numbers rapidly decline (Sastry et al., 2000). The end of the curve has a tailing phase that has received several explanations, which have been summarized by Yaun et al. (2003): lack of homogeneous population, multi-hit phenomena, presence of suspended solids, the use of multiple strains that may vary in their susceptibility to UV-C, varying abilities of cells to repair DNA mutations, and the shading effect that may have been produced by the edge of the Petri dishes used in some experiments.

As a general rule in photochemical processes, the principle of equi-effectivity of the product of fluence rate and exposure time is valid. This principle is known as the Bunsen-Roscoe reciprocity law. The principle of Bunsen-Roscoe asserts that for the effectiveness of radiation it does not matter whether the fluence is reached with high fluence rate and short exposure time or with low fluence rate and long exposure time. Sommer et al. (1996) tested the validity of this law. The principle could not be rejected for the tested virus and bacteria. However, in the eukaryotic test strains of *Saccharomyces cerevisiae*, longer exposure times with lower fluence rates led to enhanced inactivation.

Microbial cells can repair themselves from a photochemical damage. There are two types of repair, dark repair and photoreactivation. Photoreactivation means the reversal of UV damage in bacteria by illumination with visible light (Cleaver, 2003). It is catalysed by the enzyme photolyase, which uses light energy to split UV-induced cyclobutane dimmers in damaged DNA through a radical mechanism. Photolyase is a flavoprotein and contains two noncovalently bound chromophores. One chromophore is the fully reduced flavin-adenine dinucleotide ($FADH^-$), the catalytic cofactor that carries out the repair function upon excitation by either direct photon absorption or resonance energy transfer from the second chromophore (methenyltetrahydrofolate or deazaflavin) that harvest sunlight and enhances the repair efficiency. The excited flavin cofactor transfers an electron to the cyclobutane pyrimidine dimmer to generate a charge separated radical pair. The anionic ring of the dimmer is split, and the excess electron returns to the flavin radical to restore the catalytic competent $FADH^-$ form and close the catalytic photocycle (Kao et al., 2005).

1.4.4 Advantages and disadvantages

1.4.4.1 Advantages

- The equipment is relatively inexpensive and the technique is easy to use, although is subject to certain safety precautions (Bintsis et al., 2000).
- Lack of residual compounds (López-Rubira et al., 2005).
- Avoid the use of chemicals that can cause ecological problems and/or are potentially harmful to humans (Allende and Artés, 2003b).

1.4.4.2 Disadvantages

- For opaque bodies, it is only useful to decontaminate surfaces. Although some degree of inactivation could occur below the surface, the decontamination is only virtually achieved at the surface.
- When irradiating a three-dimensional object, it is necessary to ensure that all its surfaces receive adequate exposure to UV, which needs equipments with radically new designs (Gardner and Shama, 2000).

- Possibility that microorganisms repair themselves from damage. One of them is photoreactivation, which should be avoided by protecting produce from visible light.
- Possibility of shading, that is, when microorganisms readily absorb the rays, such as *Aspergillus niger*, and are present one upon another, the organisms in the lower layers are very hard to destroy in contrast to those in the upper layer (Hiramoto, 1984).

1.4.5 Effect on microbial populations

It has been proved that UV-C can inactivate pathogenic microorganisms *in vitro* as well as inoculated onto vegetable surfaces. For example, *Salmonella typhi*, *Shigella sonnei* and *Staphylococcus aureus* cultures (Chang et al., 1985) and *Salmonella* and *E. coli* O157:H7 inoculated onto agar surfaces (Yaun et al., 2003) and lettuce leaves (Yaun et al., 2004) have been inactivated by UV-C.

UV-C has been successfully used to prolong the shelf-life of MPV. According to the analysis of the literature, two possibilities to prolong the shelf-life of MPV by UV-C illumination can be distinguished. In the first one, it seems that UV-C illumination does not decrease microbial populations after treatment, but causes some damage to microbial cells that slows their growth rate during storage at low temperatures. In the second possibility, an initial reduction in microbial populations is achieved by UV-C illumination; resulting in microbial counts of treated produce that remain lower than those of untreated produce during storage at low temperatures.

Some examples fall in the first category. Mesophilic bacterial populations and yeast and fungal populations were higher in untreated slices of zucchini squash than in those exposed to 4.93 or 9.86 kJ/m^2 at the end of storage for 22 and 14 days at 5 and 10℃ respectively (Erkan et al., 2001). Treatment of MP Red Oak Leaf lettuce with up to 7.11 kJ/m^2 UV-C on each side of the produce did not reduce total aerobic bacteria, facultative aerobic bacteria and yeasts. Moreover, it resulted in about 1 log CFU/g reduction in lactic acid bacteria, but these group was not determinant for the shelf-life of this MPV. The treatment slowed down growth of total aerobic and

facultative aerobic bacteria, and yeasts; resulting in a shelf-life extension of at least two days at 5°C based on microbial growth (Allende et al., 2006).

As examples of the second category, MP Red Oak Leaf lettuce irradiated on one side with 0.41-8.14 kJ/m^2 UV-C doses reduced the psychrotrophic population by about 0.5-2 log CFU/g from the beginning of the storage at 5°C to almost the end of the study (9-10 days), resulting in a shelf-life prolongation of 2 days or even longer when the highest fluences were used. Initial reductions in coliforms and yeast populations were kept during storage, but LAB populations were not affected by the treatments and grew to counts higher than those of controls during storage, although without reaching critical levels (Allende and Artés, 2003a). Similar results were reported by Allende and Artés (2003b) for MP Lollo Rosso lettuce stored at 5°C for 9-10 days. Treatment with 4.06 or 8.14 kJ/m^2 prolonged the shelf-life of the product by three days based on microbial growth.

The discrepancy with results published for the same MPV (MP Red Oak Leaf lettuce) might be due to difficulties in the reproducibility of decontamination results.

1.4.6 Effect on sensory attributes

Since MPV are very delicate in nature, decontamination methods, such as UV-C illumination, should not impair their sensory attributes. UV-C illumination has been reported to preserve or not affect the sensory quality of MPV in most of the cases depending on the MPV, fluence and storage temperature.

For zucchini squash slices, storage temperature influenced sensory quality. After 12 days of storage at 10°C, slight UV-C damage was observed in slices treated with 4.93 or 9.86 kJ/m^2, but samples stored at 5°C did not show any UV-C induced damage up to 22 days of storage (Erkan et al., 2001). Treating MP Red Oak Leaf lettuce or Lollo Rosso lettuce with up to 8.14 kJ/m^2 UV-C did not cause immediate effects sensory quality, evaluated in terms of general appearance, aroma, texture, taste, browning and colour (Allende and Artés, 2003ab), and no effects were observed during storage of MP Red Oak Leaf lettuce during storage at 5°C for 8 days (Allende and Artés, 2003a). However, for MP Lollo Rosso lettuce stored at 5°C for 8

days, Allende and Artés (2003b) reported a beneficial effect of UV-C. Samples treated with the highest fluences (2.44, 4.07 and 8.14 kJ/m^2) preserve better their overall visual quality and presented less browning than untreated samples or samples treated with the lowest fluences (0.407 and 0.814 kJ/m^2). Based on browning, a shelf-life extension of 1 day from the sensorial point of view was reached by using the highest fluences. However, lettuce tissue became shinier when the highest fluence was applied. That may have been related to a possible induction of lignification-like processes started by the lettuce tissue to protect itself against the UV-C stress, according to the authors.

During storage of whole peppers having 90% red colour treated with UV-C up to 7 kJ/m^2 at 10℃, fruit lightness of treated samples was not different from that of controls up to 18 days. Nevertheless, after 12 days, treated samples displayed higher hue values than control fruits indicating less additional colour development (Vicente et al., 2005). Also, Allende et al. (2006) reported the effect of a two-sided UV-C treatment on the overall visual quality of MP Red Oak Leaf lettuce during storage. Fluences up to 2.37 kJ/m^2 did not cause significant differences, but 7.11 kJ/m^2 induced tissue softening and browning after 7 days of storage at 5℃.

1.4.7 Effects on the physiology of MPV

It is known that UV-C treatment induces physiological responses in vegetable tissue, some of them useful to control post-harvest losses (Shama and Alderson, 2005).

In general, UV-C illumination accelerates the respiration rate of MPV. That has been observed for squash slices (Erkan et al., 2001), MP Red Oak Leaf lettuce (Allende and Artés, 2003a, Allende et al., 2006), and MP Lollo Rosso lettuce (Allende and Artés, 2003b). As examples, the use of 2.44 kJ/m^2 increased the respiration rate from 25.6 ml CO_2/kg h (unprocessed and untreated control) to 39.9 ml CO_2/kg h, and to 46.4 ml CO_2/kg h by applying 4.06 kJ/m^2 (Allende and Artés, 2003a). For MP Lollo Rosso lettuce, the respiration rate increased from 21.1 ml CO_2/kg h to 41.8 ml CO_2/kg h when 4.06 kJ/m^2 was applied (Allende and Artés, 2003b). High respiration rates are associated with fast spoilage. All these examples however reported shelf-life extension in spite of the acceleration of the respiration caused by UV-C

treatments. Authors concluded that the prolongation of the shelf-life was related to effects on the microbial population of the MPV.

UV-C did not increase the respiration rate of MP pomegranate arils treated with 0.56 or 1.13 kJ/m^2, although the authors warned that due to the large variability observed in the measurements it is difficult to draw any conclusion of the effect of UV-C on the respiration rate of the arils (López-Rubira et al., 2005). Contrastingly, the respiration rate of two whole produce, namely broccoli heads and peppers, was decreased by UV-C doses of 7 kJ/m^2 (Vicente et al., 2005), and 10 kJ/m^2 (Costa et al., 2006) respectively.

UV-C illumination does not affect contents of sugars and acids. Concentrations of total sugars, fructose, glucose, sucrose, and malic acid have been reported to not differ between controls and irradiated samples during storage of MP zucchini squash at 5 and 10℃ for 18 days (Erkan et al., 2001) and MP Red Oak Leaf lettuce at 5℃ for 10 days (Allende et al., 2006). No significant differences in sugar content were observed between control and treated peppers during storage at 10℃ for 18 days (Vicente et al., 2005).

UV causes modification or destruction of aminoacid residues, and can lead to inactivation of whole proteins and enzymes (Hollósy, 2002). In mango nectar, Guerrero-Beltran and Barbosa-Canovas (2006) demonstrated that UV can inactivate PPO. However, when changes of enzymatic activity are studied *in vivo*, such as in the following example, they can be a consequence of a direct effect of UV, or originated by alteration of the metabolism of the produce. On this regard, the effect of UV-C depends on the enzyme. Immediately after treating broccoli heads with 10 kJ/m^2, Costa et al. (2006) detected an increase in Mg-dechelatase activity but not in chlorophylase or chlorophyll peroxidase activity. During storage at 20℃ for 6 days, Mg-dechelatase activity in treated produce was higher than in the control until the first two days of storage, then lower. Chlorophylase activity increased in control broccoli but did not change in UV-C treated samples. And peroxidase activity in both, control and treated samples increased parallelly but was lower in treated produce at day 6.

1.4.8 Effects on the nutritional and phytochemical composition of MPV

It is very well known by post-harvest experts that ultraviolet illumination acts as an elicitor of resistance mechanisms in fruit and vegetables, and thus leads to a rapid increase of stress-response compounds such as phenols, flavonoids, and phytoalexins, which have phytochemical properties. The biosynthesis of phenolic compounds is affected by UV illumination due to the increased activity of phenylalanine ammonia-lyase (Shreiner and Huyskens-Keil, 2006). Among the different controlled abiotic stresses useful to enhance the nutraceutical content of MPV, UV illumination is considered with high potential to be implemented by the industry (Cisneros-Zevallos, 2003).

Trans-resveratrol is a phytoalexin with health-promoting properties. Cantos et al. (2001) have demonstrated that UV-C can increase the concentration of phytoalexins in table grapes as much as 10-fold. Authors entitled their article as “a new functional fruit?”. Consequently, UV-C illumination could also be tested to produce a new functional vegetable. The experimental set-up used by the authors seems to have been that characteristic of a continuous UV treatment although the authors used the expression “UV irradiation pulses” to entitle their report. It should be mentioned that the experimental procedure was not completely described by the authors due to a pending patent.

UV-C illumination decreases total phenols of radiated peppers (Vicente et al., 2005). In broccoli heads, it increased total phenols after treatment, but during storage at 20°C for 6 days the total phenol concentration in controls became higher than in treated produce (Costa et al., 2006). UV-C do not degrade carotenoids of 90% red whole peppers, although it affects *de novo* synthesis (Vicente et al., 2005).

The antioxidant capacity of produce treated with UV-C increases or remains unaltered. Increments of antioxidant capacity have been described for irradiated whole peppers immediately after treatment. During storage at 10°C for 18 days, the antioxidant capacity of all peppers decreased, but after 18 days treated fruits showed more antioxidant capacity than controls (Vicente et al., 2005). Similarly, treated

broccoli heads showed increased antioxidant capacity with respect to controls after 6 days of storage at 20℃ (Costa et al., 2006). However, the antioxidant capacity of MP pomegranate was not significantly different from controls during 13 days of storage at 5℃ (López-Rubira et al., 2005).

1.4.9 Toxicological aspects

UV light (200-400 nm) can ionise only certain types of molecules under specific conditions and is generally not considered as ionising radiation (Kovács and Keresztes, 2002). Therefore, it does not pose the risks associated to gamma irradiation, although the use of the expressions “UV radiation” and “UV irradiation” could become misleading for consumers.

1.5 Intense light pulses

1.5.1 Definition

Intense light pulses (ILP) is a technique to decontaminate surfaces by killing microorganisms using short time pulses of an intense broad spectrum, rich in UV-C light. Pulsed light is produced using technologies that multiply the power many fold. Power is magnified by storing electricity in a capacitor over relatively long times (fractions of a second) and releasing it in a short time (millionths or thousandths of a second). The emitted light flash has a high peak power and wavelengths from 200 to 1100 nm (Dunn et al., 1995, 1997). The technique used to produce flashes originate, besides high peak power, which can be in the order of megawatts, a greater relative production of light with shorter bactericidal wavelengths (MacGregor et al., 1998). A special case of pulsed light treatment consists in the use of excimer UV lasers (Crisosto et al., 1998).

An early review by Barbosa-Canovas et al. (2000) stated that literature at that date came from private sources, therefore results should be confirmed by independent researchers. Nowadays, a higher amount of independently originated data exists.

1.5.2 Origin

The first works on disinfection with flash lamps were performed in Japan, and the first patent in the United States dates from 1984 (Hiramoto, 1984). Bank et al. (1990) seems to be the first work published in the scientific literature on the application of pulsed light to inactivate microorganisms. By using a UV-C light source of 40 W at maximum peak power, a 6 to 7 log decrease in viable cell numbers was achieved.

1.5.3 Units

In addition to the units used to characterize continuous UV-C processes (see section 1.4.2), the following units are specific for pulsed light processes:

- Pulse width: time interval (fractions of seconds) during which energy is delivered.
- Pulse-repetition-rate (prr): number of pulses per second (Hertz [Hz]) or commonly expressed as pps (pulses per second).
- Peak power: Watt (W), pulse energy divided by the pulse duration.

1.5.4 Inactivation mechanism

1.5.4.1 UV-C as the most important part of spectrum

Xenon flash lamps have an emission spectrum ranging from ultraviolet to infrared light. Several researchers have proved that the UV-C part of the spectrum is the most important for microbial inactivation. Rowan et al. (1999) reported that the inactivation of seven food related microorganisms was 5 to 6 log CFU/plate using a high UV flash, whereas with low UV light only 1 to 2 log CFU/plate was achieved.

Using a monochromator, Wang et al. (2005) determined the germicidal efficiency against *E. coli* as a function of wavelength over the range 230-300 nm. The results showed a maximum of inactivation around 270 nm, no measurable inactivation was observed to occur above 300 nm. Moreover the rich UV content from 220 to 290 nm in the UV spectrum provides the major contribution to inactivation.

1.5.4.2 Photothermal and/or photochemical mechanism

Considerable research has been done on the mechanism of microbial inactivation by light pulses. The lethal action of pulsed light can be due to either photothermal and/or photochemical mechanisms. Most of the authors explain their results based on the photochemical effect. For example, since Rowan et al. (1999) achieved inactivation with less than 1°C of rise in temperature, they concluded that the lethality can be attributed to the photochemical action of the shorter UV wavelengths. There is however evidence that the photothermal effect can also occur, the relative importance of each effect could depend on experimental conditions. Wekhof (2000) proposed that over ca. 0.5 J/cm^2, the disinfection is achieved through a rupture of bacteria during their momentous overheating. Later on, Wekhof et al. (2001) provided evidence of this hypothesis by showing electron-microscope photographs of flashed *A. niger* spores presenting severe deformation and rupture. A raptured top of a spore is presented as evidence of an escape of an overheated content of the spore, which became empty after such an internal “explosion” and “evacuation” of its content took place during the light pulse.

1.5.4.3 The photochemical mechanism

The mechanism of microbial inactivation by light pulses is frequently explained based on studies using continuous-wave (CW) UV light. Although similarities can be expected, also some differences could exist. Takeshita et al. (2003) compared the inactivation of *S. cerevisiae* by CW UV and broad-spectrum flash lights. DNA damage, such as formation of single strand breaks and pyrimidine dimmers was induced in yeast cells after treating with pulsed light, which were essentially the same as observed with CW UV light. However, an increased concentration of eluted protein and structural change in the flashed cells were observed only in the case of pulsed light. This suggested cell membrane damage induced by pulsed light. Flashed yeast cells showed raised and expanded vacuoles and cell membrane distortion/damage, and their shape changed to circular. On the other hand, the structure of cells did not change after illumination by CW UV light.

A mechanistic approach was applied by Wuytack et al. (2003) to study the mechanism of microbial inactivation by ILP. The authors, based on relative inactivation of *Salmonella* cells plated in selective and non-selective agars, concluded that pulsed white light inactivation should be regarded as a multitarget process. In this, structural changes to DNA would be a major reason, and damage to membranes, proteins and other macromolecules play a minor role.

Comparing the wavelength sensitivity for *E. coli* inactivation with a previously reported absorption spectra of the purine and pyrimidine bases of DNA, Wang et al. (2005) supported the hypothesis that the photochemical effect produced as consequence of the UV absorption by DNA is the major cause of microorganism inactivation by pulsed UV illumination.

1.5.4.4 Peak power dependence

Some research has been conducted to investigate if pulsed light sources really yield improved microbial inactivation rates in comparison with CW UV light sources, as it was claimed by early literature coming from private sources. A peak power effect would not be in line with the Bunsen-Roscoe law. The diversity of findings does not allow setting a definitive conclusion, although results point towards a possible peak power dependence. According to McDonald et al. (2002) several theories predict a more rapid kill of vegetative cells with pulsed light. The most probable theory postulates that the high photon flux emanating form a pulsed source simply overwhelms the cellular repair mechanisms before repair can be completed. In addition, the previous referred results reported by Wekhof et al. (2001), and Wuytack et al. (2003) seem to support a peak power dependence.

Rice and Ewell (2001) examined the peak power dependence in the UV inactivation of bacterial spores by comparing the output of a high-peak-power UV source at 248 nm from an excimer laser to a low-power CW source (254 nm) used to inactivate *Bacillus subtilis* spores. The two UV sources differed by 8 orders of magnitude in peak power. Results showed no discernible peak power effect. Therefore, it appears that the total number of photons delivered is the important parameter and not the number of photons delivered per unit of time (peak power). The results agree with the

principle of Bunsen-Roscoe. Moreover, Wang et al. (2005) concluded that the germicidal efficiency obtained with a xenon flash lamp used to inactivate *E. coli* shows no obvious difference to the published data using CW UV low-pressure mercury lamps at the same wavelength.

However, a single research group also using B. subtilis spores has reported conflicting results and conclusions. Two articles concluded, in the same line than that of Rice and Ewel (2001) that given the same fluence, no differences in the inactivation efficacy were observed when comparing the results obtained with a CW lamp that produces 3.9 mW/cm^2 on target to those obtained with two kinds of flashlamps producing in excess of 60 W/cm^2 on target, in the fluence range of 0 to 200 mJ/cm^2 (Hancock et al., 2004, McDonald et al., 2002). On the other hand, other articles concluded that pulsed UV light exhibits a minor improvement over that of the CW source over the fluence range 0 to 80 mJ/cm^2 (McDonald et al., 2000a) or that pulsed light significantly outperforms CW light in aqueous suspensions and on surfaces (McDonald et al., 2000b).

Results reported by Takeshita et al. (2003), also supported a violation of the principle of Bunsen-Roscoe. The authors compared the effect of peak power on *S. cerevisiae* cells, using 4655 and 2473 kW. These peak powers, although different, are relatively similar in comparison with the differences of several orders of magnitude used by the other authors. Their results revealed that under high peak power conditions, the killing effect and concentration of eluted protein were higher than under low peak power conditions.

1.5.4.5 Fluence threshold

MacGregor et al. (1998) and Anderson et al. (2000) reported that the higher the number of pulses the higher the killing effect. It can be observed in their results that the number of survivors as function of the number of pulses keeps constant until a certain point beyond which the inactivation starts. The same observation was reported by Fine and Gervais (2004) on viability of *S. cerevisiae* cells dried on quartz

plate, which suggested a threshold level of energy for total destruction. These findings are in line with the sigmoid pattern discussed before for CW lamps.

1.5.5 Susceptibility of microorganisms

Rowan et al. (1999) and Anderson et al. (2000) reported the following trend of susceptibility in decreasing order: Gram-negative bacteria, Gram-positive bacteria and fungal spores. Colour of the spores can play a significant role in fungal spore susceptibility. *A. niger* spores are more resistant than *Fusarium culmorum* spores, which could be because the pigment of the *A. niger* spores absorbs more in the UV-C region than that of *F. culmorum*, protecting the spore against UV illumination (Anderson et al., 2000).

1.5.6 Advantages and disadvantages

ILP has similar advantages and disadvantages than CW UV-C, but also some specific pros and cons.

1.5.6.1 Advantages

- The short pulse width and high doses of the pulsed UV source may provide some practical advantages over CW UV sources in those situations where rapid disinfection is required (Wang et al., 2005). For example, Rice and Ewell (2001), in the experiment aforementioned, needed 3 hours to deliver 10^4 J/m^2 using a CW lamp and 40 s to deliver the same total fluence using a laser with a repetition rate of 10 Hz.

1.5.6.2 Disadvantages

- As well as in CW UV, it is possible that photoreactivation occurs. Otaki et al. (2003) reported photoreactivation of *E. coli* cells, although this was lower than in CW UV illuminated cells treated with the same dose.
- Shading is also a problem in ILP applications. Anderson et al. (2000) talks about the possibility of shading effects. Fungal spores were inactivated in agar

surfaces, but spore clumps occurring naturally can have spores entrapped inside, which will be protected against the lethal action of UV.

- The ILP process can increase the produce temperature. Heat can come from the absorption of light by the food or by lamp heating. When studying the inactivation of *A. niger* spores on corn meal, Jun et al. (2003) found that some experimental factor settings resulted in sample temperatures of 120℃, which might have been due to the large amount of heat generated by the lamps, even though their device had a cooling system. The heat generated by the lamp might cause a synergist increase in lethality that can be useful for some specific applications among which MPV would unlikely be.

1.5.7 Inactivation of pathogens

Several *in vitro* studies have reported high levels of inactivation of human pathogens by ILP, but no reports about pathogen inactivation on produce surfaces seem to have been written. MacGregor et al. (1998) found that 512 µs of light (512 pulses with a duration of 1 µs and a prr of 1 Hz) resulted in 6 and 7 log CFU/g reduction of *E. coli* O157:H7 and *L. monocytogenes*, respectively. Ghasemi et al. (2003) reported a 9-log order reduction in *E. coli* and *Salmonella* after treatment with 100 pulses with each pulse providing 9 J. Krishnamurthy et al. (2004) reported a 7 to 8 log CFU/ml reduction in *S. aureus* cells suspended in buffer or agar seeded treated for 5 s.

Photosensitisers can enhance the microbicidal action of light (see 1.5.11 for definition). McDonald et al. (2000a) reported a synergism between pulsed UV light and hydrogen peroxide, which yielded a gain of nearly two logs in the inactivation of *B. subtilis* spores in comparison with the treatment of pulsed UV only. The authors did not propose the application of photosensitised UV treatment to food surfaces, but the possibility could be assayed since hydrogen peroxide *per se* has also been tested as disinfectant of MPV.

1.5.8 Decontamination and shelf-life of MPV

The inactivation of microorganisms naturally present on vegetable surfaces by ILP has been demonstrated by Hoornstra et al. (2002). The authors treated five

vegetables, namely white cabbage, leek, paprika, carrots and kale with two pulses of wide spectrum pulsed light that amounted a fluence of 0.30 J/cm^2. The reduction in aerobic count at the surface of the vegetables varied from 1.6 log CFU/cm^2 for carrots to >2.6 log CFU/cm^2 for paprika. No significant increase in the inactivation was observed after using a third pulse. Moreover, no adverse effects in sensorial quality were observed after treating of the vegetables with 3 pulses and storage at 7 and 20°C for up to 7 days with one exception, iceberg lettuce showed some discoloration after 48 hours at a storage temperature of 20°C. Their own calculations demonstrated that a reduction of 2 log CFU/cm^2 increase the shelf-life at 7°C of cut vegetables with about 4 days, which is remarkable given a treatment of only 0.4 ms of effective duration.

ILP can affect colour of treated products. Fine and Gervais (2004) compared the effect of light pulses on the inactivation of *S. cerevisiae* on black pepper and wheat flour. Colorimetric results indicated a rapid modification of product colour well before the decontamination threshold was reached and was clearly more rapid for black pepper than wheat flour. This colour modification was attributed to overheating combined with oxidation. The difference in colour modification between wheat flour and black peppers was explained by the difference in initial colour, dark products absorb more light energy than pale products.

1.5.9 Effects on the physiology of MPV

ILP can affect specific components of irradiated vegetables as well as cause physiological changes also observed due to application of CW UV. Since proteins have a strong absorption at about 280 nm (Hollósy, 2002), broad spectrum pulsed light could be useful to inactivate enzymes. Dunn et al. (1989) claimed that by using 2-5 flashes of light at a fluence of 3 J/cm^2, it is possible to inhibit potato slice browning. The polyphenol oxidase extract recovered from the treated slices exhibited less activity than that from the untreated slices. ILP could also alter the respiration rate of fruits and vegetables. Crisosto et al. (1998) tested the effect of pulsed monochromatic UV light from an excimer laser (total energy: 1 J/cm^2) for whole peach fruit decay control. Treated fruits of two varieties of peach produced

approximately 18% more CO_2 than controls, even though only 1/32 of the total fruit area was flashed.

1.5.10 Nutritional and toxicological aspects

The effect of ILP on nutritional components of vegetables has not been studied yet, neither the potential formation of toxic by-products. Since the wavelengths used for pulsed light are too long to cause ionisation of small molecules and are in the nonionising portion of the electromagnetic spectrum (Dun et al., 1995), the formation of radioactive by-products is not expected.

1.5.11 Photosensitization. Another application of light to inactivate microorganisms

Photosensitization is another technique that might be useful to decontaminate food surfaces by illumination. Photosensitization has been defined by Lukšiene (2005) as a treatment involving a photoactive compound that accumulates in microorganisms and is followed by illumination with visible light. The combination of compound and light, in presence of oxygen results in the destruction of microorganisms. After the work by McDonald et al. (2000a) described before, it is foreseeable that this definition can be expanded to include the UV part of the spectrum. The primary field of application of photosensitization has been photodynamic cancer treatment. It has been proposed as a milder alternative to the emerging non-thermal technologies for food preservation (Lukšiene, 2005). To the date, its potential application in food preservation has only been tested for the inactivation of bacteria, yeasts (Kreitner et al., 2001), and fungal food contaminants *in vitro* (Lukšiene et al., 2004, 2005). Therefore, more research is necessary to evaluate its future in food preservation.

An advantage of this technique over the simple UV inactivation is that photoreactivation can not occur. According to (Lukšiene, 2005), two ways have been proposed to explain the lethal damage of photosensitization on bacteria: breaks in both single- and double stranded DNA and damage to cytoplasmatic membrane. These damages can not be repaired by photolyase, which is affinity to cyclobutane dimmers.

1.6 Chlorine

1.6.1 General aspects

Chlorinated water consists of a mixture of chlorine gas (Cl_2), hypochlorous acid (HOCl), and hypochlorite ions (OCl^-) in amounts that vary with water pH. HOCl is the active moiety. The relative amounts of chlorine as HOCl as function of pH are 22, 78 and 100% at pHs 8.0, 7.0, and 5.5 respectively. The best compromise between activity and stability is achieved by maintaining a water pH between 6.5 and 7.5 (Suslow, 1997), which also minimizes corrosion of equipment (Parish et al., 2003). At low pH, chlorine gas is released from water. Chlorine is commercially presented in three forms: chlorine gas, calcium hypochlorite ($Ca(OCl)_2$) and sodium hypochlorite (NaOCl) (Suslow, 1997).

The terms free chlorine, reactive chlorine, and available chlorine are used to describe the amount of chlorine in any form available for oxidative reaction and disinfection. Available chlorine is the most important parameter to characterize the microbicidal capacity of chlorinated water. The term combined chlorine states for chlorine combined with ammonia or other less readily available forms of chlorine with weak antimicrobial activity such as chloramines. Total chlorine refers to the total available and combined chlorine that is present in water (Suslow, 1997).

The undissociated hypochlorite is considered the most microbicidal form of chlorine. This uncharged species is freely permeable across the plasma membrane of microorganisms and is thus able to enter the cell. Once inside the cell, the acid dissociates because the cell interior has a higher pH than the exterior (Brul and Coote, 1999); the dissociated form will then exert its antimicrobial effect. *In vitro* studies have demonstrated that the inactivation of *L. monocytogenes* in phosphate buffer is faster at low pHs (Erkmen, 2004). However, the results of the application of hypochlorites at different pHs to disinfect MPV show little or no benefit of adjusting the pH of chlorinated water to low values. Adams et al. (1989) washed lettuce with water containing 100 mg/l available chlorine adjusted to different pHs. The resulting difference in aerobic plate counts between samples treated at pH 8.8 and pH 5 was only 0.12-0.20 log CFU/g. Moreover, Delaquis et al. (1999) reported that acidification

of chlorinated water from pH 8 to pH 6 did not improve its antimicrobial effect at 4 and 47°C. It is possible that the surface of the vegetable neutralize the acidity of acidified chlorinated water.

According to Russell (2003), the primary effect of hypochlorites is believed to be either or both: 1) the progressive oxidation of thiol groups to disulphides, sulphoxides and disulphoxides, and 2) deleterious effects on DNA synthesis resulting from the formation of chlorinated derivatives of nucleotide bases; although bacterial spores do not appear to suffer direct DNA damage. Spore killing by hypochlorite appears to render spores defective in germination, possibly because of severe damage to the spore´s inner membrane (Young and Setlow, 2003).

The cytoplasmatic membrane has been proposed to be a possible target invlolved in bacterial inactivation by chlorine, since alterations in its permaeability after chlorination have frequently been described. However, Virto et al. (2005) observed membrane permeabilisation when bacteria were exposed to chlorine concentrations severalflod higher than the concentration required for cell killing. This indicates that extensive cytoplasmatic membrane damage is not a key event leading to cell death to chlorine; and suggest that more subtle events, such as uncoupling of the electron chain or enzyme inactivation either in the membrane or in the cell interior are involved in the bactericidal mechanism of chlorine. The same authours observed that the resistance of the tested microorganisms in presence of organic matter was higher in distilled water controls even though the residual chlorine concentration in the presence of organic matter was much higher than that necessary to completely inactivate the microbial population in distilled water. Therefore, the protective effect of organic matter can not be attributed solely to the chlorine consumption but maybe due to effective stabilization of some cellular structures. Other experiments reported in that article lead to the authours to propose that bacterial envelopes could play a role in cell inactivation by modulating the access of chlorine to the key targets within the cell.

As it was introduced above, one of the main drawbacks of chlorine, as well as most of the common disinfectants, is that organic matter affects its efficacy. For MPV, the finer the cut the higher the consumption of free chlorine, which is attributed to the

release of tissue juices, which in turn increases the concentration of organic material available for reaction with chlorine (Beuchat et al., 2004; Pirovani et al., 2004). For fresh cut spinach, chlorine depletion is function of initial chlorine concentration (25 to 125 ppm), water-to-produce ratio (10 to 26 l/kg) and washing time (2 to 8 min) (Pirovani et al., 2001).

Resistance of microorganisms to chlorine has been reported (Mokgatla et al., 1998). Microorganisms can develop resistance to antimicrobials when these are unproperly used, i.e. used in sublethal dosis. An Institute of Food Technologist Scientific Status Summary authored by Davidson and Harrison (2002) concluded that there is no evidence that proper use of sanitizers in food manufacturing will lead to development of resistant microorganisms. However, with increasing reliance on and used of sanitizers, the potential for emergence of such resistant microorganisms does exit. Therefore, the potential for development of resistant strains must continue to be evaluated.

1.6.2 Effect on microbial populations

The use of chlorine to decontaminate vegetables and processing water has been extensively documented. The limited efficacy of chlorine to eliminate pathogens from vegetables as well as safety concerns related to the formation of toxic by-products has triggered a search for alternative disinfectants. Chlorine washings are commonly used as reference treatment in the resultant studies, which has originated a growing body of literature about chlorine use in the frame of studies that have had as goal its substitution.

The efficacy of chlorine to kill microorganisms *in vitro* has been demonstrated by different authors. A treatment with 200 ppm of chlorine resulted in a reduction of >7.38 log CFU of *E. coli* per ml and >7.02 log CFU of *S. aureus* per ml within 30 s (Beuchat et al., 1998). However, as it was mentioned before, the presence of organic matter decreased the efficacy of chlorine and explains the lower efficacy of chlorine *in vivo*. The concentration of chlorine in solution diminishes due to reaction with organic matter; hence its microbicidal effect is reduced when used to disinfect vegetable surfaces.

The limited capacity of chlorine to inactivate human pathogens inoculated onto vegetable surfaces has been repeatedly demonstrated. Treating MP lettuce with 190 ppm free chlorine for 10 min reduced *L. monocytogenes* counts by 1.7 logs with respect to the water washed control; in MP cabbage the reduction was 1.2 log (Zhang and Farber, 1996). Counts of *Salmonella* and *E. coli* O157:H7 were reduced by about 1 log CFU/cm^2 in chlorine-treated lettuce, the inactivation occurred essentially within 1 min (Beuchat et al., 1998). Counts of *Yersinia enterocolitica* in lettuce were reduced by 2.4-2.6 log CFU/g by 100 ppm of chlorine (Escudero et al., 1999). Populations of *Salmonella baildon* in shredded lettuce and diced tomatoes were reduced by less than 1 log CFU/g when the produce was immersed for 40 s in a 120 or 200 mg/l free chlorine solution (Weissinger et al., 2000). In MP spinach, *Salmonella* reduction was around 1.2-1.4 log at 125 ppm during 8 min (Pirovani et al., 2000).

Usually, disinfectants are more effective against inoculated pathogenic microorganisms than against microflora present in whole vegetables or MPV. However, the levels of disinfection by chlorine in both cases are quite similar. Nevertheless, the degree of decontamination from spoilage microorganisms seems to be similar to that achieved by using other disinfectants discussed afterwards.

Some studies have been focused only in the decontamination of produce from spoilage microorganisms, without including storage studies. For lettuce, Adams et al. (1989) found a reduction of 1.7 log CFU/g in mesophilic aerobic plate counts after washing lettuce with chlorinated water (100 mg/l of free chlorine, 5 min). Later Nascimento et al. (2003), using 200 mg/l of free chlorine for 15 min, reported reductions in mesophilic aerobic plate counts, and moulds and yeasts of 1.85 and 1.88 log CFU/g respectively compared to water washing.

Sometimes it is possible to have lower levels of microorganisms in treated samples than in controls during storage even though no decontamination has been realized. For example, chlorination (80 mg/l of total chlorine, 3 min) of potato strips was not able to reduce counts of native mesophilic and psychrotrophic bacteria. Mesophilic and psychrotrophic counts however exhibited a lag phase up to 11 days during

storage at 4℃, while the lag phase of water washed controls lasted 5 days (Beltrán et al., 2005a). This is the example number 1.2 given in section 1.3.5.2.

Several of these studies have also shown that chlorinated produce stored at low temperatures have a longer microbial shelf-life than non chlorinated produce. After 15 days of storage at 4℃, mesophilic and psychrotrophic populations in peeled white asparagus were 2 log CFU/g lower in samples washed in chlorinated water (100 mg/l of NaOCl, 10 min) than in water washed controls. Initial LAB counts decreased 0.5 log units but were not different during storage (Simon et al., 2004). Chlorination of dry coleslaw (100 mg/l of free chlorine, 5 min) showed log reductions of 0.23-0.37 for mesophiles, 0.30-0.52 for psychrotrophs, 0.27-0.43 for lactic acid bacteria, and 0.11-0.63 for yeast and moulds. Those populations remained at lower levels in chlorinated samples than in the respective water washed controls up to 9 days at 4 or 8℃ (Clyffe-Byrnes and O´Beirne, 2005). Chlorination (80 mg/l of total chlorine, 3 min) reduced the mesophilic population of shredded lettuce by 2.1 log CFU/g compared to water washing. Total mesophilic populations in chlorinated shredded lettuce were 1.8-2.7 log CFU/g lower than in water washed controls after 14 days of storage at 4℃ (Beltrán et al., 2005b).

Sometimes the effect of decontamination of MPV with chlorinated water is lost during storage. Treatment of MP lettuce in chlorinated water (20 mg/l of free chlorine, 1.5 min) reduced mesophilic aerobic, psychrotrophic, and yeast and moulds populations by respectively 1.73, 1.14 and 1.33 log CFU/g compared to the untreated control. Mesophilic aerobic counts in decontaminated MP lettuce equalled those of the unwashed control after 2 days at 5℃ (Li et al., 2001b). This is one of the examples that can be cited regarding the case 2.4 commented in section 1.3.5.2. Chlorine (100 mg/l of free chlorine, 5 min) reduced initial total bacterial counts in shredded Irish lettuce by approximately 1-1.5 log units, and remained lower on disinfected lettuce up to day 7 at 8℃, but by day 10, there were no differences with the undipped control. For chlorinated dry coleslaw stored at 8℃, the effect of the reduction in population size was lost by day 2 (Francis and O´Beirne, 2002). Chlorination of shredded carrots (200 mg/l of free chlorine, 2 min) reduced initial total bacterial counts by approximately 1 log unit, but they reached the same levels than water washed samples after 14 days at 5℃ (Gonzalez et al., 2004). In MP sweet potatoes, 200

ppm of chorine treatment for 5 min reduced mesophilic counts by 1.73, psychrotroph counts by 0.98, and yeast and moulds counts by 0.63 logs, all reductions compared to water washing. Mesophilic counts were lower in treated samples for up to 7 days at 2 and 8℃, but psychrotroph and yeast-mould loads equalled after that storage time (Erturk and Picha, 2006).

Factors affecting the efficacy of chlorinated water are related to the kind of MPV and microbial population. Initial chlorine concentration (25-125 ppm), time (2-8 min) and the interaction between them have a significant effect on the reduction of *Salmonella hadar* populations in MP spinach, but not the water-to-produce ratio (8-26 l/kg) (Pirovani et al., 2000). Total microbial counts of MP spinach were affected by initial chlorine concentration (25-125 ppm) and washing time 2-8 min), but not by water-to-produce ratio (10-26 l/kg) (Pirovani et al., 2001), whereas the decontamination of MP lettuce was affected by initial chlorine concentration (50-150 ppm) and water-to-produce ratio (20-50 l/kg), but not by washing time (2-8 min) (Pirovani et al., 2004).

Temperature is another important factor determining the efficacy of chlorine. The use of warm (up to 50℃) chlorinated water is more effective than simple chlorination. Warm, chlorinated water reduced total aerobic populations by approximately 3 log CFU/g in lettuce washed in chlorinated (100 mg/l) water at 47℃, and 1 log CFU/g at 4℃. It is plausible that this difference in lethality was a consequence of synergy between heat and chlorine. Total aerobic and psychrotroph counts in MP lettuce decontaminated in chlorinated water and stored at 1℃ were lower than in raw lettuce until days 10 and 15 when washings were performed at 4 or 47℃ respectively (Delaquis et al., 1999). (Delaquis et al., 1999). Chlorine reduced initial microbial counts in shredded lettuce by <1 log CFU/g when washing was performed at 4℃, and by an average of 2 log CFU/g at 50℃ (Delaquis et al., 2004). Other works on the use of warm, chlorinated water are those by Li et al. (2001ab, 2002).

1.6.3 Effects on sensory quality

Chlorination can have neutral or preservative effects on the sensory quality of some MPV. For example, chlorinated coleslaw remained acceptable throughout storage for 10 days at 4℃. On the other hand, water washed samples were unacceptable after 6

days due to bad appearance and colour, and after 5 days due to aroma defects (Clyffe-Byrnes and O´Beirne, 2005). Chlorination had no effect on the sensory quality of peeled white asparagus stored at 4℃ up to 15 days (Simon et al., 2004).

Prevention of browning could be the most remarkable positive effect of chlorination on the sensory quality of MPV. Chlorination reduced edge browning of lettuce during storage at 5℃ (Fukumoto et al., 2002). In shredded lettuce, chlorinated samples stored at 4℃ under air had less browning and better visual quality score than water washed samples (Beltrán et al., 2005b). In potato strips, however, chlorination did not arrest browning when stored under vacuum at 4℃ (Beltrán et al., 2005a).

Chlorination can also produce immediate deleterious effects on the sensory quality of a MPV, although the defects can be no long lasting. For example, treatment of MP lettuce with 20 mg/l chlorine in water for 3 min immediately impaired its appearance, treated lettuce was limp compared to untreated lettuce. However, after 2 days of storage at 5℃ no differences were observed with respect to the unwashed control, which was maintained up to the end of the experiment (18 days) (Li et al., 2001b).

1.6.4 Effects on the physiology of MPV

Chlorination in some cases does not affect the respiration rate of MPV, as in the case of chlorinated potato strips and shredded lettuce (Beltrán et al., 2000ab). In contrast, the respiration rate of a coleslaw mix was lower in chlorine washed compared to water rinsed. The reason is unclear, but may be explained by the inhibitory action of the chlorine anion on enzymes involved in the respiratory process (Clyffe-Byrnes and O´Beirne, 2005).

Chlorination has shown to not affect the pH of a coleslaw mix (Clyffe-Byrnes and O´Beirne, 2005), as well as the pH and acidity of peeled white asparagus during storage at 4℃ for 15 days (Simon et al., 2004). The texture during storage at 4℃ of shredded lettuce (Beltrán et al., 2005b) and peeled white asparagus (Simon et al., 2004) was not affected by chlorination.

1.6.5 Effects on the nutritional and phytochemical composition of MPV

It seems that chlorination does not affect the nutritional and phytochemical composition of MPV. A treatment with 100 ppm NaOCl for 10 min did not affect the total sugar and ascorbic acid content of peeled white asparagus during storage at 4℃ for 15 days (Simon et al., 2004). The total phenolic and vitamin C content during storage of shredded lettuce at 4℃ for 13 days was not affected by chlorination (Beltrán et al., 2005b).

1.6.6 Toxicological aspects

There is nowadays a big concern on the use of chlorine in drinking water and produce washing solutions because of the formation of toxic by-products such as chloramines and trihalomethanes. The four chemical species known as trihalomethanes are: chloroform, bromodichloromethane, chlorodibromomethane, and bromoform. The International Agency for Research on Cancer determined that neither chloramine (WHO, 2004) nor hypochlorite salts, bromoform, chlorodibromomethane, nor halogenated acetonitriles (WHO, 1991) are classifiable as to its carcinogenicity to humans. However, bromodichloromethane is classified as possibly carcinogenic to humans (WHO, 1991) as well as chloroform (WHO, 1999), and another chlorinated disinfection by-product of importance, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (Richardson et al., 1998), also known as MX (WHO, 2004).

1.7 Electrolysed oxidizing water

1.7.1 Definition

Electrolysed oxidizing water (EOW) is created by electrolysis of diluted sodium chloride solutions in an electrolysis chamber, having free chlorine as the major disinfection factor. Salts such as KCl and $MgCl_2$ can also be used (Buck et al., 2002), but NaCl is the salt used for food processing applications. Since tap water contains chloride, it is also possible to reactivate free chlorine in tap water by electrolysis (Nakajima et al., 2004).

The most common type of electrolytic cell is a two-cell chamber, although some devices work with single-cell chambers (Venczel et al., 1997). The generation of EOW using the two-cell chamber involves reactions in a cell containing positively charged and negatively charged electrodes, respectively, separated by a membrane, and through which a very diluted salt-water solution passes. By subjecting the electrodes to direct current voltage, two types of water possessing different characteristics are generated: an acidic EOW (AcEW) and an alkaline EOW (AlEW). The first one has been the most studied due to its microbicidal properties. AcEW is produced from the anode side, and characterized by a pH <3, a high oxidation-reduction potential (ORP), about 1,150 mV, and the presence of HOCl. AlEW is produced from the cathode side, having a pH of 11.4 and low ORP, about -795 mV (Kim et al., 2000a).

Although AlEW lack of antimicrobial properties, it can be useful to pre-wash vegetables. AlEW is considered to act like a diluted sodium hydroxide solution. Thus, it would work like a surface-active agent against vegetable surfaces. Consequently, the microorganisms on the surface would be reached easier by AcEW during a sequential process (Koseki et al., 2001). AlEW can also be used after AcEW to eliminate residual chlorine odour (Lin et al., 2005).

AcEW and AlEW might also occur in the single-cell system, however, since no diaphragm is present, they might neutralize each other, resulting in a single stream of neutral EOW (NEW), also named MIOX (mixed-oxidant solution), with enhanced levels of HOCl. AcEW and NEW are also known respectively as ESW (electrolysed strong acid water) and EWW (electrolysed weak acid water) (Kiura et al., 2002). Therefore, three kind of EOW can be produced: AcEW, AlEW, and NEW (MIOX).

There are two effects described for EOW, a direct effect and a residual effect (Ongeng et al., 2006). The direct effect is when the decontamination occurs inside the electrolytic cell. By using it, it is possible to decontaminate water. The residual effect refers to when the decontamination occurs outside the electrolytic cell, which means that the EOW is produced in the cell, collected, and then used to wash the

food. The residual effect has been found to be useful for decontamination of MPV (Izumi, 1999).

Direct and residual effects can be combined to decontaminate produce and wash water in an integrated system. To this, first the EOW is produced, then used to decontaminate food commodities. In this step the food contaminates the EOW. Afterwards, the water is recycled to the electrolytic cell to be decontaminated and used subsequently again.

1.7.2 Origin

The origin of the concept of EOW is confuse. While most authors such as Venkitanarayanan et al. (1999a) stated that EOW was developed in Japan; Stevenson et al. (2004) attributed it to Russia, citing a work written in Russian by Shtannikow and Morozovla (1977). Also Morita et al. (2000) cited some articles written in Russian published in 1968.

1.7.3 Inactivation mechanism

There is no agreement about which is the primary factor contributing to the microbicidal activity of EOW. When using NEW, free chlorine is frequently the only monitored parameter (Izumi 1999; Ongeng et al., 2006). Three factors are however usually measured in order to characterize AcEW, namely concentration of free chlorine, ORP and pH. These factors are also related, which makes it difficult to independently study the role of each one. Higher levels of free chlorine and lower pH increase ORP and raise the disinfectant power of EOW. Moreover a low pH increases the disinfectant effect of chlorine.

After conducting *in vitro* experiments manipulating the ORP and residual chlorine levels, Kim et al. (2000ab) concluded that ORP may be the primary factor affecting microbial inactivation of AcEW. On the other hand, Nakagawara et al. (1998), Len et al. (2000), Koseki et al. (2001) and Kiura et al. (2002) have concluded that the main contributor to the bactericidal activity is the HOCl concentration.

Besides HOCl, other chemical species with antimicrobial activity might be present in EOW. Otherwise the microbicidal efficacy of EOW and chlorine solutions with the same concentration of available chlorine should yield the same levels of microbial inactivation. ClO_2 has been identified in EOW (Bergmann and Koparal, 2005). However, hydroxyl radical supposedly present in EOW have not been detected by electron spin resonance spectroscopy (Stan et al., 2005). Results from *in vitro* tests support the hypothesis of antimicrobial agents other than HOCl present in EOW. For example, Venczel et al. (1997) compared the inactivation kinetics of *Clostridium perfringens* spores exposed to a 5-mg total oxidant dose of MIOX or free chlorine (from NaOCl) per liter of pH-7, oxidant-demand free phosphate-buffered water. The spores exposed to the NaOCl solution exhibited much slower inactivation rates than those exposed to MIOX although the mean free chlorine concentration throughout the experiments was 4.5 mg/l in the first, and the average total oxidant concentration was 3.8 mg/l in the second.

However, reports of *in vivo* results do not agree with the referred hypothesis. Park et al. (2001) found no differences between the efficacy of AcEW and acidified chlorinated water to inactivate *E. coli* O157:H7 and *L. monocytogenes* on lettuce, when both disinfectants were adjusted to the same pH (2.5), ORP (1,130 mV) and chlorine concentration (45 ppm). It is possible that those additional compounds become quickly degraded by organic matter, and be not relevant in practice, although more evidences are necessary to draw conclusions.

Surely, chlorine is involved and the difference in pH determines which species is most important: Cl_2 below pH 3 (typical of AcEW), and HOCl and ClO^- above pH 4 (typical of NEW) (Nakagawara et al., 1998). Nakagawara et al. (1998) as well as Len et al. (2000) and Park et al. (2004) observed in *in vitro* tests that the maximum antibacterial activity of AcEW occurs around pH 4. However, as happens with chlorinated water, results from *in vivo* tests do not confirm the applicability of *in vitro* results. Yang et al. (2003) dipped MP lettuce inoculated with pathogens into 300 ppm EOW at pHs between 4 and 9. The effect of pH was not significant for *S.* Typhimurium and *L. monocytogenes*. The inactivation had two maxima for *E. coli* O157:H7, one at pH 4, and the other at pH 8.

The antimicrobial efficacy of AcEW is reduced by reaction with organic matter. Oomori et al. (2000) found that proteins and oils but not sugars decrease free chlorine and the disinfectant efficiency against *E. coli*. Interestingly for application to MPV, fine strips of chopped cabbage decreased free chlorine more quickly than large strips. The result suggests that the finer the cut of a MPV the lower the efficacy of EOW.

Similar results have been found with NEW. The efficacy of NEW to inactivate vegetable spoilage bacteria was studied by Ongeng et al. (2006) by *in vitro* tests. Populations of *P. fluorescens*, *Pantoea agglomerans* and *Rahnella aquatilis* were reduced from 8.7 log CFU/ml to undetectable levels after 45 min of exposition to NEW containing 4 mg/l of free oxidants; while populations in sterile deionised water controls were stable. When water with a biological oxygen demand of 2.7 mg O_2/l supplemented with NaCl was used to produce NEW with 5.1 mg/l free oxidants, the reduction after 45 min dropped to 5.3, 6.5 and 7.3 log CFU/ml for *P. fluorescens*, *P. agglomerans* and *R. aquatilis* respectively. This observation shows that organic matter is an important parameter that affects the residual bactericidal effect of NEW. That is in agreement with the effect of organic matter on chlorine efficacy explained before.

At cellular level, it has been observed that AcEW produces blebs and breaks in the outer membrane of *Pseudomonas aureginosa*, inactivates nitrate reductase and degrades chromosomal DNA (Kiura et al., 2002).

1.7.4 Advantages and disadvantages

1.7.4.1 Advantages

- AcEW can be prepared by the electrolysis of a diluted saline solution, without the use of any chemicals other than sodium chloride (Koseki et al., 2004). It has therefore less adverse impact on the environment (Kim et al., 2000a). Furthermore, raw materials, water and sodium chloride, are found virtually everywhere (Venczel et al., 1997).

- AcEW can be generated *on-site* (Len et al., 2000), therefore transportation and storage of potentially hazardous chemicals are not needed (Nakagawara et al., 1998).
- EOW is not only decontaminant but can also prevent enzymatic browning during storage of MPV (Koseki and Itoh, 2002).
- AcEW could be more effective to inactivate microorganisms than chlorinated solutions having the same concentration of available chlorine (Koseki et al., 2001). Consequently, the formation of chloramines and trihalomethanes should be lower.
- NEW has also the advantage of its neutral pH, therefore it does not contribute as aggressively as AcEW to the corrosion of processing equipment or irritation of hands. It is also more stable as chlorine loss is significantly reduced at pH 6-9 (Deza et al., 2003).

1.7.4.2 Disadvantage

- Organic matter decreases the efficiency of EOW (Oomori et al., 2000; Ongeng et al., 2006).

1.7.5 Effect on microbial populations

It seems that Venczel et al. (1997) was the first report in the western literature on the effect of EOW on microorganisms, specifically on inactivation of *C. perfringens* spores by NEW. Regarding AcEW, Venkitanarayanan et al. (1999b) reported the inactivation of cultures of *E. coli* O157:H7, *Salmonella* Enteriditis, and *L. monocytogenes* by approximately 7 log CFU/ml. Subsequent studies have also proved the efficacy of EOW to inactivate human pathogens both *in vitro* (Kim et al., 2000ab, Nakajima et al., 2004; Park et al., 2004) and inoculated onto vegetable surfaces (Deza et al., 2003; Sharma and Demirci, 2003).

The studies on the effect of EOW on microorganisms present in MPV have been mainly performed in Japan. Few of them have included storage tests. Izumi (1999) studied the effect of NEW on total microbial counts of the surface and macerate of MP carrots, bell peppers, spinach, Japanese radish and potatoes. Rinsing with NEW

containing 20 ppm available chlorine for 4 minutes reduced the microbial count in the macerate of the MPV as much as 1.8 log CFU/g. The treatment of carrot slices, Japanese radish shreds or diced potatoes did not yield significant ($\alpha<0.05$) reductions, while counts in chopped bell peppers were reduced by 0.4 log CFU/g. All comparisons are referred to water washed controls. The author concluded that the effect of EOW was influenced by the type and style of MPV. The surface area, anatomy and microstructure of the tissues, which differ among vegetables, as well as the type of cuts, would affect the extent of contact of EOW with microorganisms.

Some examples of use of AcEW to decontaminate MPV can be cited. AcEW reduced total aerobic plate count of MP cilantro leaves by 0.66 log CFU/g compared to water washing (Wang et al., 2004), and by more than 1 log CFU/g in whole chinjon, leafy cabbage, spinach, cucumber and snap beans. But for green peppers the reduction was just 0.9 log CFU/g. With AlEW alone, no more than 0.5 log CFU/g reduction was observed (Lin et al., 2005). The total aerobic plate count and total *Enterobacteriaceae* count of fresh-cut cilantro leaves washed with AcEW containing 16.8 mg/l free chlorine and stored at 0℃ exhibited a lag phase 4 day long, after which counts rebounded (Wang et al., 2004).

Sometimes researchers have used AlEW as a pre-treatment before disinfection with AcEW. When lettuce was washed in AlEW for 1 min and then disinfected with AcEW for 1 min, the decontamination effect on aerobic bacteria, moulds and yeasts was higher than that for the treatment of lettuce by soaking in AcEW for 10 min (Koseki et al., 2001). AcEW reduced the aerobic mesophiles present on whole cucumbers within 10 min by 1.4 log CFU/g. The reduction after washing with AlEW for 5 min and subsequently with AcEW for 5 min was 2.1 log CFU/g. These treatments reduced coliform bacteria and fungi by at least 2.1 and 2.2 log CFU/g respectively (Koseki et al., 2004). Washing with AlEW, and subsequently decontaminating with AcEW containing 40 ppm free chlorine reduced total aerobic bacteria, coliforms and psychrotrophic bacteria on MP lettuce by 1.7, 1.6 and 1.1 log CFU/g, respectively. On MP cabbage the reductions were 1.5, 1.5 and 1.0 log CFU/g respectively (Koseki and Itoh, 2001). Applying predictive microbiology to evaluate the effect of AcEW versus tap water on the shelf-life of MP lettuce and MP cabbage, those authors estimated that AcEW prolonged the shelf-life of MP lettuce by 2.29 and 1.51 days

after storage at 5 and 10℃, and of MP cabbage by 2.69 and 1.81 days at respective storage temperatures. Bacteria grew more quickly in or in MP lettuce and MP cabbage treated with AcEW than in water washed samples, which is one example of discussed in a general way in the section 1.3.5.1. In this case however, microbial populations of control samples reached the microbial limit before those of treated samples. The same trend was reported once more by Koseki and Itoh (2002) for the same MPV. These studies demonstrated that AcEW can be useful to prolong the shelf-life of some MPV.

1.7.6 Effect on sensory quality

EOW washing does not change sensory properties of vegetables immediately after treatment. Concentrations of free chlorine up to 50 ppm did not affect the surface colour of carrot slices, trimmed spinach leaves nor cucumber slices (Izumi, 1999). Deza et al. (2003) reported no adverse effects on taste, appearance or smell in whole tomatoes treated with NEW. In whole cucumbers, no changes in appearance were observed after treatment with AcEW. A residual chlorine odour after washing vegetables with AcEW with 50 ppm active chlorine was reported to be eliminated by soaking with AlEW afterwards (Lin et al., 2005). Furthermore, Park et al. (2001) found no effects of AcEW on visual quality, stem discolouration, wilting, and colour of whole lettuce leaves stored for 14 days at 4℃.

With regard to the pH, no changes in carrot slices, trimmed spinach leaves, chopped bell peppers, Japanese radish shreds and diced potatoes were detected after treatment with NEW containing 20 ppm of free chlorine (Izumi, 1999). Treatment with AcEW did not damage the surface structures of lettuce according to observations of scanning electron micrographies (Koseki et al., 2001).

Researchers expect that a decontamination treatment would be able to disinfect MPV with minimal negative impact on sensory properties. Nonetheless, a treatment that simultaneously decontaminates MPV and arrest sensory changes will have an additional advantage over others. EOW can have beneficial effects in the sensorial stability of some MPV during refrigerated storage. For example, the effect of AcEW containing 41 ppm free chlorine versus tap water on the browning during storage of

MP lettuce and MP cabbage was evaluated by Koseki and Itoh (2002). AcEW treatment decreased the progress of browning in both MPV after 5 days of storage at 5 and 10℃ under air or nitrogen. The authors suggested that since AcEW has a strong ORP potential, the enzymes responsible for browning may have been oxidized and weakened. However, it is generally easier to oxidize phenols than enzymes, so that browning should have occurred before enzyme oxidation. It is possible that the chlorine present in the AcEW have accounted for the observed browning inhibition, since chlorinated water can accomplish that role, as it was mentioned in section 1.6.3.

But EOW can also have deleterious effects. Although washing with AcEW containing 16.8 ppm free chlorine did not affect the colour of cilantro leaves, then AcEW treated samples stored at 0℃ for 14 days had higher loss of aroma than water washed samples during the period of day 8 to day 14, which might be correlated according to the authors to their high tissue electrolyte leakage (Wang et al., 2004).

1.7.7 Effects on the physiology of MPV

The physiology of vegetables can be affected by AcEW. The respiration of MP lettuce and MP cabbage have been reported to increase during storage at 5 and 10℃, after treating with AcEW (Koseki and Itoh, 2002).

Electrolyte leakage is related to cell membrane integrity, the higher the electrolyte leakage the lower the integrity of the membrane. Washing cilantro leaves with AcEW produced a higher electrolyte leakage in comparison with water washed samples. During the first 4 days of storage under MAP at 0℃, the electrolyte leakage readings experienced a sharp decrease reaching a minimum, then increased gradually although without reaching the initial values after 14 days. The authors explained that AcEW must have oxidized the cilantro cell membrane, and that the decrease in electrolyte leakage after 4 days may suggest a membrane damage recovery process (Wang et al., 2004).

1.7.8 Effects on the nutritional and phytochemical composition of MPV

No studies have been published on the effect of EOW on the nutritional properties of vegetables according to the searched literature. Since EOW is an oxidant, reactions that decrease the concentration of oxidizable nutritional compounds are expected to occur and should be studied. It is possible that ascorbic acid be oxidized by free chlorine. Oomori et al. (2000) suggested that reaction with vitamin C could have accounted for the decrease of free chlorine when washing chopped cabbage with AcEW.

1.7.9 Toxicological aspects

No evaluation of the toxicity of EOW has been reported in the scientific literature. Assuming that lower concentrations of free chlorine are necessary in EOW than in HOCl solutions to achieve similar decontamination levels (Izumi, 1999), the formation of toxic by-products derived from chlorination reactions is expected to be lower. Private informs cited by Venczel et al. (1997) on MIOX reported conflicting results, one study found that NEW produced essentially the same levels of trihalomethanes as free chlorine, while another indicated a reduction of more than 50% in total trihalomethane production for MIOX versus free chlorine.

1.8 Chlorine dioxide

1.8.1 Definition

Chlorine dioxide (ClO_2) is one of the few compounds that exists almost entirely as monomeric free radicals (WHO, 2000). It is used as a bleaching agent at paper manufacturing plants, and in public water treatment facilities to make water safer to drink. In 2001, ClO_2 and chlorite were used to decontaminate a number of public buildings following the release of anthrax spores in the United States (ATSDR, 2004). ClO_2 has received attention as a decontaminant for vegetables, largely because its efficacy is less affected by pH and organic matter and it does not react with ammonia to form chloramines, as do liquid chlorine and hypochlorites (Beuchat, 1998). According to the review by Fukayama et al. (1986), ClO_2 is a polar gas that readily dissolves in, but does not react with, water. It has a larger oxidation capacity than

that of HOCl because it can accept 2.5 times more electrons than HOCl, but its oxidizing potential is less. It will oxidize and chlorinate many organic compounds, but at a much slower rate than that associated with HOCl reactions. In most cases, oxidation reaction predominates.

Sometimes, the term "acidified sodium chlorite" appears in the scientific literature. It refers to the solution resulting from mixing a sodium chlorite solution with an organic acid. This chemical reaction produces chlorine dioxide.

1.8.2 Inactivation mechanism

The loss of permeability control was identified by Berg et al. (1986) as the primary lethal event at the physiological level of the effect of ClO_2 on bacterial cells, with non-specific oxidative damage of the outer membrane leading to the destruction of the trans-membrane ionic gradient. Young and Setlow (2003) also proposed that some type of membrane damage could cause death of *B. subtilis* spores, since ClO_2 killed spores can undergo the initial steps in spore germination, but can not go further in this process. By using confocal scanning laser microscopy, Peta et al. (2003) observed that cells of *Bacillus cereus* treated with ClO_2 exhibited surface roughness and indentations, and were elongated, in contrast with control cells, which were uniform rods with smooth surfaces. Elongation of cells might be result of inhibition of division and associated metabolic damage.

There are no specific reports on the susceptibility of different microorganisms to ClO_2. Reina et al. (1995) found that among the groups of microorganisms present in water used to hydrocool cucumbers, the order of susceptibility was lactic acid bacteria > total enterobacteriacea > total aerobes > total yeasts > total moulds. Winniczuk and Parish (1997) reported that *Saccaromyces* was more susceptible than *Lactobacillus*, *Leuconostoc* and *Gluconobacter*. The susceptibility reported by Han et al. (1999) when ClO_2 gas was tested to sanitize tanks was: yeast (mixture of *Candida* spp. and *S. cerevisae*) and moulds (*Eurotium* spp. and *Penicillium* spp.) > *Leuconostoc mesenteroides* > *Lactobacillus buchneri*. These results do not agree with Reina et al. (1995).

1.8.3 Advantages and disadvantages

1.8.3.1 Advantages

- Its efficacy is less affected by pH and organic matter as do liquid chlorine and hypochlorites.
- It does not react with ammonia to form chloramines (Beuchat, 1998).
- Because gas has greater penetration ability than liquid, ClO_2 gas is more effective for surface sanitation than aqueous compounds (Han et al., 2001b).

1.8.3.2 Disadvantages

- Produce applications of ClO_2 needs on-site generation, specialized worker safety programs, and closed injection systems for containment of concentrate leakage and fumes from volatilisation (Suslow, 1997).

1.8.4 Effects on nutritional and phytochemical composition

It is known that ClO_2 can react with phenols (Napolitano et al., 2005). Since many phytochemicals are phenols, it is possible that ClO_2 has an impact on the content of these compounds. As ClO_2 is an oxidant, nutrients such as ascorbic acid could be easily oxidized.

1.8.5 Toxicological aspects

The non-formation of chlorinated toxic by-products is one important advantage of ClO_2 over HOCl. Unlike the other disinfectants, the major ClO_2 by-products, chlorite and chlorate ions, are derived from decomposition of the disinfectant as opposed to reaction with precursors, with no direct formation of organohalogen by-products. As opposed to chlorine, which reacts via oxidation and electrophilic substitution, ClO_2 reacts only by oxidation; this explains why it does not produce organochlorine compounds (WHO, 2000). ClO_2 can react to give HOCl, hence the possibility that halogenated aromatic products or chloramines derivatives are formed after ClO_2 is consumed on the surface of MPV can not be excluded. Moreover, chlorinated phenols have been detected during studies of ClO_2 oxidations of phenols *in vitro*

(Napolitano et al., 2005). However, in view of scientific evidence, halogenated products can be expected to be formed only at very low concentrations. Sorlini et al. (2005) found that ClO_2 produced 98% less trihalomethanes than chlorine when added to surface waters coming from ten different sources in Italy.

Tsai et al. (2001) could not detect any residue of chlorine dioxide, chlorite or chlorate in potatoes stored in an atmosphere with ClO_2. According to authors´ estimations, the ClO_2 in potatoes could have theoretically reached as much as 14 ppm, but results showed that the combined amount of chlorite and chlorine dioxide was less than 0.07 ppm and that of chlorate also less than 0.07 ppm.

Since ClO_2 is used in drinking water, there are studies assessing its toxicity and that of its by-products, mainly chlorite, in water. Studies have found no adverse effects in humans living in areas with ClO_2-disinfected water. Such studies can however not be considered conclusive due to methodological problems, according to the US Environmental Protection Agency (2000). Based on the inadequate availability of information, the International Agency for Research on Cancer determined that sodium chlorite is not classifiable as to human carcinogenicity (IARC, 1991), and chlorine dioxide as well (ATSDR, 2004).

A recent study showed that a solution of ClO_2 and its by-products ClO_2^- and ClO_3^- administrated to rats during 90 days at a dosage estimated to be 120 times higher than the human dosage was not toxic. The toxicity was evaluated in terms of weight gained, food utilization efficiency, indexes of blood and serum, liver/body weight and kidney/body weight ratios, and histopathological examination of liver and kidney (Qingdong et al., 2006).

1.8.6 Aqueous chlorine dioxide

1.8.6.1 Effect on microbial populations

ClO_2 solutions have limited efficacy to decontaminate MPV. Zhang and Farber (1996) reported that treating MP lettuce and MP cabbage with 5 ppm ClO_2 for 10 min reduced *L. monocytogenes* populations by 0.8 log CFU/g in both MPV. Populations

of the same microorganism inoculated onto bell peppers were reduced by 3.7 log CFU/5g when surface was not injured and by 0.44 when injured, after treatment with 3 mg/l ClO_2 solution (Han et al., 2001b).

Singh et al. (2002a) reported that washing MP lettuce inoculated with *E. coli* O157:H7 for 5 min with 10 mg/l ClO_2 reduced counts by 1.20 log CFU/g, an additional wash for 5 min produced more decontamination but not a third one. This shows that ClO_2 liquid is effective to decontaminate MPV and it also shows the utility of sequential washing. Furthermore, another study found that 15 min exposure of shredded lettuce or baby carrots to 20 mg/l of aqueous ClO_2 caused reductions of 1.7 and 2.5 log CFU/g of *E. coli* O157:H7 respectively (Singh et al., 2002b).

Also chlorine dioxide delivered to the produce from acidified sodium chlorite can decontaminate MPV. Acidified sodium chlorite reduced populations of *E. coli* O157:H7 on Chinese cabbage by 3.0 log CFU/g, while distilled water caused only about 1.0 log CFU/g reduction (Inatsu et al., 2005).

The use of ClO_2 solutions to decontaminate and to prolong the shelf-life of produce has yielded mixed results. Holding cucumbers in water with up to 105 ppm ClO_2 for 15 min had very little effect on the numbers of yeasts, moulds and lactic acid bacteria (Costilow et al., 1984). Reina et al. (1995) concluded that ClO_2 added to recycled hydrocooling water has little effect upon microorganisms on or in cucumbers, however, conclusions can depend on the criterion used to evaluate data. They reported 1.0 log CFU/ml reduction in total aerobes and >2.9 log CFU/ml reduction in lactic acid bacteria using 0.85 ppm ClO_2. Reductions of >2.5 and >6 log CFU/ml in total enterobacteriaceae and total yeasts respectively were reached with 1.33 ppm ClO_2. Finally as much as 3.46 log reduction in total aerobes and 1.32 log in total moulds were reached using 5.13 ppm. Cucumbers hydrocooled in water with 5.1 ppm ClO_2, then stored at 10 to 12℃, did however not have a longer storage life than just water washed cucumbers.

A more recent study reported 4.2, 1.6 and at least 1.2 log reductions for mesophilic bacteria, yeasts and moulds on shredded lettuce after treatment with 5 ppm ClO_2 for 5 min. During storage at 4℃ for 9 days, samples washed for 5 min with 3 or 5 ppm

ClO_2 had lower counts of mesophilic bacteria, yeasts and moulds than water washed controls (Rodgers et al., 2004).

1.8.6.2 Effects on sensory quality

There is no evidence that liquid ClO_2 can damage the sensory attributes of MPV. Shredded lettuce treated with 5 ppm ClO_2 for 5 min could not be differentiated from controls by using triangle tests (Rodgers et al., 2004). Similarly, acidified sodium chlorite did not change the colour of Chinese cabbage leaves (Inatsu et al., 2005).

1.8.7 Chlorine dioxide gas

The use of ClO_2 gas has been primarily limited to pulp bleaching in the paper industry (Han et al., 2003). As it was mentioned before, Han et al. (2001b) hypothesized that gaseous ClO_2 may be more effective for surface sanitation than aqueous compounds. Their results showed the validity of their statement.

1.8.7.1 Factors affecting the efficacy of ClO_2 gas

Han et al. (2001a) modelled the effect of factors determining the inactivation of *E. coli* O157:H7 on green peppers by ClO_2 gas. The order of significance from the most important to the least was: gas concentration (0.1 to 0.5 mg/l), time (7 to 135 min), relative humidity (55 to 95%) and temperature (5 to 25°C). Moreover, a synergistic behaviour between ClO_2 gas concentration and relative humidity was found. The reported effects of gas concentration, time and relative humidity are consistent with results presented by Jeng and Woodworth (1990) and Han et al. (1999). Westphal et al. (2003) demonstrated that *Bacillus thuringiensis* spores swell with increasing relative humidity. Swelling of a spore increases the diameter of channels for access of gases into spores and may account for the greater kill effectiveness of spores by gas-phase ClO_2 at very high relative humidity.

1.8.7.2 Effect on microbial populations

The first article on the use of ClO_2 gas as disinfectant was published by Jeng and Woodworth (1990) in an attempt to find a substitute of ethylene dioxide gas as

sterilant of medical products. They proved that ClO_2 gas can inactivate spores of different *Bacillus* species.

Subsequent studies have proved that gaseous ClO_2 is effective to kill microorganisms present on vegetable surfaces. Working with *E. coli* O157:H7 inoculated onto surface-injured bell peppers, Han et al. (2000a) found that 1.24 mg/l ClO_2 gas reduced bacterial populations by 6.45 log CFU/g, while the reduction after water washing was 1.5-1.7 log CFU. A subsequent study (Han et al., 2000b) showed that the decontamination with ClO_2 gas from microorganisms located on green pepper surface is impaired by surface injuries. Afterwards, the same research group (Han et al., 2001b) showed that gaseous ClO_2 is more effective than aqueous ClO_2 to inactivate *L. monocytogenes* inoculated onto both injured and uninjured green pepper surfaces, which confirmed their hypothesis on the superiority of gas phase ClO_2 over its liquid counterpart. Yet, the efficacy of gaseous ClO_2 is lower when microorganisms are located in injured tissues with respect to uninjured tissues.

Regarding MPV, Singh et al. (2002b) reported that 15 min exposure of shredded lettuce or baby carrots to 1 mg/l of gaseous ClO_2 caused reductions of 2.1 and 3.0 log CFU/g of *E. coli* O157:H7, respectively. Populations of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* Typhimurium inoculated onto MP lettuce were reduced 6.9, 5.4, and 5.4 log respectively by 0.44 mg/l ClO_2 for 3 hours (Lee et al., 2004). Sy et al. (2005b) evaluated gaseous ClO_2 as a sanitizer for killing *Salmonella*, *E. coli* O157:H7 and *L. monocytogenes* on fresh and MPV. Reductions resulting from a 4.1 mg/l treatment were 3.13-4.42 log CFU/g for MP cabbage, 5.15-5.88 log CFU/g for MP carrots, 1.53-1.58 log CFU/g for MP lettuce, 4.33 log CFU/tomato and 1.94 log CFU/onion.

There is scarce information on the effect of ClO_2 gas on the spoilage microbiota of vegetables, with mixed results. Sy et al. (2005b) reported no significant differences in yeast and mould population of tomatoes and onions treated with up to 4.1 mg/l ClO_2 for 20-25 min. However, 8.0 mg/l ClO_2 applied for 120 min to different small fruits produced positive results on yeast and mould populations, reductions of 2.06-2.32, 4.07-4.16 and 2.56 log CFU/g for blueberries, strawberries and raspberries were found respectively (Sy et al., 2005a).

1.8.7.3 Effect on sensory quality

Different reports have shown that gaseous ClO_2 treatments can decrease the sensory quality of MPV, which surely depends on time-concentration combinations. The careful selection of treatment conditions seems to be necessary to decontaminate MPV without causing damage to produces. Furthermore, it is possible that ClO_2 gas could be successfully applied only to a limited number of commodities, or coupled with a pre-treatment.

Gaseous ClO_2 can damage sensory attributes of MPV during treatment. For example, the colour of MP lettuce changed during 15 min exposure at 0.75 mg/l gaseous ClO_2, and 10 or 15 min to 1.0 mg/l. This may have been due to the oxidation of chlorophyll. Milder treatment conditions did not cause this defect (Singh et al., 2002b).

Sy et al. (2005b) evaluated appearance, colour, aroma and overall quality of several MPV treated with 1.4 mg/l ClO_2, subsequently stored at 10℃ up to 10 days. Browning of MP cabbage and MP lettuce, and slight whitening in colour of MP carrots was observed immediately after treating. Symptoms increased at higher ClO_2 concentrations.

Sy et al. (2005b) also reported that MP cabbage, MP lettuce, and MP carrots treated with ClO_2 received worse scores than controls for all attributes and storage times. The aroma and overall quality of MP cabbage treated with ClO_2, reached unacceptable scores immediately after treatment, while controls were unacceptable after 7 days for all the evaluated attributes. MP lettuce treated with ClO_2 was rejected by the panel after 3 days due to bad ratings in all tested attributes, while controls were rejected because of the same reasons only after 10 days. The overall quality of MP carrots treated with ClO_2 dropped below the rejection limit at day 10, when controls were still acceptable.

Nevertheless, positive results have also been published. Lee et al. (2004) kept MP lettuce treated with ClO_2 gas under storage at 4℃ for 18 days, finding no visible quality difference between untreated and ClO_2 gas treated samples.

In whole produce, Sy et al. (2005b), treated tomatoes and onions with 1.4 mg/l ClO_2, and stored them at 21°C for up to 10 and 31 days respectively. Panel scores for appearance, colour, aroma and overall quality of tomato were generally better in ClO_2 treated than in control samples throughout the study. The aroma and overall quality of control and treated produce was rejected after 7 days; additionally controls were also rejected because of bad appearance. However, the authors attributed the decreased sensory ratings to overripening and decay caused by moulds. Considering the efficacy of this technique to reduce *Salmonella*, they concluded that it is promising in for commercial application to tomatoes. Regarding onions, all the evaluated attributes were below the acceptability limit after 31 days for control and treated samples, except for the aroma of controls. Giving the efficacy of gaseous ClO_2 to kill *Salmonella* without greatly compromising sensory quality, the authors recommend investigating ClO_2 gas treatment of onions on a large scale.

1.9 Ozone

1.9.1 Definition

Ozone (O_3) results from the rearrangement of atoms when oxygen molecules are subjected to high-voltage electrical discharge (Khadre et al., 2001). Ozone is the highly unstable triatomic molecule that is formed by addition of an oxygen atom to molecular diatomic oxygen. When a free oxygen atom encounters molecular oxygen, it combines to form the ozone molecule (Güzel-Seydim et al., 2004).

1.9.2 Inactivation mechanism

Ozone is a strong oxidant and disinfectant. Ozone can react with contaminants directly as molecular ozone or indirectly as ozone-derived free radical such as •OH and $\cdot H_2O$ (Koseki and Itoh, 2001). Ozone decomposes in solution in a stepwise fashion, producing in turn hydroperoxyl ($\cdot HO_2$), hydroxyl (•OH), and superoxide ($\cdot O_2^-$) radicals. The hydroxyl radical is an important transient species and chain-propagating radical. The reactivity of ozone is attributed to the great oxidizing power of these free radicals (Kim et al., 1999). Different authors have proposed diverse targets for the bactericidal action of ozone: double bonds of unsaturated lipids in the

cell envelope, a general oxidation of protoplasm, interference with the respiratory system and damage to the genetic material (Kim et al., 1999). Young and Setlow (2004) favoured the idea that spore killing by ozone is due to some type of damage to the spore´s inner membrane, although the identity of this damage was not clear. There is a little agreement among researchers regarding the sensitivity of different microorganisms to ozone, which is mainly consequence of the difficulty to compare results from different sources (Kim et al., 1999; Khadre et al., 2001).

1.9.3 Advantages and disadvantages

1.9.3.1 Advantages

- Ozone is one of the most potent sanitizers known.
- Excess ozone auto-decomposes rapidly to produce oxygen, an thus it leaves no residues in food.
- The sanitizer is active against all forms of microorganisms at relatively low concentrations.
- The gas does not appreciably react with water; therefore it forms a true physical solution (Khadre et al., 2001).

1.9.3.2 Disadvantages

- Because of its instability, ozone must be generated at the usage site.
- Because of the strong oxidizing power of ozone, metal and other types of surfaces with which it comes into contact are subject to corrosion or other deterioration (Beuchat, 1998).
- Its efficacy is affected by organic matter (Khadre et al., 2001). The interference of several food components on the microbicidal efficacy of ozone has been reported (Restaino et al., 1995; Güzel-Seydim et al., 2004).
- Decomposition of ozone is so rapid in the water phase of foods that its antimicrobial activity is restricted to surfaces.
- The mechanisms of decomposition of ozone are complex processes, therefore, it may be difficult to generalize that a particular concentration of

ozone at a given rate will always be effective in inhibiting a definite concentration of microorganisms in a food product.

- Ozone detection and destruction systems and respirators are needed for the safety of workers in food processing facilities (Kim et al., 1999).
- Microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to sanitizer (Mahapatra et al., 2005).

1.9.4 Effects on the nutritional and phytochemical composition of MPV

Ozone washing reduced the vitamin C content of shredded lettuce (Beltrán et al., 2005b). In fresh-cut celery however, 0.03 and 0.08 ppm of ozone in washing water arrested vitamin C oxidation in fresh-cut celery during storage at 4℃ up to 9 days (Zhang et al., 2005). According to the authors, inhibition of PPO and delay of tissue metabolism by ozonated water could account for this effect.

Beltrán et al. (2005b) studied the effect of washing with 20 mg O_3/l on the phenolic content of shredded lettuce. No effect of ozone was observed in phenolic content and several individual phenolic compounds after treatment or during storage at 4℃ for 13 days. Chlorogenic and isochlorogenic acids, the compounds whose biosynthesis has been associated with wound-induced browning in iceberg lettuce midribs, increased their content regardless the treatment (ozonation or water washing), suggesting that the biochemical mechanism of action used by ozone to prevent browning is not related to inhibition of phenolic metabolism.

1.9.5 Toxicological aspects of the application of ozonated water to MPV

There is no conclusive evidence that ozone can cause mutagenic or carcinogenic effects in humans (Kim et al., 1999). However, ozone toxicity to workers is a major concern (Güzel-Seydim et al., 2004). Ozone is intended to substitute use of chlorine. Sorlini et al. (2005) found that ozone produced 98% less trihalomethanes than chlorine when added to surface waters used for drinking water production in real potabilisation plants. Trihalomethances can be formed by reaction of ozone with organic matter present in non potabilised water. Nevertheless, ozone applied to

waters with high bromide concentration can generate bromate, which then can form trihalomethanes.

1.9.6 Liquid ozone

1.9.6.1 Effect on microbial populations

In spite of its theoretically high microbicidal power, ozone has shown mixed efficacy to reduce pathogen populations on vegetable surfaces, perhaps because it is fast degraded by the organic matter of the vegetable. Singh et al. (2002b) achieved 1.42 and 1.80 log CFU/g reductions in *E. coli* O157:H7 after treating respectively MP lettuce and baby carrots with ozonated water (16.5 mg/l) for 10 min. Only 0.59 log reductions were possible after treating alfalfa seeds for 3 min at 14.3 mg/l (Singh et al., 2003). In contrast, counts of *L. monocytogenes* and *E. coli* O157:H7 in shredded lettuce were reduced from 6.0 log CFU/g to <1 log CFU/g by washing 5 min with ozonated water containing 3 ppm ozone (Rodgers et al., 2004).

Ozonated water has also shown mixed effectiveness to reduce microorganisms naturally present in MPV. Ozonated water containing 5 ppm of ozone reduces viable aerobes in MP lettuce by 1.5 log CFU/g, and yeasts and moulds by about 1 log CFU/g within 10 min, while water washing controls did not affect those microbial populations (Koseki et al., 2001). The same treatment applied to whole cucumbers produced a reduction of 0.7 log CFU/cucumber (Koseki et al., 2004). The same ozone concentration used to wash shredded lettuce for 5 min reduced mesophilic bacteria, yeasts and moulds by 4.0, 1.6 and >1.4 log CFU/g respectively. MP lettuce treated with ozonated water with 2.5-7.5 mg/l of ozone had a 0.6-0.8 log reduction in aerobic plate counts and a treatment with 7.5 mg/l of ozone reduced the psychrotroph counts by approximately 0.5 log, all data are compared with rinsing with distilled water (Garcia et al., 2003). Zhang et al. (2005) reported increasing decontamination levels of total bacterial counts with increasing concentrations of ozone in water used to wash fresh-cut celery, with a maximum reduction of 1.69 log CFU/g at 0.18 ppm. After washing fresh-cut lettuce with ozonated water (20 mg/l), Beltrán et al. (2005b) found that the total mesophilic population was reduced by 1.6 log units compared to water washing. Ketteringham et al. (2006) washed pre-cut

green peppers with aqueous ozone at 3.95 mg/l without obtaining significant decreases in aerobic plate counts. The inefficacy of ozone in this example is assigned by the authors to the leaching of organic material from the cut surfaces to the ozone solution. This organic material degrades ozone, therefore decreases the antimicrobial capacity of the solution. Moreover, they stated that some microorganisms may have migrated into the deeper tissue or become protectively attached during the span of five days between cutting and washing. Ozonated water was also unsuccessful to reduce microbial populations of potato strips (Beltrán et al., 2005a).

MPV decontaminated by ozonated water and stored at 4°C have been shown to have longer shelf-life than their untreated counterparts, from the microbiological point of view. Counts of mesophilic bacteria in ozonated MPV remained lower than in controls in the cases of shredded lettuce for up to 9 days (Rodgers et al., 2004) and 13 days (Beltrán et al., 2005b); and fresh-cut celery for up to 9 days (Zhang et al., 2005). For ozonated MP potato, mesophilic bacteria counts were not different from controls during storage, however, no decontamination was observed due to treatment (Beltrán et al., 2005a).

1.9.6.2 Effect on sensory attributes

Washing with ozone does not only reduce microbial counts but also maintains the sensory quality of MPV, in spite of being a powerful oxidizer. Lettuce surface is not affected by ozonated water according to observations using microscopy (Koseki et al., 2001). The shelf-life of MP lettuce washed with ozone and stored at 4°C was 4 days longer than chlorinated samples (Garcia et al., 2003). Fresh-cut cilantro leaves washed with ozonated water and stored at 0°C up to 14 days had the same colour than leaves washed with tap water. The typical cilantro aroma was maintained with a higher score by the ozone treatment at day 14. The ozonated samples exhibited better overall quality retention during storage. The leaves appeared to be near the fresh or initial conditions of the cilantro, with a green and fresh appearance, no yellowing or dehydration and no trace of off-odour (Wang et al., 2004). The evaluation of the sensory quality (colour, visible structural integrity, and general appearance) of fresh-cut celery treated with ozonated water up to 0.18 ppm during

storage at 4°C showed that ozone was beneficial in maintaining the sensory quality (Zhang et al., 2005). Potato strips treated with ozonated water (20 mg/l) stored under vacuum at 4°C during 14 days showed no evidence of browning, maintained the full typical aroma and a very firm and turgid texture (Beltrán et al., 2005a). Samples of MP lettuce washed with ozonated water and stored in air maintained an excellent visual quality during storage at 4°C up to 13 days without significant differences compared to the initial visual quality, and no browning was observed. In contrast, washed water controls decreased visual quality and browned sharply after 5 days. Moreover, ozone did not affect the texture of MP lettuce (Beltrán et al., 2005b).

1.9.6.3 Effects of ozonated water on the physiology of MPV

The respiration rate of MPV in some cases becomes altered after washing with ozonated water. Zhang et al. (2005) reported that the respiration rate of fresh-cut celery was decreased with increasing concentrations of ozone in water. On the other hand, based in the evolution of O_2 and CO_2 concentrations during storage at 4°C, Beltrán et al. (2005a) concluded that washing with ozonated water did not alter the respiration rate of potato strips. Similarly, measuring the effect of ozonated water on the respiration rate of MP lettuce, Beltrán et al. (2005b) found no differences with respect to water washed controls.

Washing with ozonated water did not alter electrolyte leakage of fresh-cut cilantro during storage, compared to tap water washed control; therefore it did not affect the membrane integrity of the plant cell (Wang et al., 2004). Zhang et al. (2005) reported lower PPO activity during refrigerated storage of fresh-cut celery treated with ozonated water than in controls.

1.9.7 Gaseous ozone

1.9.7.1 Factors affecting the efficacy of ozone gas

As in the case of gaseous ClO_2, O_3 gas must have more penetrability than its aqueous counterpart, hence being more effective than disinfectant solutions. Han et al. (2002) studied the factors affecting the efficacy of O_3 gas to kill *E. coli* O157:H7 inoculated onto green bell pepper surface. The order of significance of the factors

affecting O_3 decontaminant efficacy from the most important to the least was: ozone concentration (2-8 mg/l), exposure time (10-40 min), and relative humidity (60-90%), in the ranges indicated. Moreover, a synergism was found between gas concentration and RH. These results are similar to those commented for gaseous ClO_2 in the section 1.8.7.1 of this chapter. Temperature may be another important factor for ozone gas treatment, but it was not studied by these authors because of limitations of their experimental conditions. Also Li and Wang (2003) found that germicidal efficiencies increased as RH increased for four microorganisms, which could be related to the higher number of radicals from O_3 reaction with more water vapour at higher RH.

The shape of the inactivation curve for the microbial inactivation with ozone differs with the microorganism. Kowalski et al. (2003) studied the kinetics of the inactivation of a strain of *E. coli* in an enclosed flow-through system, and modelled also data previously published for the inactivation of *B. cereus* with ozone. Survival data on *E. coli* was fitted to a two-stage curve (a fast inactivation part and a tailing part) with a shoulder. For *B. cereus* however, data was fitted to a two-stage curve but no significant shoulder was evident.

Li and Wang (2003) found the following order of susceptibility of microorganisms from the most susceptible to the least: *E. coli* (Gram-negative), *Candida famata* (yeast), *Penicillium citrinum* spores (mould) and *B. subtilis* spores (Gram-positive). Ozone dosage differences for 80% microorganism inactivation were as high as 40 times between both extremes.

1.9.7.2 Effect on pathogenic microorganisms and sensory quality

There are very few studies about the application of ozone in gas phase to decontaminate MPV. Singh et al. (2002b) achieved 1.79 and 2.64 log CFU/g reductions in *E. coli* O157:H7 populations after treating respectively MP lettuce and baby carrots with gaseous O_3 (7.6 mg/l) for 15 min. Decolouration of lettuce leaves was observed after treatment with 5.2 or 7.6 mg O_3/l for 10 and 15 min. Han et al. (2002) reported 7.35 log reduction (log CFU/5g) of *E. coli* O157:H7 inoculated onto

green bell pepper surface after treatment with 8 mg/l O_3 at 90% relative humidity for 25 min.

1.10 Final commentary

Current available literature does not permit to choose the “ideal” decontamination method for shelf-life prolongation. Each decontamination method has its own advantages and disadvantages. Moreover, it is likely that some of them be suitable for a limited number of commodities. Industry and governments considering aspects such as the economics, not addressed in this review, would decide their final implementation.

CHAPTER 2

Factors affecting the inactivation of microorganisms by intense light pulses[2]

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Chapter 2. Factors affecting the inactivation of microorganisms by intense light pulses

2.1 Abstract

The aim of this part of the thesis was to determine the influence of several factors on the inactivation of microorganisms by intense light pulses (ILP). Microorganisms on agar media were flashed 50 times under different conditions and their inactivation measured. Microorganisms differed in sensitivity to ILP but no pattern was observed among different groups. Several enumeration methods to quantify the effect of ILP were investigated and showed relevant differences, covering effect and photoreactivation accounted for them, the strike method yielded the most reliable results. Higher decontamination efficiencies were obtained for Petri dishes located close to the strobe and inside the footprint. Decontamination efficacy decreased significantly at contamination levels > 6.85 log. After 13 successive treatments, no resistance to ILP could be demonstrated. Media warming up depended on the distance from the lamp and the number of flashes. For an industrial implementation: the position and orientation of strobes in an unit will influence the lethality, products should be flashed as soon as possible after contamination occurs, a cooling system should be used for heat sensitive products and flashed products should be light protected. No resistant flora is expected to develop. Conclusions derived from this work will allow a better implementation of this decontamination technique at industrial level.

Keywords: intense light pulses, ultra-violet, inactivation, decontamination, pulsed UV light, food, pulsed white light, minimally processed.

2.2 Introduction

As it was defined in chapter 1, section 1.5.1; intense light pulses (ILP) is a novel technique proposed to decontaminate surfaces by killing microorganisms using short time high frequency pulses of an intense broad spectrum, rich in UV-C light. It has potential application for the treatment of food, packages, medical devices, packaging and processing equipments for the food, medical and pharmaceutical industries; and water and air (Dunn et al., 1997). The following names have been used by different authors to denominate techniques that can be included in the definition of ILP: pulsed UV light (Sharma and Demirci, 2003), high intensity broad spectrum pulsed light (Roberts and Hope, 2003), pulsed UV disintegration (Wekhof, 2000) (this term does not comprehend all kinds of ILP treatments), pulsed light (Rowan et al., 1999) and pulsed white light (Marquenie et al., 2003). This technology is grouped by food scientists in the frame of the called non-thermal technologies, which are intented to produce stable and safe food products without those damages provoked by heating. It has been demonstrated that UV light is the most important part of the spectrum for microbial inactivation (Rowan et al.,1999; Wang et al., 2005).

The literature on ILP is actively growing but a gap between basic and applied research is notorious in its application to food decontamination. Additionally, the applied research about ILP is frequently supported using the basic research obtained from the study of the effects of continous UV, however, despite of the spectrum of the light used in ILP studies have a very important UV component, both mechanisms of microbial inactivation are not necessary equivalents. As example, MacGregor et al. (1998) wrapped their plates in aluminium foil after flashing to avoid photoreactivation, although this phenomenon has not been reported for ILP treated microbes.

As it was summarized in chapter 1, section 1.5.7; *in vitro* studies by MacGregor et al. (1998), Rowan et al. (1999) and Anderson et al. (2000) showed the capability of ILP to achieve high lethality on bacteria and mould spores, and Roberts and Hope (2003) showed its capacity to inactivate viruses important in therapeutic biological products.

On the other hand, *in vivo* studies have been focused on specific applications. Huffman et al. (2000) reported the inactivation of *Klebsiella terrigena*, the parasite

Cryptosporidium parvum and strains of polio and rotavirus in water. Hoornstra et al. (2002) achieved more than 2 log reductions in the aerobic count of four from five vegetables after two flashes, while Marquenie et al. (2003) found no effect of ILP on the suppression of *Botrytis cinerea* rotting of strawberries. Jun et al. (2003) modelled the inactivation of *Aspergillus niger* spores on corn meal, reporting up to 4 log reduction, that the shorter the distance and the higher the time and voltage the higher the lethality, and too high sample temperatures under some experimental conditions. Similar conclusions regarding distance, time and temperature have been reported by Sharma and Demirci (2003) when modelling the inactivation of *Escherichia coli* O157:H7 on alfalfa seeds, and additionally finding that the thicker the seed layer the lower the inactivation. Fine and Gervais (2004) failed to achieve 1 log reduction in the natural flora of wheat flour and black pepper, yet the treatment burned both products.

More extensive basic research is needed to understand how ILP affects microorganisms in order to make the best profit of this decontamination technique. This study therefore (1) includes a critical evaluation of enumeration methods to determine the number of survivors of ILP, verifying the existence of the photoreactivation phenomenon in flashed microorganisms, (2) extensively surveys the lethal effect of ILP on a wide variety of micro-organisms, (3) investigates how the relative position of the sample with respect to the strobe affects the lethality of the process, (4) the influence of the size of microbial populations on the level of decontamination by ILP, (5) the possible development of resistance to ILP and (6) the heating of the supporting medium by ILP treatment; emphasizing the implications of the results for the food processing industry.

2.3 Materials and methods

2.3.1 Intense light pulses device

The test assembly consisted of a housing, a stroboscope and a lamp. The housing was a rectangular parallelepiped stainless steel box (length: 60 cm, width: 40 cm, depth: 30 cm). The stroboscope was a 100 W apparatus (UV ST100-IE, Sysmat Industrie, France), pulse duration of 30 µs and pulse intensity of 7 J. The stroboscope has two modes for controlling flash trigger, a manual mode or an

automatic mode, in the first each flash is generated by pressing a switch, in the second successive flashes at a frequency of 15 Hz are triggered after pressing the switch and stopped when the switch is pressed once more. The lamp was a 14 cm cylindrical Xenon flash lamp (Hamamatsu, Shizuoka-ken, Japan), with an emitted spectrum ranging from UV-C to infrared, with 50% of the light in the UV region. Figure 2.1 shows the spectral distribution of the light as reported by the fabricant. The lamp was horizontally placed at 1 cm of the axis of a stainless steel parabolic reflector of 15.5 cm of arc, 14 cm of length and 10 cm of width; and this reflector was horizontally placed without protruding at the centre of the ceiling oriented along the longitudinal axis. A mechanical device allowed changing the distance between sample and probe by moving the shelf where plates were placed.

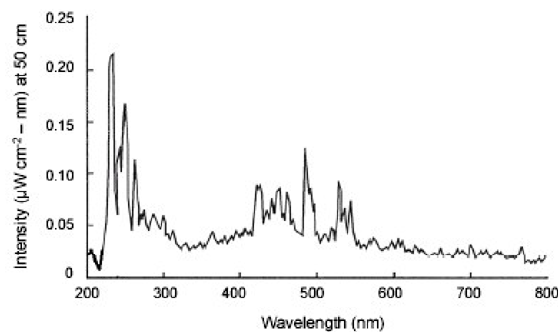

Figure 2.1. Spectral distribution of the used Xenon lamp. Source: Hamamatsu (1999).

2.3.2 Standard treatment

A standard treatment was used in the experiments, unless noted otherwise, using a distance between the strobe and the surface of spread inoculated Petri dishes of 8.4 cm, and 50 flashes per treatment; every flash was manually started at a rate of one pulse/second. The lethality of the process was evaluated by using the formula: Inactivation = log (N_0) – log (N), where N_0 and N are respectively the numbers of microorganisms before and after the treatment.

All the experiments were carried out in triplicate using the same culture on the same day for all three trials to avoid sample variability.

2.3.3 Microbial strains and spore preparation

The species of microorganisms used in these experiments, their origin, culture media and incubation temperature are summarized in table 2.1.

Table 2.1. Origin and culture conditions of the used microorganisms

Name	Code[1]	Media		Temp.[4] (°C)
		Liquid[2]	Solid[3]	
Aeromonas hydrophila	LMG 3771	BHI	NA	30
Alicyclobacillus acidoterrestris	ATCC 49025	OSB	OSA	44
Aspergillus flavus	CBS 131.61 10/8/9		PDA	30
Bacillus cereus	LMG 6924	BHI	NA	30
Bacillus circulans	Isolated from potato puree of a refrigerated meal	BHI	NA	30
Botrytis cinerea			PDA	30
Brochotrix thermosphacta 1	Isolated from cut degreased ham	BHI	NA	30
Candida lambica	Isolated from mixed lettuce stored at 7°C	SAB	YGC	30
Clostridium pasteurianum	LMG 3285	TGM	NA	37
Clostridium perfringens	LMG 11264	TGM	NA	37
Clostridium sporogenes	LMG 8421	TGM	NA	37
Enterobacter aerogenes	LMG 2094	BHI	NA	37
Escherichia coli	LMG 8223	BHI	NA	37
Klebsiella oxytoca	LMG 3055	BHI	NA	37
Lactobacillus sake subsp. *carnosis*	Isolated from cooked ham	MRSB	MRSA	30
Leuconostoc mesenteroides	NRRL 1335	MRSB	MRSA	30
Listeria monocytogenes	LMG 13305	BHI	NA	30

Table 2.1. Continuation.

Name	Code[1]	Media		Temp.[4] (°C)
		Liquid[2]	Solid[3]	
Photobacterium phosphoreum	LMG 4233	BHI	NA	30
Pseudomonas fluorescens	LMG 1794	BHI	NA	30
Rhodotorula mucilaginosa	Isolated from mixed lettuce stored at 7°C	SAB	YGC	30
Salmonella Typhimurium	LMG 10396	BHI	NA	37
Shewanella putrefaciens	LMG 2250	BHI	NA	37
Shigella flexnii	LMG 10472	BHI	NA	37
Staphylococcus aureus	LMG 8224	BHI	NA	37
Yersinia enterocolitica O:9	WS 24/92	BHI	NA	37

[1] Culture collection codes: ATCC: American Type Culture Collection, Rockville, Maryland, USA. CBS: Centraal Bureau voor Schimmelcultures, Baarn, The Nederlands. LMG: Laboratorium Microbiologie, Universiteit Gent, Belgium. NRRL: Northern Regional Research Laboratory, USDA, Peoria, Illinois, USA. WS: Bakteriologishes Institut, Süddeutsche Versuchs- und Forschungsanstalt für Milchwirtschaft, Techische Universität München, Freising-Weihenstephan, Germany.
[2] Liquid media codes and sources: BHI: Brain heart infusion (Oxoid, Hampshire, England); MRSB: De Man, Rogosa and Sharpe broth (Oxoid); OSB: Orange serum broth (Beckton Dickinson, Cockeysville, USA); SAB: Sabouraud liquid medium (Oxoid); TGM: Thioglycolate medium (BBL, Le Pont De Claix, France).
[3] Solid media codes and sources: MRSA: De Man, Rogosa and Sharpe agar (Oxoid); NA: Nutrient agar (Oxoid); OSA: Orange serum (Beckton Dickinson); PDA: Potato dextrose agar (Oxoid); YGC: Yeast Glucose Choramphenicol (Sanofi Diagnostic Pasteur, Marnes-La-Coquette, France).
[4] Incubation temperatures.

Conidia suspensions were obtained by growing moulds on Potato Dextrose Agar at 30°C. *Aspergillus flavus* produced sufficient spores after 6 days, *Botrytis cinerea* after 3 weeks. The slants were afterwards washed with 5 ml of physiological saline solution (PSS) (1 g peptone (Oxoid) and 8.5 g NaCl (Vel, Leuven, Belgium) per litre of water) with 0.01% Triton X-100 (Sigma, USA). This suspension was filtered to

remove mycelium fragments and then centrifuged at 3700 g. Finally, the detergent was replaced by a 10 mM phosphate buffer pH 7.2 and the spores were used immediately.

Bacteria endospore suspensions were obtained plating a 24 h culture and incubating during 4 days to get sporulation. The plates were washed with 10 ml of PSS and this suspension was heated at 80°C for 10 min to kill vegetative cells. *Alicyclobacillus acidoterrestris* was incubated during 5 days, the culture was washed with 10 ml of sterile apple juice and this suspension was heated during 20 min at 70°C. The heat-shocked endospore suspensions were stored at 0-2°C and checked by microscopy before starting experiments to assure that they had not germinated.

Serial dilutions were done by transferring 1 ml of culture to 9 ml of PSS.

2.3.4 Enumeration methods

Three methods to determine the number of survivors were tested in this study:

2.3.4.1 Incubation method

15±0.1 g of the appropriate agar for each microorganism (table 2.1) contained in Petri dishes was rubbed with 0.1 ml of the different dilutions of a 24 h culture. After drying during one hour on the laboratory table to avoid light attenuation due to PSS, the plates were flashed, then immediately wrapped in aluminium foil (unless otherwise noted) to prevent photoreactivation and incubated without further treatment during 48 h. Then the separate colonies were counted. The limit of detection was one colony.

2.3.4.2 Strike method

The strike method is similar to the incubation method but after flashing, 0.1ml of physiological saline solution with 0.5% Tween 20 (Merck, Hochenbrunn, Germany) was brought on the agar and this fluid was spread thoroughly. Then the plates were wrapped in aluminium foil, incubated during 48 h and then the separate colonies were counted. The limit of detection was one colony.

2.3.4.3 Plating method

In the plating method, the agar (15.0±0.1 g) was immediately after flashing aseptically removed from the dishes, and weighted and mixed with PSS in a Stomacher bag making a 10-fold dilution. This suspension was homogenised thoroughly by means of a Colworth Stomacher 400 (Steward Laboratory, London, UK) and 10-fold dilutions were prepared, plated, incubated during 48 h, and the individual colonies were afterwards counted. The limit of detection was 10 colonies.

2.3.5 Sensitivity of microorganisms to ILP

For this assay, the microorganisms showed in table 2.1 were flashed using the standard treatment and survivors were enumerated according to the plating method on the appropriate agar. The lower the degree of inactivation the more resistant was the microorganism. From these results, one bacterium was chosen as indicator microorganism.

2.3.6 Evaluation of enumeration method

2.3.6.1 Enumeration method reliability

To compare the reliability of the plating method and the incubation method to enumerate survivors of ILP, plates containing the appropriate agar were inoculated with *Enterobacter aerogenes*, *Lactobacillus sake* or *Listeria monocytogenes*, flashed according to the standard treatment and survivors were enumerated by the incubation method or the plating method. Shading effect and photoreactivation experiments were designed in order to explain the observed differences. All the enumeration methods included an one-hour drying step between inoculation and flashing to let agars absorb PSS, which could have interfered with the light transmission towards cells; although it is possible that during that time some cells undergone division, they should likely have been in stationary phase.

2.3.6.2 Shading effect

Agar medium plates inoculated with *L. monocytogenes* (10^6 CFU/cm²) were flashed according to the standard method and the survivors were enumerated with the incubation method or the strike method. Triplicates were performed.

2.3.6.3 Photoreactivation

Foods and food samples are exposed to light during processing and microbiological analyses respectively. This experiment was designed to evaluate the effect of sunlight and artificial light on the reactivation of damaged cells under conditions that simulate what could occur during real-life conditions. To test the possibility that photoreactivation occurs, agar medium plates inoculated with *L. monocytogenes* were flashed according to the standard method and subjected to two tests. In the first, three dishes were wrapped with aluminium foil immediately after flashing, and three others were placed on the laboratory table and let them get illuminated by sunlight during four hours. The same scheme was used in the second test, but with fluorescent light coming from eight fluorescent lamps sited 2.5 m above the dishes, normal conditions occurring in a microbiological analysis laboratory. The survivors were enumerated by the incubation method.

2.3.7 Influence of the relative position between sample and lamp on the decontamination efficacy

The effect of vertical distance between microorganisms and lamp was studied. To that, *L. monocytogenes* spread inoculated on agar was flashed at vertical distances between agar surface and lamp ranging from 3 to 27 cm, according to the strike method.

The effect of the placement of microorganisms at a fixed vertical distance from the lamp but different positions in a plane was also studied. To that, 12 agar media in plates were inoculated with *L. monocytogenes* and placed on a 43 cm x 32 cm flat shelf in a 3 x 4 arrangement and flashed. The experiment was done at 6 cm and 13 cm vertical distances between shelf and lamp and the inactivation at different

positions on the shelf was measured for each distance by the strike method. Data were collected in triplicate.

2.3.8 Influence of the population size on decontamination efficacy

To determine the influence of the bacteria population size on the decontamination efficacy of ILP, several agar media in Petri dishes were inoculated with 0.1 ml of the 10-fold dilution of a 24 h culture of *L. monocytogenes* and stored at 7°C, temperature in which this bacterium can grow. Plates were taken out at determined times, flashed and survivors were enumerated by means of the plating method. As an agar medium, Nutrient Agar at which 5.12% of NaCl (a_w = 0.970) was used to ensure the presence of a significant lag phase in the growth curve.

2.3.9 Development of resistance

To evaluate the possibility of building resistance to ILP, three subcultures from a *L. monocytogenes* culture were taken and three dilutions prepared out of each one, these subcultures were flashed and enumerated by the strike method. After an incubation time of 48 h and survivors' enumeration, one colony from each subculture and dilution was picked up, cultured during 24 h in Brain Heart Infusion broth, plated and flashed again; the procedure was performed 13 times. The significance of the linear regression model (inactivation = D x repetition + E) was evaluated by an F-test.

2.3.10 Media heating

To examine the heating of the surface and the distribution of heat throughout the agar, three optical fibers (Reflex, Quattro model, Aims Optronics, Belgium) linked to a computer with the appropriate software (Noemi Assistant 1.5) were inserted through the bottom of Petri dishes completely filled with fresh Nutrient Agar (agar layer 12 mm high) whose colour is pale yellow and placed at a certain depth in the agar (1, 5 and 10 mm from the surface, measured by a caliper). The plates were treated with 50 flashes generated manually, or 195, 495 and 990 flashes generated automatically; and the computer registered the temperature increase during treatment. The distance between the agar surface and the lamp was 8.4 cm. Each data point represents the mean of three measurements.

2.3.11 Statistical analyses

Data were analysed by using SPSS 11.0 (SPSS Inc., Chicago, USA), with $\alpha=0.05$. A t-test was used to compare pairs of means; when multiple mean comparisons were done, one-way Anova and the Duncan test were used.

2.4 Results

2.4.1 Sensitivity of microorganisms to ILP

Table 2.2 summarizes the results obtained in the sensitivity assay, inactivation levels ranged from 1.2 to >5.9 log CFU/cm^2. No clear pattern could be observed regarding the sensitivity of the different groups of microorganisms and very high inactivation levels make impossible statistical comparisons to differentiate sensitivities. Rowan et al. (1999) reported that Gram-positive bacteria are more resistant than the Gram-negatives although the pattern they presented is not very sharp; the same trend was reported by Anderson et al. (2000) but comparing only the resistance to ILP of three bacteria. *L. monocytogenes* was chosen as an indicator organism for the rest of the experiments because this microorganism was among the most resistant to ILP and poses an important problem in the food industry. Regarding the results presented for conidia inactivation, it is possible that during the one hour drying step of the cultures before flashing, some conidia had germinated. Since vegetative cells are generally more sensitive to inactivation treatments than spores, part of the observed reduction in the population of *A. flavus* and *B. cinerea* could actually be accounted by the killing of vegetative cells coming from germinated spores; therefore the correspondent results have to be evaluated with precaution.

2.4.2 Evaluation of enumeration method performances

2.4.2.1 Enumeration method reliability

Once the indicator microorganism (*L. monocytogenes*) was selected, a protocol had to be stipulated to measure the degree of inactivation by ILP. The only method that appears in the literature to test ILP lethality on agar surfaces is the incubation method (Dunn et al., 1997; MacGregor et al., 1998; Rowan et al., 1999; Anderson et

Table 2.2. Initial counts and inactivation (log CFU/cm²) after 50 flashes at 8.4 cm for different groups of microorganisms.

Microorganism	Initial count	Inactivation ± S.D.
Gram-negative spoilers		
Photobacterium phosphoreum	4.8	>4.4*
Pseudomonas fluorescens	5.6	4.2±1.0
Shewanella putretaciens	5.1	3.9±0.8
Gram-positive spoilers		
Alicyclobacillus acidoterrestris	5.7	>5.2*
Bacillus circulans	4.5	>4.1*
Brochotrix thermosphacta	3.7	3.1±0.3
Lactobacillus sake	5.0	2.5±0.2
Leuconostoc mesenteroides	5.0	4.0±0.8
Enterobacteriaceae		
Enterobacter aerogenes	5.4	2.4±0.5
Klebsiella oxytoca	5.1	4.2±0.6
Gram-negative pathogens		
Aeromonas hydrophila	5.5	2.3±0.3
Escherichia coli	5.3	4.7±1.3
Salmonella Typhimurium	5.4	3.2±0.7
Shigella flexnii	5.1	3.8±0.9
Yersinia enterocolitica	4.8	3.9±0.5
Gram-positive pathogens		
Bacillus cereus	3.4	>3.0*
Clostridium perfringens	3.3	>2.9*
Listeria monocytogenes	5.0	2.8±0.4
Staphylococcus aureus	5.5	>5.1*
Yeasts		
Candida lambica	3.4	2.8±0.4
Rhodotorula mucilaginosa	3.2	>2.8*
Conidia		
Aspergillus flavus	5.2	2.2±0.1
Botrytis cinerea	4.1	1.2±0.1
Bacterial spores		
Alicyclobacillus acidoterrestris	3.3	2.5±0.4
Bacillus circulans	5.7	3.7±0.3
Bacillus cereus	6.3	>5.9*

* When the inactivation was greater than the detection limit, SD could not be calculated.

al., 2000). However, when the degree of contamination of a food needs to be measured, the plating method is applied. The enumeration method affected the quantification of the level of inactivation due to ILP significantly ($\alpha \leq 0.05$). The inactivation measured for *E. aerogenes* by means of the incubation method was 4.9±0.4 log CFU/cm² while by the plating method was 3.6±0.6 log CFU/cm²; the results for *L. sake* were respectively 3.5±0.0 and 2.5±0.0, and for *L. monocytogenes*

3.6±0.2 and 2.4±0.1. Therefore, the incubation method yields a lethality at least 1 log CFU/cm² higher than the plating method. The results of the experiments designed to explain this difference are presented in the next two sections.

2.4.2.2 Shading effect

When the shading effect was tested, the incubation method yielded in a first test an inactivation level of 4.3±0.2 log CFU/cm² and the strike method 3.4±0.6 log CFU/cm², in the second test the results were respectively 4.7±0.2 and 3.8±0.7; and in the third one 4.5±0.3 and 3.4±0.4, all the experiments yielded statistically significant differences ($\alpha \leq 0.05$), which shows that the inactivation was in average 1 log CFU/cm² higher when measured by the incubation method than by the strike method.

The average size of a bacterium is 1 µm x 3 µm assuming that it has a rectangle form. This means that a monolayer of bacteria will have 3 x 10^7 bacteria/cm^2. To test the shading effect, the Petri dishes were inoculated with 10^6 CFU/cm². Although its spreading was done carefully, it is practically impossible to avoid some overlapping. This implies that during flashing, those bacteria located on top will be easily reached by the light and are consequently inactivated, whilst those that are covered will have a greater chance to survive the treatment due to the screening of the light by the bacteria located on top, although at high fluence rates the light might be able to penetrate deeper. Since in the incubation method the survivors are not further treated after flashing, if two or more surviving microorganisms are situated very close to each other on the surface of the agar they will develop as a single colony, which will lead to an overestimation of the lethality of the process. However, when microorganisms are once more spread after flashing, as it is done by the strike method, they become separated from each other (although the efficiency of this separation is not necessarily 100%), and develop separately. Consequently more survivor colonies are counted and the higher inactivation measured by the incubation method in comparison to the strike method can be attributed to the shading effect. For publications on ILP, it is therefore important to realize that the incubation method does not reflect the reality on food products. Moreover it is possible that levels of inactivation from *in vitro* tests reported in the literature have been overestimated due to the limitations of the incubation method.

2.4.2.3 Photoreactivation

The photochemical effects of UV light on some living beings, including the microorganisms, can be reversed by illumination with longer wavelengths, especially visible light, a repairing mechanism called photoreactivation (Cleaver, 2003). As consequence, when a sample treated with UV illumination is exposed to common light (sunlight or fluorecent lamps), the survivor counts will be higher than those from samples stored at darkness immediately after flashing. Since the lethality of ILP is caused at least partly by the photochemical effect of the UV light, it can be deduced that photoreactivation can occur after flashing, which was observed in this chapter. Photoreactivation is a well known phenomenon in continuous UV treatments (see chapter 1, section 1.4.4.2) but it has never proved to occur after ILP treatment. Some authors (MacGregor et al.,1998; Rowan et al., 1999; Anderson et al. 2000) have however taken the precaution of wrapping their Petri dishes in aluminium foil to avoid it.

To test the possibility that photoreactivation occurs, ILP treated plates were exposed for 4 h to sunlight or fluorescent light before wrapping in aluminium foil for further incubation. Non exposed plates were incubated as control. The inactivation measured in the test using sunlight was 4.3±0.3 log CFU/cm² in immediately wrapped Petri dishes and 4.0±0.2 log CFU/cm² in those wrapped after 4 h of illumination. When fluorescent lamps were used, the respective results were 4.7±0.2 and 4.4±0.2 log CFU/cm². Only in the experiment with lamps the differences were statistically significant ($\alpha \leq 0.05$) and the inactivation was 0.3 log CFU/cm² higher in the plates immediately wrapped after flashing.

It is realistic to claim that photoreactivation must have been present during the implementation of the plating method, since this was not performed in darkness, while in the strike method the plates suffered light exposition only the time necessary to pour the solution of Tween 20 and spread it. Moreover, the plating method is time consuming and difficult to perform in complete absence of light, since the agar has to be separated from the Petri dish, weighted, diluted with PSS, homogenised by the

Stomacher, a sample has to be taken out, serial dilutions prepared, plated, agar has to be poured and let solidify. Hence, both the incubation method and the plating method have shown to have important disadvantages when applied for the enumeration of ILP treated samples. Considering that the most reliable method is the one that yields the lowest lethality, one can say that with the incubation method the lethality is overestimated with about 1 log, whilst with the plating method it is impossible to eliminate photoreactivation, resulting in a part of the damaged cells that are able to repair themselves (0.3 log). The strike method has not these disadvantages, although it can suffer of an incomplete separation of all cells. Therefore it is recommended as the most reliable enumeration method for studies on ILP.

This part of the thesis shows the existence of photoreactivation after ILP treatment, which has two practical consequences. From the experimental point of view, plates that are not kept in darkness after plating will lead to an underestimation of the killing effect or a high variability as noted in the pioneer work by Kelner (1949). From an industrial point of view, it could be important to keep treated products in darkness at least the first hours after treatment or take into consideration photoreactivation when estimating the shelf-life. In what extend photoreactivation might have influenced results reported in the literature on *in vivo* experiments needs to be evaluated.

2.4.3 Influence of the relative position between sample and lamp on the decontamination efficacy

2.4.3.1 Vertical distance

Figure 2.2 shows the relationship between the inactivation and the vertical distance from lamp to sample. Distance clearly influenced negatively the inactivation efficacy of ILP; the longer the distance the lower was the inactivation. The results can be fitted to the equation show in Figure 2.2. Sharma and Demirci (2003) and Jun et al. (2003) reported also similar equations to model the effect of distance from agar to strobe on the level of inactivation.

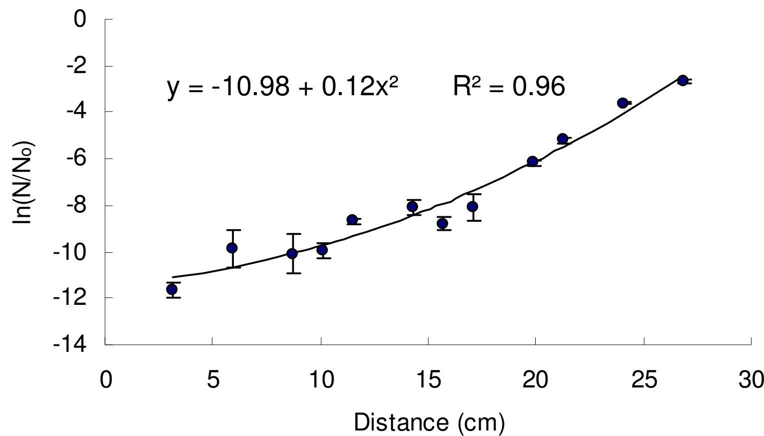

Figure 2.2. Effect of the distance between the strobe and the surface of spread inoculated Petri dishes on the inactivation of *Listeria monocytogenes* after 50 flashes. Bars indicate ± SD. N_o and N: number of microorganisms respectively before and after flashing. Solid line is the trend line.

2.4.3.2 Position with respect to the lamp

Figure 2.3 illustrates the effect of the position of Petri dishes on a shelf on the inactivation of *L. monocytogenes* at two vertical distances from the lamp. Results can be divided in two groups, those from the two central Petri dishes and those from plates placed at the periphery. At 6 cm, the inactivation (log reductions) for the first group was very high (>5) and for the second was low (0.0-1.0). At 13 cm, the inactivation for the first group was between 3.2 and 4.0 and for the second between 0.5 and 2. At both distances the inactivation is higher directly below the lamp than at the borders of the shelf. When comparing both distances, the inactivation directly below the lamp decreases with the distance, but increases at the borders of the shelf.

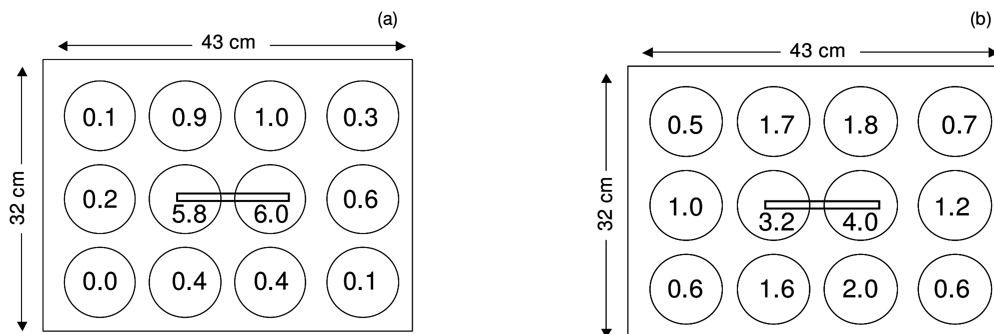

Figure 2.3. Effect of the relative position of inoculated Petri dishes on a tray, on the inactivation of *Listeria monocytogenes* after 50 flashes at 6 cm (a) and 13 cm (b). Numbers inside the Petri dishes are log CFU/cm² reductions attained in each dish. The rectangle at the centre of each panel represents the lamp.

If a number of food pieces are going to be treated and the lamp is placed very close, those pieces located near to the lamp will be efficiently decontaminated, but the others will undergo almost no decontamination. If we can put the group of samples farther form the lamp, the decontamination will be less intense in those samples located closer to it but the rest of the samples will be also decontaminated. This effect has important practical consequences from an industrial point of view, the position and orientation of strobes in an industrial decontamination unit will influence in a great extension the decontamination efficacy.

2.4.4 Influence of the population size on decontamination efficacy

When foods are contaminated by bacteria, the cell population is initially low and become higher during time. In this work, the experiment on the effect of the population size on the inactivation efficiency of ILP was performed to simulate what occurs in real food systems, where the effect of population size and state of growth can not be separated.

Bacteria can possibly react differently to ILP depending on the growth stage and population size. Figure 2.4 shows the changes in the level of inactivation as a function of the number of microorganisms present on the surface of an agar medium as a result of surfacial growth. According to results of the multiple mean comparison test, the inactivation was greater between the lag and early stationary phases. After 138 h the counts had increased to approximately 6.85 log CFU/cm^2 and the inactivation decreased significantly. When after 289 h the count reached approximately 7.5 log CFU/cm^2, the inactivation kept constant at approximately 1.2 log CFU/cm^2.

The drop in inactivation efficiency from a specific density of microorganisms on (6.85 log CFU/cm^2) can be explained by the shading effect. During growth, bacteria are situated on top of each other in several layers forming a colony. Bacteria located on the upper layers receive the light directly and are easily inactivated. On the other hand, bacteria on the bottom layers are protected by those on the upper layers, which screen the incident light, and as a result the survivor numbers are higher. When bacteria arrived to the stationary phase only the bacteria on the upper layer

are inactivated and a large and constant part of the contaminating population will survive the treatment, resulting in a constant put poor inactivation. It is also possible that cells in lag, log and early stationary phases are more susceptible than cells in late stationary phase. Experiments trying to separate both factors should yield more basic information on this regard; nevertheless the results reported hereby reflect better a practical situation. These results show that when ILP is implemented in the industry one must take into account the contamination degree of products. Heavily contaminated products can be less efficiently decontaminated due to the shading effect. The highest efficiency of the ILP will be reached if the food products are flashed as soon as possible after the processing steps where contamination can occur. In the case of fluid products, the same shading effect can be expected at high cell populations as was noted by Gashemi et al. (2003), a thoroughly mixing should alleviate this effect.

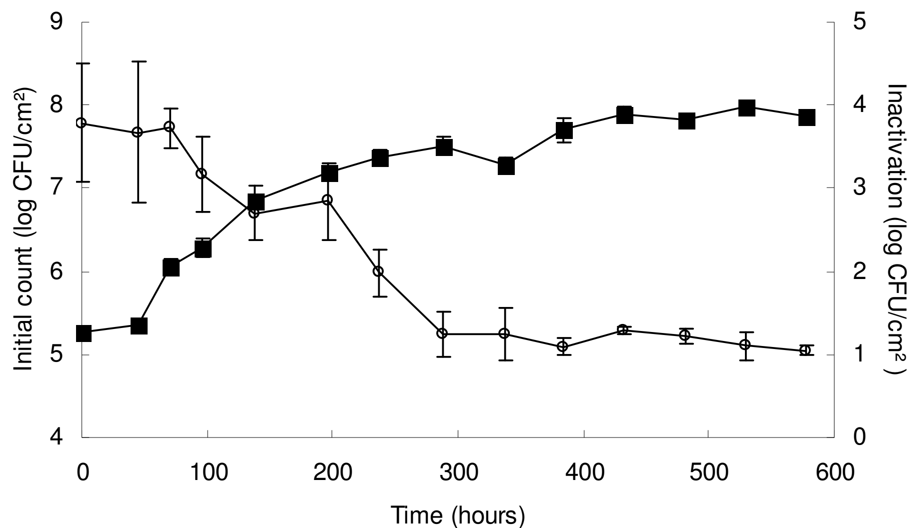

Figure 2.4. Mixed effect of the state of growth and population size on the inactivation of *Listeria monocytogenes* after 50 flashes at 8.4 cm. Initial counts (■), log reductions (○). Bars indicate ± SD.

2.4.5 Development of resistance

To implement a decontamination technology in the food industry it is important that microorganisms do not develop resistance towards the decontamination method, as in this way a resistant house flora can develop hampering long term efficiency of the technique.

In Figure 2.5, an example of the inactivation of cells of one strain of *L. monocytogenes* after successive flashing with the corresponding regression line is given. The multiple correlation coefficient examined by a F-test was equal to 0 for the three strains. This means that there was no correlation between the dependent variable, the inactivation, and the independent variable, the number of times that the flashing procedure was applied. This was the case for all tested dilutions. Since the degree of inactivation of *L. monocytogenes* achieved by flashing was independent of the successive flashing, culturing, plating and flashing again, no resistance to ILP was demonstrated in these experiments. Until now, no other reports on the development of resistance to ILP are available.

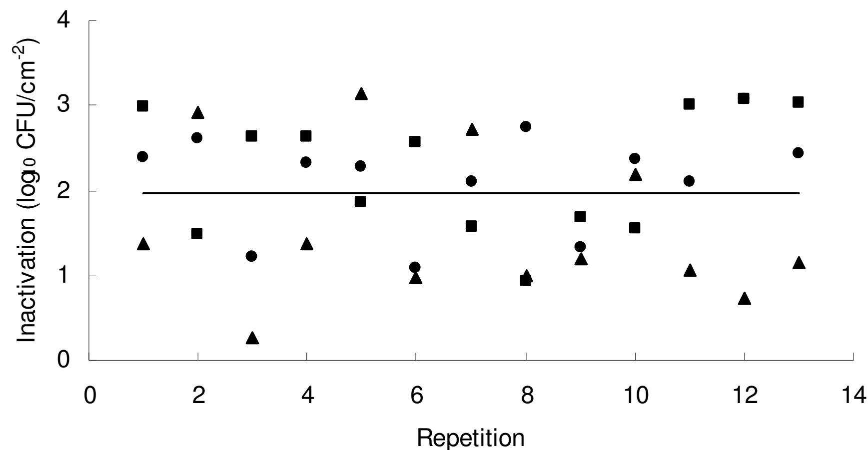

Figure 2.5. Effect of repetitive flashing (50 flashes at 8.4 cm) on the inactivation of one strain of *Listeria monocytogenes*. Equal symbols represent that the cell treated in a specific repetition was isolated from a colony from the previous repetition represented by the same symbol. The trend line was obtained from all the showed data.

2.4.6 Media heating

Due to the presence of visible and infra-red light together with UV light in the spectrum emitted by the lamp, the surface of the product can warm up during flashing, which can be a disadvantage of this decontamination method already mentioned in chapter 1, section 1.5.6.2. The lamp itself produces heat, which can reach the product surface by natural convection. The thermic effects of the

illumination and lamp heating can not be separated in the kind of device used for these experiments. The results presented in Figure 2.6 show clearly that both, the number of flashes and the location of the sensor on a particular depth in the agar influence the temperature increase; the higher the number of flashes and the lower the depth, the higher the measured temperature increase. The increase of the temperature at 10 mm depth was low in comparison with that at 1 mm. At a depth of 10 mm the temperature difference after 990 flashes was 0.8℃, whereas at a depth of a 1 mm this difference amounted 12.2℃. The link between the temperature increase and the depth in the agar is exponential and depends on the number of flashes. Table 2.3 shows the corresponding equations.

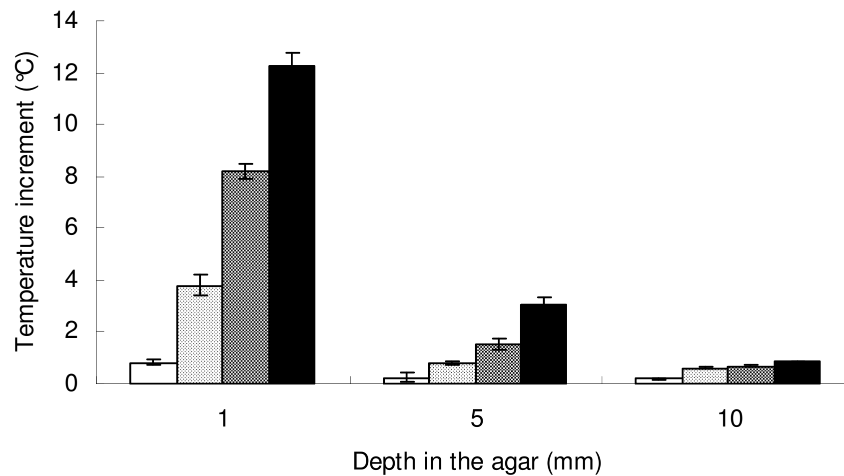

Figure 2.6. Effect of the number of flashes and the depth of the agar on the temperature increment of agars flashed at 8.4 cm from the strobe. Bars indicate ± SD. 50 flashes, ░ 195 flashes, ▓ 495 flashes, ■ 990 flashes.

Table 2.3. Equations and determination coefficients for the relationship between the temperature increment (y, ℃) and the depth of the agar (x, mm) for different number of flashes, at 8.4 cm of distance.

Number of flashes	Equation	R^2
50	$y = 0.80\ x^{-0,66}$	0.99
195	$y = 3.60\ x^{-0,82}$	0.97
495	$y = 8.32\ x^{-1.08}$	0.99
990	$y = 13.42\ x^{-1.11}$	0.96

In the literature, Anderson et al. (2000) investigated the temperature increase during flashing and they reported a negligible rise in temperature after it, although the measuring method was not described, while Krishnamurthy et al. (2004) reported about 20°C increment after flashing phosphate buffer and Baird-Parker agar during 20 s. It had been interesting to measure the temperature increase in the surface of the agar, where microorganisms actually form colonies, but it was impossible to place the sensor at a depth less than 1 mm without breaking the agar. To estimate the temperature increase in the surface of the agar, the equations given in table 2.3 can be used. For example, at 0.001 mm from the agar surface, 50 flashes increase the temperature by 76°C, which will account for the photothermal effect modelled in this article. Nutrient agar is a pale yellow agar medium when solid, according to the spectrum of the light emitted by the lamp (Figure 2.1) no special effect of the colour on the energy absorption by the agar could be expected. Despite of differences can be expected between the temperature increase in agar and specific food surfaces, the warming up of samples should be taken into consideration for industrial applications. ILP has especially potential as a minimal preservation technique, influencing as less as possible the organoleptical and nutritional quality of the treated product. Heating should be controlled in heat-sensitive products, such as minimally processed fruits and vegetables, although it could work additive or synergistically for treatement of other commodities.

2.5 Conclusions

Microorganisms differed in sensitivity to ILP but no pattern was observed among different groups. Differences in the performance of three methods to enumerate survivors with *in vitro* studies were detected. The incubation method seems to overestimate the killing efficacy, and the strike method yielded the most reliable results. Shading effect and photoreactivation accounted for the differences in the performance of enumeration methods. For an industrial implementation: the position and orientation of strobes in an unit will influence the lethality, products should be flashed as soon as possible after contamination occurs, a cooling system should be used for heat sensitive products and flashed products should be light protected. No

resistant flora is expected to develop. Conclusions derived from this work will allow a better implementation of this decontamination technique at industrial level.

Chapter 3 continues the exploration of the application of ILP to foods by using *in vitro* tests, and study the application of ILP to prolong the shelf-life of MPV.

CHAPTER 3

Intense light pulses decontamination of minimally processed vegetables and their shelf-life[3]

[3] *Redrafted from: Gómez-López, V.M., Devlieghere, F., Bonduelle, V., Debevere, J., 2005. Int. J. Food Microbiol. 103:79-89.*

Chapter 3. Intense light pulses decontamination of minimally processed vegetables and their shelf-life

3.1 Abstract

Intense light pulses (ILP) is a new method intended for decontamination of food surfaces by killing microorganisms using short time high frequency pulses of an intense broad spectrum, rich in UV-C light. This work studied in a first step the effect of food components on the killing efficiency of ILP. In a second step, the decontamination of eight minimally processed (MP) vegetables by ILP was evaluated, and thirdly, the effect of this treatment on the shelf-life of MP cabbage and lettuce stored at 7°C under modified atmosphere packaging was assessed by monitoring headspace gas concentrations, microbial populations and sensory attributes. Proteins and oil decreased the decontaminating effect of ILP, whilst carbohydrates and water showed variable results depending on the microorganism. For this reason, high protein and fat containing food products have little potential to be efficiently treated by ILP. Vegetables, on the other hand do not contain high concentrations of both compounds and could therefore be suitable for ILP treatment. For the eight tested MP vegetables, log reductions up to 2.04 were achieved on aerobic plate counts. For the shelf-life studies, respiration rates at 3 kPa O_2 and 7°C were 14.63, 17.89, 9.17 and 16.83 ml O_2/h kg produce for control and treated cabbage, and control and treated lettuce respectively; used packaging configurations prevented anoxic conditions during the storage times. Log reductions of 0.54 and 0.46 for psychrotrophic count were achieved after flashing MP cabbage and lettuce respectively. Psychrotropic count of treated cabbage became equal than that from control at day 2, and higher at day 7, when the tolerance limit (8 log) was reached and the panel detected the presence of unacceptable levels of off-oddours, while control never reached 8 log in total psychrotrophic count and were sensory acceptable until the end of the experiment (9 days). In MP lettuce, psychrotrophic count of controls reached rejectable levels at day 2, whilst that of treated samples did after 3 days. Both samples were sensory unacceptable at day 3, controls because of bad overall visual quality (OVQ), off-odour and leaf edge browning and treated samples due to bad OVQ; browning inhibitors might be proposed to preserve OVQ. Yeasts and lactic acid bacteria counts were low in all the samples. It seems that ILP

treatment alone under the conditions used in this work does not increase MP vegetables shelf-life in spite of the reduction in the initial microbial load.

Keywords: intense light pulses, minimally processing, fresh-cut vegetables, decontamination, shelf-life.

3.2 Introduction

Fresh vegetables have been traditionally prepared for consumption at home or at restaurants by washing, trimming, cutting, peeling and so on immediately or few hours before serving. When industry started to carry out these processes, a new type of product was created, called minimally processed (MP) vegetables. Although between processing and consumption a span of several days occurs, consumers still want to have fresh or fresh-like vegetables on their dishes or pans, in spite of MP vegetables have a shorter shelf-life than their intact counterparts. Processing of vegetables promotes a faster physiological (Brecht, 1995) and microbial (Brackett, 1987) degradation of the product in comparison with the raw commodities, and increases the risk of outbreaks (Alzamora et al., 2000). Industry has to overcome these problems with limited tools since preservation methods should avoid impairing the fresh or fresh-like attributes of the product. In order to slow down a faster physiological degradation, the MP industry can apply modified atmosphere packaging and refrigeration (King et al., 1991). The latter is also necessary to slow down microbial proliferation, although the shift to psychrotrophic microorganisms has to be taking into account. In order to prevent microorganisms from reaching undesirable levels in MP vegetables, contamination should be minimised and initial counts before storage can be decreased by using decontamination treatments, which are limited by their effects on product quality. Washing with chlorinated water has been traditionally applied to decontaminate vegetables, but several reports have questioned its efficacy (see chapter 1, section 1.6.2). Furthermore studies have shown that toxic compounds are generated when chlorine reacts with organic matter (Richardson et al., 1998), including possible carcinogenic compounds such as those mentioned in chapter 1, section 1.6.6. As a consequence, several innovative approaches have been experimented to decontaminate MP vegetables. The bulk of them have been devoted to eliminate pathogens (Beuchat, 2000, Parish et al., 2003), and little research has been performed about spoilage microorganisms and the effect of these methods on the sensory attributes of the MP vegetables and their nutritious value and shelf-life (Li et al., 2001b, Allende and Artés, 2003a,b).

Intense light pulses (ILP) is a novel decontamination method for food surfaces that could be suitable for disinfecting MP vegetables. This technique appears recurrently

in literature reviews (Fine and Gervais, 2003; Señorans et al., 2003; Parish et al. 2003), commented as "having potential for future use" (Alzamora et al., 2000) or "imminent commercial applications" (Ohlsson, 2002), but the literature on this subject is scarce, especially in experiments on food surfaces, and much of the information comes from industry sources, therefore, independently conducted research is needed (FDA, 2000). ILP kills microorganisms using short time high frequency pulses of an intense broad spectrum, rich UV-C light. The mechanism of microorganism inactivation is revised in chapter 1, section 1.5.4. Since the ILP decontamination effect seems to depend on the light absorption by microorganisms, certain food components could also absorbe the effective wavelengths and decrease the efficiency of this treatment.

ILP has been used to successfully inactivate *Escherichia coli* O157:H7 on alfalfa seeds (Sharma and Demirci, 2003a) and *Aspergillus niger* spores on corn meal (Jun et al., 2003). Regarding shelf-life of MP vegetables, Hoornstra et al. (2002) achieved more than 2 log reductions in aerobic counts on selected vegetables, and calculated, without showing experimental data, that a reduction of 2 log almost increases the shelf-life at 7°C of cut vegetables with about 4 days.

Consequently, the present study was designed to (1) study the influence of food components on the decontamination efficiency of ILP, (2) the decontamination effect of ILP on several MP vegetables, and (3) its effect on the shelf-life of two MP vegetables stored under equilibrium modified atmosphere packaging (EMAP) (Jacxsens et al., 1999a) and refrigeration, evaluating the microbial as well as the sensory quality.

3.3 Materials and methods

3.3.1 ILP device

ILP processing was done using the device described in chapter 2, section 2.3.1. The manual mode was used for *in vitro* experiments, and the automatic mode for *in vivo* ones.

3.3.2 Effect of food components on the decontamination efficiency of ILP

3.3.2.1 Media preparation

To examine the influence of several components of foods on the decontamination efficiency of ILP; oil, starch, water and proteins were added to nutrient agar (Oxoid, CM3). In order to test the effect of oil, maize oil (Maïsolie, Derby) was autoclaved separately during 15 minutes at 121°C. After cooling down, the oil was poured at 45°C in the liquid agar medium in which 0.5% Tween 20 was also aseptically added. The mixture was shaken carefully by inversion of the bottle. Then Petri dishes were poured. Plates with concentrations of 0% (control), 1% and 10% of oil were prepared. Water soluble starch (217820, Beckton Dickinson) was chosen as an example of soluble polysaccharides. This ingredient was autoclaved in a concentrated solution, was added to autoclaved agar, shaken and plates were poured. Plates with concentrations of 0% (control), 1% and 10% were prepared. To test the influence of the presence of proteins, casein (WP I, Dairsco, 02/2001) was autoclaved separately in powder form during 30 minutes at 121°C. The autoclaved powder was added to the autoclaved agar medium and after careful stirring a homogeneous mixture was obtained which was poured into plates. To investigate the influence of a humid surface on the decontamination efficiency of ILP, inoculated agar medium was spread with 0.1ml and 1 ml of water, immediately before flashing. Controls were inoculated nutrient agar plates at which no extra compound was added. Experiments were done in triplicate.

3.3.2.2 Inoculation, treatment and enumeration

The prepared Petri dishes with 15 ml of agar medium were spread inoculated with 0.1 ml of the 10-fold dilution of a 24-hour culture. Studied microorganisms were the Gram-positive *Listeria monocytogenes* (LMG 13305), the Gram-negative *Photobacterium phosphoreum* (LMG 4233) and the yeast *Candida lambica* (own isolate from mixed lettuce at 7°C, PR9). *L. monocytogenes* as well as *P. phosphoreum* were cultivated at 30°C in Brain Heart Infusion broth (CM 225, Oxoid) while *C. lambica* was cultivated in Sabouraud liquid medium (CM 147, OXOID) at 30°C.

After one hour drying, the plates were treated with 50 flashes at 8.4 cm from the strobe. The agar was removed from the dishes in a sterile manner in the laminar flow hood and mixed with physiological saline solution making a 10-fold dilution. This blend was mixed thoroughly by means of a Colworth Stomacher 400 (Steward Laboratory, London, UK) and 10-fold dilutions were prepared, plated on Nutrient Agar, incubated during 48h at 30℃, and the individual colonies were afterwards counted. For the experiments with casein, selective media had to be applied, as autoclaving of the casein did not result in a sterile product: for *L. monocytogenes*, Listeria selective agar base (Oxoid, CM 856) with the Listeria selective supplement Oxford medium (Oxoid, SR140E) was used. *P. phosphoreum* was plated on agar to which crystal violet had been added to a concentration of 0.1 mg by 100 ml agar. *C. lambica* was plated on Yeast Glucose Chloramphenicol agar (64104, Sanofi Diagnostics Pasteur, Marnes-La-Coquette, France).

3.3.3 Decontamination effect of ILP on several MP vegetables

3.3.3.1 Processing of the vegetables

Six types of vegetables were bought in a in a local wholesale company where produce are daily freshly delivered (Van Landschoot, Gent, Belgium), stored at 7℃ and processed within one day: spinach (*Spinacia olaracea* L.), celeriac (*Apium graveolens* var. *rapaceum*), green bell pepper (*Capsicum annuum* L.), soybean sprouts (*Glycine max* L.), radicchio (*Cichorium intybus* var. *foliosum* L.), carrot (*Daucus carota* L.), iceberg lettuce (*Lactuca sativa* var. *capitata* L.) and white cabbage (*Brassica oleracea* var. *capitata* L.). Processing depended on the vegetable: spinach, radicchio and lettuce were shredded in 1 cm pieces and peppers were shopped in 2-4 cm x 1-2 cm pieces with a sharp knife; celeriac was manually peeled and then grated in 0.3 x 0.3 x 3-5 cm sticks, peeled carrots were grated in 0.3 x 0.3 x 2 cm sticks and cabbage was shredded in 1 mm thick pieces using a Compacto Kitchen Cutter (Philips, Eindhoven, the Netherlands). Soybean sprouts were used whole. Vegetables were immersed in tap water for 1 min and dried for 1 min by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland). At least 3 units of each vegetable were used to make a pool of each.

3.3.3.2 ILP treatment

For ILP treatment, an amount of sample (see table 3.1) was evenly and aseptically distributed on a sterile 14 x 21 cm tray and placed at 12.8 cm from the strobe. The tray dimension fitted with the size of the window of the lamp holder; below it the illumination must be maximal. Treatments were done in two cycles; in the first cycle, samples were treated during 45 or 180 s, equivalent to 675 and 2700 pulses. Then the vegetables were immediately turned upside down on another sterile tray and a second cycle with the same duration than the first was started. Finally, the produce was immediately sampled under aseptic conditions and total aerobic counts were performed according to 3.3.4.3. Untreated samples were used as controls. Treatments were performed in duplicate.

Table 3.1. Log reductions (CFU/g) achieved in mesophilic aerobic counts after treating minimally processed vegetables by intense light pulses at a distance of 12.8 cm.

Type of fresh-cut produce	Type of cut	Sample size (g)[a]	Time of processing (s/side)[b] 45	180
Spinach	Shredded	15	0.34	0.90
Celeriac	Grated	30	0.21	-
Green paprika	Chopped	80	0.37	0.56
Soybean sprouts	Whole	40	0.65	0.65
Radicchio	Shredded	25	0.66	0.79
Carrot	Grated	30	1.67	1.64
Iceberg lettuce	Shredded	30	1.24	1.97
		19	1.29	2.04
White cabbage	Shredded	30	0.64	0.84
		11	1.03	1.64

[a]Samples very evenly distributed on a 0.14x0.21 m^2 surface. [b]Samples were treated at both sides of a plane.

3.3.4 Effect of ILP on the shelf-life of MP cabbage and MP lettuce

In the present as well as the rest of the shelf-life studies presented in this thesis, the same basic protocol was followed. First, untreated and treated samples were

produced in order to determine the respiration rate of the products. The packaging configurations required to achieve an equilibrium modified atmosphere were then calculated based on the results of the respiration rate tests. Finally, the shelf-life experiment itself was performed with two kinds of samples, untreated and treated, stored in the designed packages.

For ILP experiments, white cabbage or iceberg lettuce were processed, 30 g per treatment, as indicated in 3.3.3.1, flashed 45 s/side according to 3.3.3.2 and immediately packaged using aseptic conditions under EMAP designed according to results previously obtained as indicated in section 3.3.4.1.

3.3.4.1 Respiration rate measurements

The respiration rate of the MP control and flashed cabbage and lettuce was measured by means of a closed method (Mannapperuma and Singh, 1994). Thirty grams of MP vegetables were placed in airtight glass jars (635 ± 11 ml) in triplicate. The jars were flushed with a mixture of 12 kPa O_2, 4 kPa CO_2 and 84 kPa N_2 as initial gas atmosphere by means of a gas packaging unit (gas mixer, WITT M618-3MSO, Gasetechnik, Germany; gas packaging, Multivac A300/42 Hagenmüller KG, Wolfertschwenden, Germany). Air products (Air Liquid, Amsterdam, The Netherlands) supplied the gases. Jars were stored at 7°C and a gas sample was taken periodically through an airtight septum and analysed by gas chromatography (MicroGC M200, columns: molecular sieve 5A PLOT at 35°C and Paraplot Q at 45°C (Agilent, DE, USA)) and helium as gas carrier (Air Liquide, Luik, Belgium). Data were processed according to Jacxsens et al. (1999a) to estimate O_2 consumption at 7°C and 3 kPa O_2. Means were compared with a t-test (SPSS 12.0, SPSS Inc., Chicago, USA), with α=0.05.

3.3.4.2 Packaging of the MP vegetables

MP lettuce and cabbage were packaged in designed bags, then a gas mixture was injected into the bags as initial gas atmosphere by the gas packaging unit described in section 3.3.4.1, in quadruplicate. The applied packaging films (Hyplast N.V., Hoogstraten, Belgium) were experimental films with a high permeability for oxygen at 7°C and 90% relative humidity, and were selected based on their oxygen

permeability. The packaging configurations were designed by using the method validated by Jacxsens et al. (1999a) and shown in table 3.2. Gas samples were periodically taken during the shelf-life experiment and analysed by gas chromatography (see 3.3.4.1) to assure that anoxic conditions were never reached.

Table 3.2. Package design and packaging films, for control and intense light pulses treated minimally processed lettuce and cabbage.

Type of fresh-cut product	MP Iceberg lettuce		MP white cabbage	
	Control	Treated	Control	Treated
Respiration rate[a]	9.17±0.17[d]	16.83±1.33[e]	14.63±2.25[d]	17.89±1.46[d]
Fill weight (Kg)	0.90	0.90	0.90	0.90
Package area (cm^2)[b]	15.5 x 15.5	16.5 x 16.5	16.5 x 16.5	15 x 20
Required permeability for O_2[c]	2290	3538	3562	3542
Applied permeability for O_2[c]	2290	3529	3529	3529

[a] Average ± standard deviation, (ml O_2/(Kg.h)) at 7°C and 3 kPa O_2. [b] Length x width. [c](ml O_2/m^2.24h.atm) at 7°C. [de] Within the same MPV, means with different superscript are statistically different (α≤0.05).

3.3.4.3 Microbiological analysis of spoilage microorganisms

Microbiological counts were done by taking 30 g of sample from one bag and mixing it with 270 ml peptone saline solution (8.5 g/l NaCl (Vel 8605) and 1 g/l peptone (Oxoid, L34)) in a sterile Stomacher bag, and homogenisation for 60 s with the Colworth Stomacher. Tenfold dilution series were made in peptone saline solution for plating. Three bags were sampled for control and treated samples and for each storage day. Specifications published by Debevere (1996) were used to establish the end of the microbial shelf-life, which are: 8 log CFU/g for psychrotrophs, 7 log CFU/g (plus sensory analysis) for LAB and 5 log CFU/g for yeasts.

The following media and incubation conditions were used to enumerate the proliferation of the spoilage microorganisms: Plate Count Agar (Oxoid, CM325) for aerobic plate count (APC), pour plated and incubated at 30°C for 3 days as for total aerobic psychotropic count, but incubated at 22°C for 5 days; de Man-Rogosa-Sharpe medium (Oxoid, CM361) with 0.14% sorbic acid (Sigma, S-1626) for lactic acid bacteria, pour plated, overlaid with the same medium and incubated aerobically at 30°C for 3 days; Yeast Glucose Chloramphenicol agar (Biorad, 64104**)** with 50 mg/l (Tournas et al., 2001) chlortetracycline (Difco, 233331) to enumerate yeasts, spread plated and incubated at 30°C for 3 days.

3.3.4.4 Evaluation of sensory quality

Sensory evaluation was performed the same day of the microbiological analyses by a semi-trained panel of 6 persons, who evaluated specific sensory attributes. Attributes potentially determining the sensory shelf-life of each produce were selected according to results from preliminary experiments and previous reports (Kader et al., 1973, López-Gálvez et al., 1997, Fan et al., 2002, Allende and Artés 2003a, Allende et al., 2004).

After taken samples for microbiological analyses and pH, samples were transferred to closed plastic recipients coded with random numbers. Samples taken from six bags were evaluated, three with treated samples and three with untreated samples. The appraisal was performed in a special room with individual booths. The first part of the evaluation (odour, flavour) was judged under red light; under daylight the rest of the attributes were evaluated. The end of the shelf-life from the sensory point of view was reached when at least one of the mean scores was above the middle point of the respective scale (Botta, 1995).

3.4 Results and discussion

3.4.1 Effect of food components on the decontamination efficiency of ILP

Figure 3.1 reflects the influence of several food components on the decontamination efficiency of ILP. The presence of proteins or oils had a strongly pronounced impact on the decontamination by ILP. Increasing levels of oil and protein reduced the killing

efficiency of ILP for the three tested microorganisms. For example, the obtained decontamination effect of about 1.5 log CFU/cm^2 for *Candida lambica* was totally reduced in the presence of 10% oil or 10% casein. Since proteins have strong absorption at about 280 nm as well as at higher wavelengths of the UV-B region, and lipids with isolated or conjugated double bonds absorb UV (Hollósy, 2002), it is possible that part of the illumination that would have killed microorganisms in these experiments had been absorbed by proteins and oils, decreasing the effective illumination dose on microorganisms. When water or carbohydrates were added to the medium, the conclusions are less coherent. In the presence of water, *C. lambica* was much more sensitive to ILP whereas *P. phosphoreum* showed exactly the opposite behaviour; *L. monocytogenes* was not influenced by water. Mimouni (2000) also reports a better inactivation of *A. niger* on a moist environment than on a drier environment. Starch had no influence on the decontamination of *P. phosphoreum*, whereas *C. lambica* and *L. monocytogenes* became more susceptible to ILP in the presence of starch. It seems that proteinaceous or fatty foods are inappropriate for decontamination by ILP. On the other hand, foods high in carbohydrates but poor in fat and proteins, such as most fruits and vegetables seems to be very suitable for it. Hence, MP vegetables might be good candidates for decontamination by ILP.

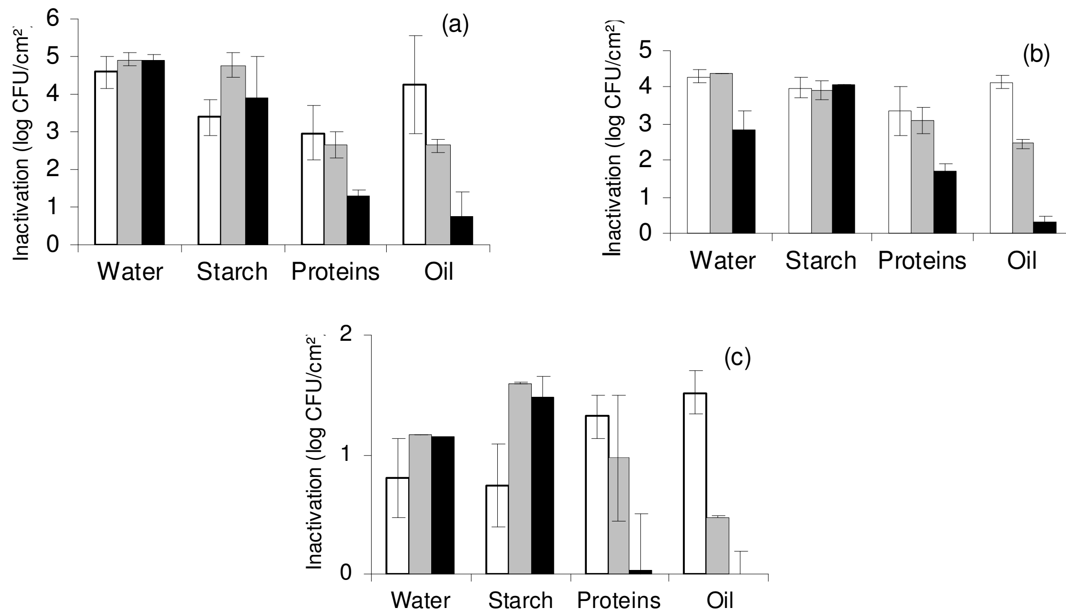

Figure 3.1. Effect of food components on the killing effect of intense light pulses (50 flashes, 8.4 cm from the strobe) over *L. monocytogenes* (a), *P. phosphoreum* (b) and *C. lambica* (c). Water: control □, low level: 0.1 ml ■, high level: 1 ml ■. Starch: control: □, low level: 1% ■, high level: 10% ■ (v/v). Proteins: control: □, low level: 1% ■, high level: 10% ■ (w/v). Oil: control: □, low level: 1% ■, high level: 10% ■ (v/v). Error bars indicate SD.

3.4.2 Decontamination effect of ILP on several MP vegetables

Table 3.1 shows the log reductions achieved after treating several MP vegetables at treatment times up to 45 and 180s/side at 12.8 cm distance from the strobe. Log reductions were between 0.21 and 1.67 at 45 s/side, and between 0.56 and 2.04 at 180 s/side. Slightly higher reductions (from 1.6 for carrots to >2.6 for paprika) were obtained in aerobic counts by Hoornstra et al. (2002) but using only 2 pulses (0.15 Joule/cm^2 per flash). Sharma and Demirci (2003a) achieved a population reduction of *E. coli* O157:H7 of 4.89 log on a 6.25 mm thick layer of alfalfa seeds treated for 90 s (270 pulses) at 8 cm distance, but only 1.42 log reduction at 13 cm; Jun et al. (2003) obtained a log reduction of 2.95 after treating *A. niger* spores inoculated on corn meal up to 100 s at 13 cm distance from the strobe. Marquenie et al. (2003) found no suppression of fungal development by treating *Botrytis cinerea* inoculated on strawberries up to 250 s with equipment similar to that used here. Although differences between experimental conditions advise prudence in making comparisons, it can be concluded that the maximum population reductions obtained in this work are in the usual range for this kind of treatments.

Overcoming sample heating was a problem in these experiments, limiting the processing time and the proximity of the sample to the lamp. Treatments longer than 45 s/side heated samples excessively, hence results obtained for 180 s/side provided in table 3.1 are only indicative of the potential of this technique to decontaminate vegetables. Such a long treatments are not useful to treat samples without a serious impairment of quality unless an effective cooling system could be incorporated to the equipment. Even equipments with blowers such as that used by Jun et al. (2003) can cause sample heating as these authors found that some experimental factor settings resulted in corn meal sample temperatures of 100°C.

The differences in log reduction among samples showed in table 3.1 are difficult to explain, but might be related to different resistances of the natural microbial populations of each vegetable (as demonstrated by Anderson et al. (2000) for several pathogens and spoilage fungi), the location of microorganisms on and into the samples (shadow effect by different structures) and/or protective substances of the specific vegetable. No pattern is observable related to the kind of processing (i.e.

shredded versus grated), shape (foliar versus grated) or sample size. Differences between decontamination results of several vegetables, as reported by Hoornstra et al. (2002), were also difficult to explain.

The high log reduction achieved after treating grated carrots up to 45 s/side was remarkable. However, samples showed obvious signs of dehydration probably due to the absorption of light and consequent heating, plus a possible effect of a dry environment (see revision in chapter 5, section 5.2). After 5 s immersed in water, carrot sticks recovered a fresh-like appearance, but this rehydration step would complicate the industrial application of ILP, and the carrot tissue could have been damaged during dehydration. Therefore, they were disregarded in subsequent experiments. Since relatively high decontamination was observed after treating MP lettuce and MP cabbage (table 3.1), and those MPV are relatively common in the market, they were selected for subsequent studies. However, no criterium exists to predict a prolongation of shelf-life of a MPV as function of the level of decontamination (see chapter 1, section 1.3.3).

A shadow effect was observable when comparing the reduction in contamination between sample sizes of 11 and 19 grams versus 30 g (lettuce and cabbage). Eleven and 19 g corresponded to the maximum number of vegetable pieces possible without overlapping. The 30 g corresponded to a sample size considered practical to the later performed shelf-life studies, given the necessity of processing high amounts of sample using a small capacity equipment. In this case, overlapping occurred although it was minimised.

3.4.3 Effect of ILP on respiration rates of MP vegetables

Table 3.2 shows the respiration rates of control and flashed MP vegetables as well as the package design used for these commodities in shelf-life studies. Measuring respiration rates is fundamental to design the appropriate packaging configuration to reach an EMAP. ILP increased the respiration rate of lettuce more than 80%, but that for cabbage was not significantly affected, revealing interspecific differences in susceptibility. To date, no report is known on the effect of ILP on MPV physiology. Moreover, from these results no distinction can be made between possible

photothermal and photochemical effects. However, since the lamp used in this work is rich in the UV-C part of the spectrum, some similarities may be established between the results found in this thesis and those obtained using continuous UV-C. Research summarized in chapter 1, section 1.4.7 has shown that continuous UV-C can accelerate the respiration rate of MPV. Therefore, it is likely that the increment of lettuce respiration rate after ILP is related to the UV-C part of the used light spectrum. It is known that UV-C affects plant cells, causing damage to DNA, tissue and photosynthetic apparatus among other effects (Stapleton, 1992) that may alter vegetable respiration. By taking into consideration the change in respiration rate after ILP treatment, the packaging configurations used in these experiments allowed that gas concentrations inside packages were out of anoxic conditions during the shelf-life studies.

3.4.4 Microbiological spoilage of the minimally processed vegetables

When samples were processed for shelf-life studies, 0.54 log reductions were achieved in MP white cabbage for psychrotrophic count and 0.46 in MP Iceberg lettuce, reductions on yeasts counts were not statistically significants ($\alpha \leq 0.05$). Lactic acid bacteria (LAB) counts were always below the detection limit of the method (10 CFU/g) in both products. Gram-positive bacteria are uncommon in lettuce. King et al. (1991) reported that 97.3% of their bacterial isolates from lettuce were Gram-negative rods, mainly *Pseudomonas*. Moreover Barriga et al. (1991) explained the poor growth of LAB in refrigerated lettuce by the competition with other populations with higher growth rates at low temperatures and a better adaptation to lettuce. Jacxsens et al. (2004) stated that LAB are not so important in the spoilage of green leafy vegetables, but during storage anoxic conditions can favour their growth. LAB are part of the natural microflora of cabbage (Carr et al., 2002), but they are present in very low levels, and storage conditions may have not favoured their growth.

Psychrotrophic and yeasts counts during the storage of control and flashed MP cabbage are presented in Figure 3.2. At day 2, the benefit of the decontamination achieved by ILP was lost, control and treated samples yielded then the same counts, similar result was observed in chapter 5, section 5.4.6 (ClO_2 treatment of MP lettuce and MP cabbage, but not MP carrots) and chapter 6, section 6.4.4 (NEW treatment

of MP cabbage). From day 7 on flashed samples had the highest psychrotrophic count until the end of the experiment. The possibility that the growth rate of microorganisms in decontaminated vegetables stored at 7℃ is consistently faster than that in non-treated samples deserves careful study because could hamper the application of some decontamination techniques. This finding is in line with the comments expressed in chapter 1, section 1.3.5.1. As an example, Li et al. (2001b) studied the changes in the natural microflora of Iceberg lettuce treated in warm, chlorinated water, and during storage at refrigeration temperatures. In spite of the obtained reductions of 0.34-1.27 logs in the psychrotrophic count following treatments, these counts were higher in treated samples after 4 days storage at 5℃ and 2 days storage at 15℃. It is possible that some decontamination treatments make vegetable tissues become weaker and more susceptible to microbial degradation. Psychrotrophs reached the limit of acceptability (8 log CFU/g) at day 7 in treated samples, whilst controls were acceptable during the 9 days of the experiment; yeasts counts never reached the acceptability limit (5 log CFU/g). Therefore, ILP did not extend the microbial shelf-life of MP cabbage stored under MAP at 7℃.

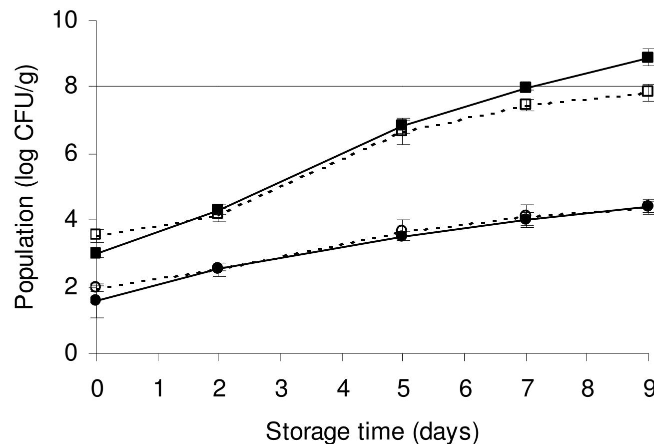

Figure 3.2. Psychrotrophic count and yeasts (log CFU/g ± SD) on untreated and intense light pulses treated (45 s/side, 12.8 cm) MP white cabbage. Control, psychrotrophs (--□--), flashed, psychrotrophs (—■—), control, yeasts (--○--), flashed, yeasts (—●—). Horizontal line indicates the shelf-life limiting number of 10^8 CFU/g for psychrotrophs. Error bars indicate ± SD.

Figure 3.3 shows the psychrotrophic count and yeast counts during the storage of control and flashed MP lettuce. Psychrotrophic count of treated samples kept lower than that for the controls during the 5 days of the experiment, whilst yeasts count of treated lettuce were higher, although still very limited, than that of controls at the end of the experiment. Control samples reached the acceptability limit before or at day 3, while treated ones did after the third day. From the microbiological point of view, gaining one extra day seems to be possible by using ILP, taking also into account that yeasts counts were low.

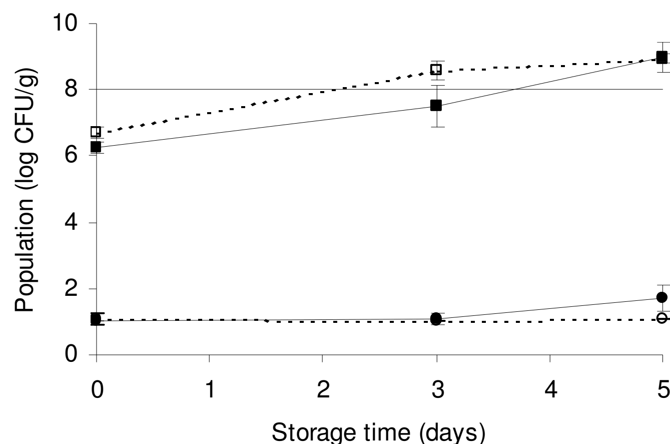

Figure 3.3. Psychrotrophic count and yeasts (log CFU/g ± SD) on untreated and intense light pulses treated (45 s/side, 12.8 cm) MP Iceberg lettuce. Control, psychrotrophs (--□--), flashed, psychrotrophs (—■—), control, yeasts (--○--), flashed, yeasts (—●—). Horizontal line indicates the shelf-life limiting number of 10^8 CFU/g for psychrotrophs. Error bars indicate ± SD.

3.4.5 Sensory quality

The shelf life of perishable food products should always be established by combining the microbial shelf life and the sensory shelf life. Table 3.3 shows the evolution of six relevant sensory attributes of control and flashed MP white cabbage stored on MAP at 7℃ up to 9 days. The presence of off-odours limited the shelf-life of the treated samples to 7 days (score > 3.0), the rest of the tested parameters were always below the rejection limit for both kind of samples up to 9 days. A distinctive off-odour described as "plastic" was obvious immediately after treating samples by ILP, which faded during the next hours according to preliminary studies. For this reason the shelf-life experiment was continued in spite of the early rejection of the treated

samples. The detected off-odour is expected to disappear before the product is consumed, and it was in fact no detected from the second day of storage on. From these data, the shelf-life of the treated product based on sensory quality would be 7 days, equal to the microbiological shelf-life.

Table 3.3. Sensory evaluation of untreated and treated with intense light pulses MP white cabbage stored under MAP at 7°C.

Quality attributes		Time (days)				
		0	2	5	7	9
Off-odour[a]	Control	2.5±1.5	1.8±1.0	2.2±1.1	2.3±1.0	2.1±0.9
	Treated	**3.4±1.6**	2.3±1.2	3.0±1.2	**3.4±1.5**	**3.5±1.3**
Taste[b]	Control	1.6±0.7	1.4±0.5	1.8±0.9	1.7±0.9	2.5±0.7
	Treated	2.1±1.0	1.8±0.6	2.1±1.1	2.7±0.9	2.7±0.8
OVQ[c]	Control	1.0±0.0	1.6±1.0	2.4±0.9	2.9±1.4	4.0±1.4
	Treated	1.0±0.0	1.6±0.9	3.3±1.4	3.8±1.8	4.9±2.2
Sogginess[a]	Control	1.0±0.0	1.3±0.5	1.4±0.5	1.3±0.5	1.4±0.5
	Treated	1.0±0.0	1.3±0.5	1.3±0.5	1.3±0.5	1.3±0.5
Browning[a]	Control	1.0±0.0	1.0±0.0	1.2±0.4	1.4±0.8	2.2±0.8
	Treated	1.0±0.0	1.1±0.3	1.4±0.7	2.2±0.9	2.7±1.5
Dryness[a]	Control	1.1±0.2	1.5±0.5	2.1±0.9	1.8±0.7	2.1±0.8
	Treated	1.1±0.2	1.5±0.5	2.1±0.9	1.7±0.5	2.1±0.9

Average ± S.D. Numbers in bold are scores above the acceptability limit. [a]Off-odour, sogginess, browning and dryness scores: 1=none, 5=severe. [b]Taste score: 1=fresh, 3=acceptable, 5=spoiled. [c]Overall visual quality score: 1=excellent, 5=fair, 9=extremely poor.

The results obtained for the evolution of eight relevant sensory attributes in control and flashed MP Iceberg lettuce stored on MAP at 7°C up to 9 days are provided in table 3.4. In this case, the shelf-life given by sensory characteristics was limited by off-odours, overall visual quality (OVQ) and leaf edge browning for control samples, and only by OVQ for flashed ones, all these parameters reached unacceptable scores at day 3. How much browning accounts for the bad OVQ score was not determined in this work, but it is known that browning is the major cause of quality loss reported in MP lettuce, and leaf surface browning and cut edge browning defects have been demonstrated to contribute with a decreased OVQ in MP lettuce (López-Gálvez et al., 1996). The choice of not using antibrowning agents in this experiment

was taken in view of the possibility that ILP could inactivate polyphenol oxidase (PPO) (Dunn et al., 1989), since it is known that this enzyme is responsible for lettuce browning after tissue injury (Cantos et al., 2001). However, according to obtained results for leaf browning, it seems that no significant PPO inactivation occurred. Therefore, treating MP lettuce with antibrowning agents before ILP flashing can be recommended in order to avoid that this defect limits the shelf-life of the product.

Table 3.4. Sensory evaluation of untreated and treated with intense light pulses MP Iceberg lettuce stored under MAP at 7°C.

Quality attributes		Time (days)		
		0	3	5
Off-odour[a]	Control	1.7±1.1	**3.1±1.3**	**4.3±1.0**
	Treated	2.3±1.3	2. 8±1.4	**3.7±1.1**
Taste[b]	Control	1.3±0.6	2.4±1.1	**3.8±1.2**
	Treated	1.8±1.0	2.2±1.3	2.6±1.4
OVQ[c]	Control	1.5±0.7	**5.8±1.5**	**6.9±0.9**
	Treated	1.6±0.8	**5.5±1.9**	**6.9±1.6**
Sogginess[a]	Control	1.1±0.2	2.2±1.4	**3.5±0.9**
	Treated	1.2±0.4	2.2±1.3	**3.2±1.0**
Leaf edge browning[a]	Control	1.4±0.6	**3.7±1.0**	**3.6±0.7**
	Treated	1.2±0.4	2.9±1.2	**3.3±1.1**
Leaf surface browning[a]	Control	1.1±0.2	2.5±0.8	2.6±1.1
	Treated	1.2±0.4	2.8±0.8	**3.1±1.2**
Translucency[a]	Control	1.3±0.8	2.0±0.9	2.3±0.9
	Treated	1.4±0.8	2.1±0.9	2.0±0.9
Wiltness[a]	Control	1.1±0.3	1.4±0.5	2.4±1.2
	Treated	1.1±0.2	1.4±0.6	2.6±0.8

Average ± S.D. Numbers in bold are scores above the acceptability limit. [a]Off-odour, sogginess, leaf edge browning, leaf surface browning, translucency and wiltness scores: 1=none, 5=severe. [b]Taste score: 1=fresh, 3=acceptable, 5=spoiled. [c]Overall visual quality score: 1=excellent, 5=fair, 9=extremely poor.

3.5 Conclusions

This study gives evidence that proteinaceous or oily foods are inappropriate for decontamination by ILP. On the other hand, foods high in carbohydrates such as

fruits and vegetables seem to be more suitable for it. This study also provides new data about the effect of ILP to decontaminate MP vegetables. An increase of the respiration rate of MP vegetables after ILP treatment as well as shelf-life studies of ILP treated MP vegetables are reported for the first time. ILP did not prolong the shelf-life of MP white cabbage or MP iceberg lettuce. However, from the microbial point of view, one extra storage day at 7℃ was achieved for MP Iceberg lettuce. It is therefore suggested that the application of an antibrowning treatment in combination with ILP would increase the total shelf-life of MP iceberg lettuce.

After testing the possibility of ILP to prolong the shelf-life of MPV, the focus of the study shifted in chapter 4 to the study of chemical disinfection methods to prolong the shelf-life of MPV.

CHAPTER 4

Decontamination of minimally processed vegetables by gaseous and liquid chlorine dioxide and neutral electrolysed oxidising water[4]

[4] *Redrafted from: Gómez-López, V. M., Devlieghere, F., Ragaert, P., Chen, L., Ryckeboer, J., Debevere, J. (Submitted September 2006).*

Chapter 4. Decontamination of minimally processed vegetables by gaseous and liquid chlorine dioxide and neutral electrolysed oxidising water

4.1 Abstract

Decontamination methods can be used to prolong the short shelf-life of minimally processed vegetables (MPV) as long as they do not impair sensory quality. The goal of this research was to test the efficacy of gaseous and aqueous ClO_2, and neutral electrolysed oxidising water (NEW) to decontaminate MPV, and the impact of these methods on sensory quality. Aerobic plate counts (APC) and sensory triangle tests were performed to evaluate the effect of ClO_2 gas or the effect of immersion in different decontaminant solutions for different treatment times. ClO_2 gas at concentrations of 0.4-1.8 mg/l, ca. 90% relative humidity and 10-15 minutes yielded >1 log reduction in minimally processed (MP) Iceberg, Lollo Bionda and Lollo Rosso lettuces, white cabbage and carrots, but not in bell peppers. Moreover it darkened Iceberg lettuce and white cabbage ($\alpha \leq 0.05$). Washing with 20 mg/l of ClO_2 for 20 minutes was ineffective to reduce significantly ($\alpha \leq 0.05$) APC of MP Iceberg lettuce and MP white cabbage, but yielded >1 log reduction in carrots. The longer the treatment time and the higher the ClO_2 concentration, the higher the decontamination effect for MP carrots ($\alpha \leq 0.05$). MP Iceberg lettuce was sensory impaired by washing with 20 mg/l of ClO_2 for 5 minutes ($\alpha \leq 0.05$), but not MP cabbage nor MP carrots. Washing with NEW containing ca. 40 ppm of free chlorine for 20 minutes yielded >1 log reduction in MP Iceberg lettuce, MP white cabbage and MP carrots; in general, the higher the free chlorine concentration the higher the decontamination ($\alpha \leq 0.05$) but the effect of time was not significant ($\alpha \leq 0.05$); a treatment up to 5 minutes did not damage their sensory quality ($\alpha \leq 0.05$). ClO_2 gas and free chlorine depletion was slower in lettuces, suggesting a role of cross cut sections in the degradation of active decontaminant substances. Decontamination with gaseous ClO_2 seems suitable to be tested to prolong the shelf-life of MP Lollo Rosso and MP Lollo Bionda lettuces, and MP carrots; and it might be also applicable to MP Iceberg lettuce and MP white cabbage treated with antibrowning agents. ClO_2 liquid might be recommendable to treat MP carrots, and NEW for MP Iceberg lettuce, MP white cabbage and MP carrots. Gaseous ClO_2 was more effective than liquid ClO_2.

Keywords: minimally processing, decontamination, chlorine dioxide, electrolysed oxidising water.

4.2 Introduction

Minimally processed vegetables (MPV) are a heterogeneous group of commodities prepared and handled by single methods to maintain their living fresh state and nutritional and sensory quality while providing convenience to consumers and ensuring food safety. The processing operations eliminate every inedible part and reduce their several weeks or months shelf-life to a very short shelf-life (Artés and Allende, 2005). The combination of good manufacture practices, chilling storage and modified atmosphere packaging is used to preserve quality. Spoilage of MPV has two components: the action of microorganisms growing in and on the product, and the physiological changes of the product due to wounding and ageing. A decontamination method that could slow down microbial spoilage could be useful for shelf-life extension of MPV as long as it does not impair the sensory quality of the product and it does not increase the rate of physiological deterioration. Therefore, searching novel methods for MPV decontamination has to comply both requisites; it has to be efficient enough to decontaminate but mild enough to not affect sensory quality or accelerate physiological deterioration. Furthermore, food safety should be assured and nutritional quality preserved. Fumigation or washing with ClO_2 and washing with electrolysed oxidising water are among the novel methods potentially useful to decontaminate MPV with the aim to prolong their shelf-life.

ClO_2 is a powerful oxidiser useful to decontaminate MPV when used in solution as well as in its gas stage. Several articles summarized in chapter 1, section 1.8.7 have reported the microbicidal efficacy of gaseous ClO_2, that ClO_2 gas is more effective than the aqueous form, and that uninjured surfaces are easier to decontaminate than injured ones. For example, Han et al. (2000a) found that 1.24 mg/l ClO_2 gas reduced bacterial populations by 6.45 log CFU/g, while the reduction after water washing was 1.5-1.7 log CFU/g. ClO_2 gas can however damage the sensory properties of some MPV, as it was explained in chapter 1, section 1.7.8.3. A treatment with gaseous ClO_2 can induce browning in MP lettuce and MP cabbage (Sy et al., 2005b). The efficacy of liquid ClO_2 to decontaminate vegetables has been tested by different authors, as summarized in chapter 1, section 1.8.6.1.

Electrolysed oxidising water is generated in an electrolytic cell from tap water or a sodium chloride solution pumped into it (Nakajima et al., 2004). As it was already explained (chapter 1, section 1.7.1), two types of electrolytic cells are described. One type has a membrane between the electrodes separating alkaline electrolysed water from acidic electrolysed water, which are produced at different sides of the membrane. Both waters are collected as separated streams. Most of the literature on decontamination with electrolysed water deals with the use of the acidic water. The other kind of cell has no membrane between the electrodes. Therefore the water is collected as a single stream, having a neutral pH; the present work deals with this kind of water, namely neutral electrolysed oxidising water (NEW). Nonetheless, NEW can be also generated using the first kind of electrolytic cell by redirecting a part of the product formed at the anode into the cathode chamber (Deza et al., 2003).

The efficacy of NEW to disinfect fresh-cut vegetables has been reported by Izumi (1999), who found log reductions as high as 1.8 on trimmed spinach leaves compared to a water washed control, and some other authors, as it was reviewed in chapter 1, section 1.7.5.

Since the bulk of the research on decontamination of vegetables by the above mentioned methods has been performed on pathogenic microorganisms and a limited amount of vegetable matrixes with little emphasis in sensory quality, the goal of this research was to assess the decontaminant efficacy of gaseous and liquid ClO_2 and of NEW on the natural spoilage microflora of some MPV as well as their potential deleterious effect from the sensory point of view.

4.3 Materials and methods

4.3.1 Processing of the vegetables

Six types of vegetables were bought in a in a local wholesale company where produce are daily freshly delivered (Van Landschoot, Gent, Belgium), stored at 7°C and processed within one day: carrot (*Daucus carota* L.), Iceberg lettuce (*Lactuca sativa* var. *capitata* L.), Lollo Bionda lettuce (*Lactuca sativa* var. *crispa*), Lollo Rosso lettuce (*Lactuca sativa* var. *crispa*), white cabbage (*Brassica oleracea* var. *capitata*

L.) and green bell pepper (*Capsicum annuum* L.). Lettuces were shredded in 1-2 cm strips with a sharp knife. Carrots, cabbage and bell peppers were processed as indicated in chapter 3, section 3.3.3.1. Vegetables intended to gas treatment were immersed in tap water for 1 min and dried for 1 min by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland). The washing protocol for vegetables intended to dipping treatments is indicated in the correspondent section.

4.3.2 Method for ClO_2 gas treatment for small amounts (SA) of sample

A closed cabinet of 48 litre of capacity made of plastic and glass was used in these experiments. One hundred grams of MPV were placed into the cabinet forming monolayers on perforated shelves and processed at 25±3°C and 90% relative humidity. A thermohygrometer (Digitron 2020R, Devon, England) was used to measure relative humidity and temperature. A 1000 mg/l solution of ClO_2 was prepared by diluting a stock solution (Vernagene, Bolton, UK) from which the concentration had been previously determined by the method indicated below. ClO_2 was separated from the solution by a stripping column at a flow rate of 100 ml of solution/min, and the liberated gas was lead to the cabinet by fans. The stripping column was operated up to 10 min, then the samples were left in the cabinet for 5 min more. Three experiments per MPV were performed. Results are compared to washed but no fumigated samples.

4.3.3 Method for ClO_2 gas treatment for big amounts (BA) of sample

The same assembly described before was used for these experiments, but the ClO_2 gas was supplied in another way to the cabinet. Two kg of MPV were placed into the cabinet whose relative humidity was adjusted at approximately 91% by a flow of wet air. A 1000 mg/l solution of ClO_2 was prepared and warmed up to 48°C, then ClO_2 was stripped from it by air bubbling (4 l/h) and led by the same air stream to the cabinet where perforated plastic pipes together with cabinet tumbling procured an homogeneous contact between the sanitizer gas and MPV pieces. Treatment was performed at 22±2°C and took 10 min, including 30 s of stripping, ClO_2 concentration inside the cabinet was monitored by taking gas samples at regular intervals. When the pre-set treatment time was completed, the cabinet was opened and samples

were taken for the studies. At least two experiments per MPV were performed. Results are compared to washed but not fumigated samples.

4.3.4 Determination of ClO_2 in solution

The concentration of ClO_2 in solution was determined by an iodometric method. 5 ml of ClO_2 solution was mixed with 5 ml of buffered 7% (w/v) KI (Sigma-Aldrich, Steinheim, Germany) solution pH 7, in an Erlenmeyer flask. The mixture was titrated with a 0.01N sodium thiosulfate solution (Aldrich, WI, USA) to a clear colourless endpoint, using soluble starch (Difco, Becton Dickinson, Meylan, France) as indicator.

4.3.5 Determination of ClO_2 in air

To determine the concentration of gaseous ClO_2, samples were taken out of the cabinet by means of an air sampling pump (Gylair 3, Sensydine, England) for up to 15 s. Two impingers were serially placed between the cabinet and the pump, so that the air sample was scrubbed through a buffered 7% (w/v) KI solution pH 7. The contents of the impingers were quantitatively transferred to an Erlenmeyer. Then, 3 ml of 6N HCl (Merck, Darmstadt, Germany) was added. The mixture was titrated with a 0.01N sodium thiosulfate solution to a clear colourless endpoint, using soluble starch as indicator.

4.3.6 Dipping treatments

Washing treatments of MPV were performed by immersing 10 g of sample in 200 ml of each disinfectant solution (1:20 ratio) in a sterile 400 ml Stomacher bag with gentle, continuous agitation using a shaker (OS10, Ika-Werke, Staufen, Germany) at 120 rpm, for 1, 5, 10, and 20 min, at 26±2°C. At the end of the contact time, the treatment liquid was drained off, and the treated samples were immediately diluted with PSS (see microbiological analysis) and analysed for microorganisms. A control with tap water was run up to the same time. Moreover, samples of non-washed MPV were taken for comparison. Three experiments per MPV were performed.

4.3.7 Production of aqueous disinfectants

Aqueous ClO_2 solutions at 5, 10, or 20 mg/l were prepared by diluting a commercial solution (Vernagene, UK) with tap water.

NEW was produced by a bench-top electrolyser (Ecodis 0,20.2-4A/2, Schoten, Belgium) consisting of an electrolytic cell with two anodes and one cathode, without a separating membrane. NEW with different free chlorine concentrations can be produced in this device by regulating the flow rate of incoming water or very diluted salt solution, the current and voltage supplied to the electrodes, and by feeding the cell with different concentrations of salt solutions. For this research, two kinds of NEW´s were used, one with a free chlorine concentration of 4.9-5.1 mg/l (pH 7.7) produced by feeding the cell with tap water at 6 l/h and setting the voltage at 15 V and the current at 0.65 A; the other with a free chlorine concentration of 38-43 mg/l (pH 8.3) produced by feeding the cell with a 0.05% sodium chloride (VWR, Fontenay sous Bois, France) solution at 6 l/h and setting the voltage at 15 V and the current at 1.70 A. Tap water used as control contained 0.05-0.09 mg/l free chlorine and had pH 7.3.

4.3.8 Free chlorine measurement

Free chlorine concentration in NEW was determined according to the DPD method by using a kit HI9133 and corresponding photometer for free and total chlorine measurements (HANNA instruments Inc., Woonsocket, RI, USA).

4.3.9 Aerobic plate count

The methods described in chapter 3, section 3.3.4.3 were used for total aerobic plate count. Samples were taken and mixed with PSS as fast as possible to dilute the remaining disinfectants. The standard size of sample (30 g) was used except for the experiments where MPV were treated in Stomacher bags, where the complete 10 g of sample were taken.

4.3.10 Sensory analysis

Triangle tests were conducted in order to detect differences due to treatments, using 14 experienced panellists (Botta, 1995). The SA method was used to treat MPV in order to detect potential deleterious effects caused by gaseous ClO_2. In order to assess the effect of liquid ClO_2, MPV were treated for 5 min with 20.0 mg/l of ClO_2; for NEW, the conditions 5 min of exposure and 40 mg/l free chlorine were used. These conditions were selected because they allowed decontamination within a relatively short treatment time. Washing treatments were performed by immersing 80 g of MPV in 1.6 litre liquid (1:20 ratio) in a bowl moved by an orbital shaker operated at 120 rpm, then spun dried by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland) for up to 15 s and stored at 7°C for 3-4 hours before evaluation.

4.3.11 Statistical analysis

Data were analysed for mean differences among different treatment conditions for each MPV with the Duncan's test and Brown-Forsythe's test (for non homocedastic groups) by using the statistics program SPSS 12.0 (SPSS Inc., Chicago, USA), with α=0.05. Significant differences in triangle tests were established according to Botta (1995) with α=0.05.

4.4 Results and discussion

4.4.1 ClO_2 gas treatment

Two set-ups were used to study the effect of gaseous ClO_2 on MPV, one to treat small amounts (SA) of samples, and the other for bigger amounts (BA) enough to perform future shelf-life studies. To fumigate surfaces, the contact between the surface and the gas is essential. The SA method was used to perform the first exploratory experiments, in which it was possible to place a monolayer of MPV to ensure surface-gas contact. However, cabinet tumbling was necessary to treat bigger amounts of sample, enough to perform future shelf-life studies. That made impossible to use the stripping column, which can not be subject to tumbling. Consequently, the BA method was also implemented. Furthermore, because the microbicidal efficacy of gaseous ClO_2 increases with the relative humidity, high

relative humiditys were used in both cases (Jeng and Woodworth, 1990; Han et al., 1999, 2001a).

Both, the SA (table 4.1) and the BA (table 4.2) methods yielded >1 log reduction on APC of different MPV except for green bell pepper, as compared to water washing. There is no information available on the minimum logarithmic reduction that can result in shelf-life prolongation (see chapter 1, section 1.3.3), as it was discussed in chapter 1, section 1.3.3. Since most of the aqueous decontamination treatments revised in chapter 1 reduce the spoilage microflora of a MPV by about one log, a treatment capable to produce 1 log reduction might be promising.

Table 4.1. Effect of gaseous ClO_2 treatment for small sample sizes (100 g) for 15 min on the aerobic plate count (log CFU/g) of several MPV.

MPV	[ClO_2] (mg/l)	% RH	T (℃)	Log reduction*
Iceberg lettuce	0.62±0.08	90.6±0.2	27.8±0.6	1.02±0.70
Lollo Bionda lettuce	0.37±0.13	90.1±0.3	24.8±0.3	1.42±0.45
Lollo Rosso lettuce	0.52±0.05	90.2±0.4	22.6±0.2	1.49±0.29
White cabbage	0.41±0.03	90.8±0.7	22.3±0.1	1.37±0.32
Green bell pepper	0.61±0.02	90.0±0.3	27.1±0.1	0.75±0.41

Results are means **± SD of three experiments.** * Log reduction = log (count of water washed sample) – log (count of fumigated sample).

Table 4.2. Effect of gaseous ClO_2 treatment for big sample sizes (2 Kg) for 10 min on the aerobic plate count (log CFU/g ± SD) of several MPV.

MPV	n[1]	Peak [ClO_2] (mg/l)	% RH	T (℃)	Log reduction[2]
Iceberg lettuce	4	1.82±0.53	90.6±0.1	20.7±1.4	1.11±0.27
Lollo Rosso lettuce	2	1.11±0.94	90.7±0.4	19.5±0.1	1.33±0.50
White cabbage	3	1.64±0.39	90.6±1.6	21.6±2.4	1.06±0.25
Carrots	4	1.32±0.18	92.1±1.3	22.9±3.5	1.34±0.44

[1] Number of experiments. [2] Log reduction = log (count of water washed sample) – log (count of fumigated sample).

Results are reported taking into consideration the sample size treated by each method because of the importance of the ratio total amount of ClO_2/sample size,

which determines the decontamination efficacy and ClO_2 consumption in a given treatment. In table 4.2, peak ClO_2 concentrations are given as reference point, the actual values monitored through treatment time are given in Figure 4.1. It can be observed that MP carrots and MP cabbage consumed ClO_2 before the programmed 10 minutes of treatment which was not the case for MP lettuces, possibly because of their smaller area of cut surface in comparison with those of MP cabbage and MP carrots. It seems that the degradation of ClO_2 by cut fresh tissue is faster than that by intact tissue, as it will be discussed later. It is known that ClO_2 is unstable and it is also absorbed by plastic and glass, which are the material used to make the treatment cabinet. Tests ran with the empty treatment cabinet showed that these effects were negligible for up to 10 min, therefore the observed decrease can be only accounted to degradation by MPV. From the practical point of view, a complete consumption of ClO_2 is desirable to avoid the necessity of implementing exhausting systems and scrubbers in order to not release this gas to the environment, which can be accomplished by using appropriate time-concentration combinations.

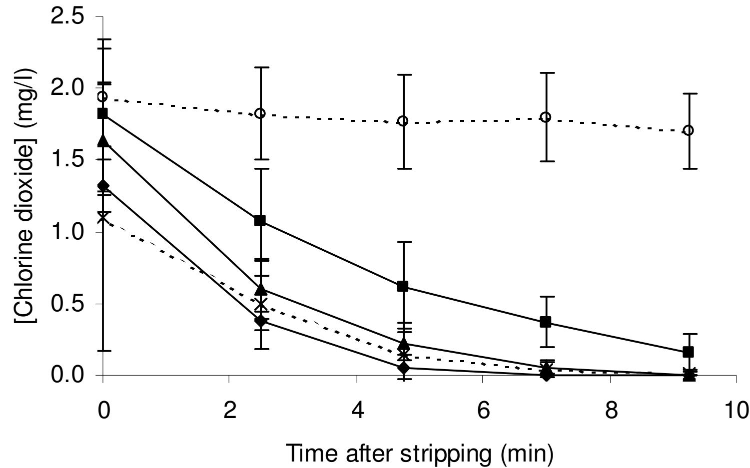

Figure 4.1. Concentration of ClO_2 gas during the treatment of four MPV by the method for big sample sizes. Empty chamber (--o--), MP carrots (—◆—), MP cabbage (—▲—), MP Iceberg lettuce (—■—), MP Lollo Rosso lettuce (--x--). Points represent means of three experiments, and bars indicate ± SD.

The levels of decontamination achieved in these experiments suggest that ClO_2 gas could be a good alternative to decontaminate MP lettuces, MP cabbage and MP carrots. However, the method has also some important drawbacks. In order for a

decontamination treatment to be potentially successful in prolonging the shelf-life of a MPV, it should not only decrease the spoilage microflora of the MPV but it should also not impair its sensory quality. Subjective examination of MP cabbage and MP lettuce revealed browning after treatment, moreover MP lettuce lost green colour. Results of triangle tests have shown that the differences between untreated and treated samples can be objectively perceived (table 4.3). Moreover, preliminary experiments have shown that after applying drastic treatment conditions, ClO_2 gas treatment provokes browning of white asparagus, bleaching of radicchio leaves, and white spots appeared on Lollo Rosso and Lollo Bionda leaves; likewise the green area of trimmed lettuce leaves retracted in direction from bottom to apex. Similar results regarding MP cabbage and MP lettuce browning after treatment with ClO_2 have also been reported by Sy et al. (2005b), and the MP lettuce decolourisation has been reported by Singh et al. (2002b) as well, who suggested that the oxidation of chlorophyll can account for this defect. Since phenol oxidation by ClO_2 has been reported (Napolitano et al., 2005), MP cabbage and MP lettuce browning may be due to oxidation of phenols. Therefore it is advisable to test the suitability of antibrowning agents, such as ascorbate salts or L-cysteine, which could minimise this deleterious effect of ClO_2. That possibility was tested in chapter 5. Another alternative consists in testing different ClO_2 concentration-time combinations, which could help to find a suitable level of decontamination without impairing sensory properties.

Table 4.3. Results of triangle tests on the effect of different decontamination treatments on the sensory quality of several MPV. n=14.

MPV	ClO_2 gas	ClO_2 liquid	NEW
Iceberg lettuce	+ [a]	+	–
White cabbage	+	–	–
Carrots	[b]	–	–
Lollo Bionda lettuce	– [a]	[b]	[b]
Lollo Rosso lettuce	–	[b]	[b]
Green paprika	–	[b]	[b]

[a] Panel detected (+) or did not detect (-) statistically significant differences ($\alpha \leq 0.05$) between untreated and treated samples. [b] The corresponding MPV was not treated by the matching method.

4.4.2 ClO_2 liquid treatment

Table 4.4 shows the results of the decontamination of three MPV by ClO_2 liquid at different concentrations and contact times with respect to raw material. No significant differences ($\alpha \leq 0.05$) were observed in the results for MP lettuce and MP cabbage in spite of the high logarithmic reductions achieved in some conditions such as 20 mg/l of ClO_2 and 20 minutes. For both MPV the variability of the results was in some cases very high, therefore possibly hampering an industrial application because in some cases the decontamination would be high and in others too low. Moreover, the respective controls with water washing exhibited relatively high reductions, therefore most of the decontamination could be accounted to the washing itself but not to a biocide effect of the ClO_2.

Table 4.4. Logarithmic reduction (log CFU/g) after washing several MPV with aqueous chlorine dioxide with respect to raw material*. Results are means ± SD of three experiments.

		Time (min)			
MPV	[ClO_2] (mg/l)	1	5	10	20
Iceberg lettuce[j]	0	0.35±0.39	0.18±0.13	0.08±0.32	0.32±0.13
	5	0.13±0.04	0.58±0.13	0.23±0.24	0.57±0.19
	10	0.28±0.12	0.11±0.42	0.49±0.25	0.67±0.32
	20	0.17±0.22	0.50±0.44	0.72±0.28	0.78±0.66
White cabbage[j]	0	0.46±0.39	0.48±0.13	0.77±0.26	0.83±0.12
	5	0.83±0.14	1.08±0.18	1.14±0.28	1.01±0.09
	10	0.58±0.20	0.95±0.44	1.11±0.45	1.12±0.23
	20	0.77±0.58	1.39±0.65	1.29±0.70	1.28±0.44
Carrots	0	0.49±0.08[a]	0.53±0.23[ab]	0.72±0.28[abc]	0.77±0.06[abc]
	5	0.92±0.20[cd]	1.11±0.14[de]	1.17±0.09[de]	1.12±0.19[de]
	10	0.86±0.18[bcd]	1.13±0.19[de]	1.39±0.18[efg]	1.37±0.16[ef]
	20	1.34±0.09[e]	1.69±0.24[fgh]	1.71±0.21[gh]	1.89±0.24[h]

* Raw material counts: 5.67±0.09 log CFU/g for lettuce, 4.40±0.32 log CFU/g for cabbage and 4.95±0.52 log CFU/g for carrots. [a-i] For all time-concentration combinations within MP carrots, values sharing a common letter are not significantly different ($\alpha \leq 0.05$) according to the Duncan's test. [j] For all time-concentration combinations within each MPV, means are not significantly different ($\alpha \leq 0.05$) according to the Brown-Forsythe's test.

For MP carrots, time and ClO_2 concentration effects were statistically significant ($\alpha \leq 0.05$), the longer the treatment time and the higher the ClO_2 concentration, the higher the decontamination effect. The interaction of these two factors was not significant. The differences were not significant when comparing reductions due to treatment with ClO_2 levels of 5 and 10 mg/l for up the same times, but they were when comparing those ClO_2 levels and water washing or 20 mg/l for up to respective times. When results are analysed with respect to the decontamination achieved by using water washing up to respective times, at least one logarithmic reduction can be accounted to the biocide effect of the ClO_2 at concentrations of 20 mg/l and times equal or higher than five minutes.

Singh et al. (2002b) used a similar experimental set-up than that used in the present work to wash MP Romaine lettuce and baby carrots with 20 mg/l of free chlorine for 15 minutes, reporting respectively 1.72 and 2.54 log reductions of inoculated *E. coli* O157:H7 compared to the initial population, and respectively 0.78 and 1.39 log reductions compared to the counts obtained after washing with sterile deionised water. Those results are comparable to those reported hereby.

A triangle test (table 4.3) showed that samples of MP carrots treated with 20 mg/l of ClO_2 for up to 5 minutes could not be differentiated by the panel from samples washed with tap water for up to the same time ($\alpha \leq 0.05$), the same result was found for MP cabbage but not for MP lettuce. Rodgers et al. (2004) however reported no detectable differences when MP lettuce was washed with ClO_2 for 5 min, but using only a concentration of 5 ppm.

These results show that this treatment can decrease the microbial load without damaging sensory attributes when applied to MP carrots. Yet, washing carrot pieces can result in important losses of nutrients due to leaching. Therefore, an evaluation of the impact of this kind of treatment on the nutritional quality of shredded carrots is strongly advised. It could also be possible to test other kind of carrot cuts, which although not so common in the market as grates, could be less exposed to leaching.
As it was mentioned before, Han et al. (2001b) demonstrated that gaseous ClO_2 is more efficient than the liquid form to decontaminate vegetable surfaces. However,

from the practical point of view, the implementation of a fumigation system in an industrial facility is more complicated than changing the sanitizer applied to wash water. Taking this into consideration, ClO_2 liquid might be a good alternative as long as it could be suitable for some MPV.

Furthermore, the efficacy of gaseous and aqueous ClO_2 can be compared based on data of tables 4.2 (gaseous) and 4.4 (liquid), taking into account some considerations. Gaseous ClO_2 treatment was performed during 10 min at concentrations lower than 2 mg/l. Therefore, the results of 10 min treatment time at an aqueous ClO_2 concentration of 5 mg/l are comparable to those of the fumigation. Also, the log reductions reported in table 4.2 were calculated with repect to water washed samples, while those from table 4.4 were computed with respect to raw material. The results with respect to water washed (corresponding to 0 mg ClO_2/l) are 0.15, 0.37, and 0.45 log CFU/g for MP lettuce, cabbage and carrots respectively. Although the applied ClO_2 gas concentrations were lower than those applied in solution, its decontamination efficacy was at least 3 times higher than those attained in solution. As conclusion, gaseous ClO_2 was more effective than liquid ClO_2 to decontaminate MP Iceberg lettuce, MP white cabbage, and MP carrots. These results are in line with those reported for green bell pepper by Han et al. (2001b).

4.4.3 NEW treatment

The results of the treatment of three MPV with NEW with different free chlorine concentrations for different washing times are shown in table 4.5. There were found statistically significant differences ($\alpha \leq 0.05$) for the three MPV. The effect of the free chlorine concentration was significant but not that of the time. In general, the higher the free chlorine concentration the higher the decontamination. However, in the specific case of MP lettuce, similar reductions were reached using 4.9 and 39 ppm free chlorine. It is possible that the highest feasible decontamination of MP lettuce by this method was already reached using NEW with just 4.9 ppm free chlorine. Also Izumi (1999) found that a treatment with NEW containing 15 ppm of free chorine decreased significantly the aerobic plate count of trimmed spinach leaves, but 30 and 50 ppm of free chlorine did not increase the decontamination significantly, moreover,

no significant effect was reported on the aerobic plate count of carrot slices and cucumber slices treated at 15 and 50 ppm free chlorine.

Table 4.5. Logarithmic reduction (log CFU/g) after washing several MPV with neutral electrolysed oxidising water with respect to raw material*. Results are means ± SD of three experiments.

MPV	[Free chlorine] (ppm)	Time (min) 1	5	10	20
Iceberg lettuce	0.09[1]	0.14±0.29[a]	0.50±0.23[abc]	0.22±0.42[ab]	0.29±0.45[ab]
	4.9[2]	0.88±0.34[bc]	1.12±0.27[c]	0.57±0.18[abc]	0.92±0.25[bc]
	39[3]	0.90±0.10[bc]	0.67±0.44[abc]	1.05±0.35[c]	1.05±0.70[c]
White cabbage	0.06[1]	0.36±0.05[a]	0.51±0.15[ab]	0.41±0.10[ab]	0.43±0.17[ab]
	5.0[2]	0.41±0.13[ab]	0.55±0.08[ab]	0.43±0.30[ab]	0.54±0.19[ab]
	38[3]	0.54±0.21[ab]	0.78±0.25[bc]	0.96±0.23[cd]	1.16±0.41[d]
Carrots	0.05[1]	0.39±0.05[a]	0.47±0.08[ab]	0.56±0.27[ab]	0.57±0.33[abc]
	5.1[2]	0.30±0.14[a]	0.26±0.35a[a]	0.37±0.26[a]	0.60±0.08[abc]
	43[3]	0.71±0.13[abc]	0.94±0.47[bcd]	1.08±0.10[cd]	1.23±0.06[d]

* Raw material count: 5.71±1.17 log CFU/g for lettuce, 4.60±0.17 log CFU/g for cabbage and 5.02±0.79 log CFU/g for carrots. [1] Tap water, [2] NEW produced from tap water, [3] NEW produced from a 0.05% NaCl solution. [a-d] For all time-concentration combinations within each MPV, values sharing a common letter are not significantly different (α≤0.05).

There was a markedly slower depletion of free chlorine (Figure 4.2) in the NEW used to treat MP lettuce in comparison with the sharp decline observed after one minute of treatment of MP cabbage or MP carrots. From the point of view of an industrial application, that means that NEW can be used to decontaminate relatively large amounts of MP lettuce without losing decontamination capacity. The larger cut area of MP cabbage and MP carrots in comparison with that of MP lettuce could explain this difference. Oomori et al. (2000) reported that fine strips of chopped cabbage consumed 50 ppm free chlorine of acidic electrolysed water within 10 minutes, while a level above 40 ppm was maintained when the same experiment was carried out using larger strips. Interestingly, the ClO_2 depletion (Figure 4.1) was also slower in Iceberg lettuce in comparison with the rest of the tested MPV, which suggests a similar role of the cut surface on ClO_2 gas degradation.

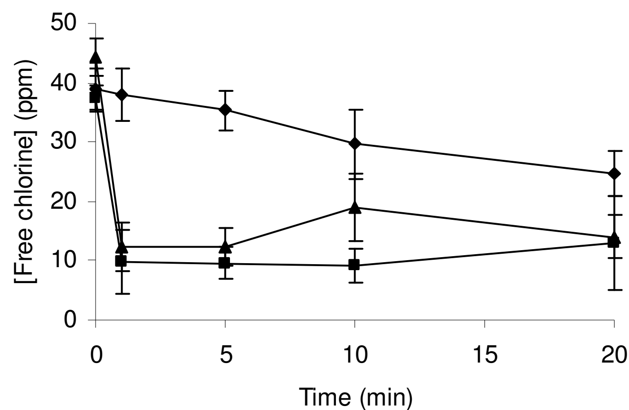

Figure 4.2. Concentration of free chlorine after treating several minimally processed vegetables with neutral electrolysed oxidising water originally containing 38-43 ppm of free chlorine MP carrots (—▲—), MP lettuce (—◆—), MP cabbage (—■—). Bars indicate ± SD.

The decontamination levels found in this work agree with those reported by Izumi (1999), although there are differences in the experimental set-ups. That author rinsed five types of MPV with tap water (control) or NEW containing 20 ppm free chlorine for four minutes and reported between 0.2 and 0.4 log reduction except for spinach, whose reduction was 1.8. These results are similar to those obtained in this work (0.2-0.8) when dipping the MPV for one minute in NEW containing 38-43 ppm free chlorine. However, it is difficult to make comparisons when the variability among similar experiments is high. For example, also Izumi (1999) reported data from two separate experiments. In both cases the author rinsed carrot slices with NEW with 50 ppm free chlorine for four minutes, using tap water as control. One of the experiments yielded 0.3 log reduction with no statistical difference and the other a statistically significant 1.0 log reduction, both at $\alpha \leq 0.05$.

Although in the present work more than one logarithmic reduction was attained at a free chlorine concentration of 38-43 mg/l, part of this decontamination can be accounted to a simple water washing. When compared to water washing, the highest logarithmic reductions were between 0.7 and 0.8 for the different MPV, still good enough taking into consideration the simplicity of the production of the NEW.

The results of the sensory evaluation (table 4.3) show that the panel was unable to detect differences between water washed MPV and samples washed with NEW with 30 mg/l of free chlorine ($\alpha \leq 0.05$), for 5 minutes, therefore NEW does not impair the

sensory quality of these foods. A strong off-odour was detected after washing MP cabbage with NEW, but it disappeared before the sensory evaluation session (3-4 hours), which is short enough for an industrial application.

In general, it seems that the simple production of NEW from tap water could be enough to decontaminate MP lettuce but not enough to decontaminate MP cabbage and MP carrots, making it necessary to add minimal amounts of sodium chloride to water in order to achieve good levels of decontamination.

In the three tested methods, the highest decontamination tended to be in carrot pieces. It has been proven that biofilms (Norwood and Gilmour, 2000), attachment (Liao and Sapers, 2000) or internalisation (Takeuchi and Frank, 2000) protect microorganisms from the action of sanitizers, all these processes require time. The microbial flora of MPV may reflect that of the vegetable in the growing field as well as contamination during processing (Garg et al., 1990). Field contamination is reduced by the peeling step that is giving to carrots (Garg et al., 1990) therefore, a substantial amount of MP carrots microflora would likely come mainly from processing and would consist in microorganisms that have not had time enough to attach or to form biofilms, therefore, MP carrots are easier to decontaminate. Moreover, microorganisms that could have been internalised into the carrot will get exposed after grating.

4.5 Conclusions

In conclusion, fumigation with ClO_2 seems suitable to be tested to prolong the shelf-life of Lollo Rosso and Lollo Bionda lettuces, and carrots, and could be also applicable to Iceberg lettuce and white cabbage if finding a suitable antibrowning agent. ClO_2 liquid could be tested in carrots. When comparing gaseous and aqueous ClO_2, the first was more effective in reducing APC of MP Iceberg lettuce, white cabbage and carrots, since lower concentrations applied during 10 min attained higher log reductions. With regards to NEW, it could be tested in Iceberg lettuce, white cabbage and carrots. Since a reduction on initial counts does not necessarily lead to a prolongation of the shelf-life of MPV, it is necessary to perform storage studies to test if these methods can indeed prolong the shelf-life of MPV.

In this chapter the decontaminant efficacy of gaseous ClO_2 was shown, as well as its capability to induce the browning of MP lettuce and MP cabbage. Chapter 5 includes results of experiments performed with the aim to prolong the shelf-life of MPV by using gaseous ClO_2. Additionally, a way to inhibit browning is explored.

This chapter also shows the decontaminant efficacy of NEW. Chapter 6 includes results of experiments performed with the aim to prolong the shelf-life of MPV by using NEW.

CHAPTER 5

Shelf-life extension of minimally processed vegetables by gaseous chlorine dioxide[5]

[5] *Redrafted from: Gómez-López, V.M., Devlieghere, F., Ragaert, P., Debevere, J. Int. J. Food Microbiol. (Accepted); and Gómez-López, V.M., Ragaert, P., Jeyachchandran, V., Debevere, J. Devlieghere, F.*

Chapter 5. Shelf-life extension of minimally processed vegetables by gaseous chlorine dioxide

5.1 Abstract

Gaseous ClO_2 was evaluated for effectiveness in prolonging the shelf-life of minimally processed (MP) carrots, lettuce and white cabbage. Carrots were peeled, grated, washed, spun dried and separated in two portions, one to be treated with ClO_2 gas and the other to remain untreated for comparisons. Lettuce and cabbage were shredded, washed, and separated in two batches; the batch to be decontaminated was immersed in a 0.5% L-cysteine solution, the other was not. Then both batches were spun dried. The decontamination of MP carrots, MP lettuce and MP cabbage was independently performed in a cabinet at 90-91% relative humidity and 22-28°C for up to 10 min, at maximal registered ClO_2 concentrations of 1.33, 1.74 and 1.29 mg/l for MP carrots, MP lettuce and MP cabbage respectively. Untreated and treated samples were then stored under modified atmosphere packaging at 7°C. Changes in O_2 and CO_2 headspace concentrations, microbiological and sensorial quality, and pH were followed during storage. ClO_2 affected the respiration rate of MP cabbage significantly ($\alpha \leq 0.05$), and decreased the pH of MP carrots and MP cabbage significantly ($\alpha \leq 0.05$). The decontamination levels (log CFU/g) achieved with ClO_2 for MP carrots were 1.88, 1.71 and 0.66 for aerobic plate count (APC), psychrotrophs and yeasts respectively. The respective reductions for MP lettuce and MP cabbage were 0.84, 0.88 and 0.64; and 0.25, 0.27 and 0.46. ClO_2 treatment did not cause initially any significant ($\alpha \leq 0.05$) alteration of the sensory attributes, except for off-odour to MP lettuce. No browning was observed after treating MP lettuce and MP cabbage, which might be accounted to the pre-treatment with L-cysteine. O_2 and CO_2 concentrations during storage kept within a safe range. For MP carrots, a lag phase of at least 2 days was observed for APC, psychrotrophs, and lactic acid bacteria only in treated samples. After that APC and psychrotroph count increased parallelly in untreated and treated products; no sensorial parameter was a shelf-life limiting factor. The shelf-life extension was limited to one day due to the restricted effect of the ClO_2 treatment on yeast counts. For MP lettuce and MP cabbage, APC and psychrotroph counts reached in treated samples higher levels than in the reference ones before the third day. Reference and treated samples of

MP lettuce were sensorially unacceptable after 4 days, while MP cabbage remained sensorially acceptable during at least 9 days. ClO_2 failed to prolong the shelf-life of MP lettuce and MP cabbage. Nevertheless, it might be promising to prolong the shelf-life of grated carrots. The results also proved that it is possible to arrest browning caused by ClO_2. Since gaseous ClO_2 has been previously recommended to produce safer MP cabbage, its use together with a browning inhibitor might be feasible for industrial implementation.

Keywords: chlorine dioxide, minimal processing, fresh-cut, decontamination, shelf-life.

5.2 Introduction

In order for a disinfectant to kill microorganisms, a good contact between the disinfectant and the microorganisms should be guaranteed. Most of the research on the efficacy of disinfectants has been performed with bacteria freshly inoculated onto surfaces, where the contact with disinfectants is relatively easy. As it was however discussed in chapter 1, section 1.3.1, microorganisms can also be located in irregularities of the vegetable surface (Adams, 1989), underneath it (Takeuchi and Frank, 2000, 2001), strongly attached (Liao and Sapers, 2000) or forming biofilms (Fett and Cooke, 2005); which make the activity of disinfectants more difficult.

Chlorine dioxide (ClO_2) is a strong oxidizing and sanitizing agent that has a broad and high biocidal effectiveness (Beuchat, 1998). Because gas has greater penetration ability than liquid, ClO_2 gas may be more effective for surface sanitation than aqueous ClO_2 (Han et al., 2001b) or other aqueous sanitizers.

Studies on the efficacy of gaseous ClO_2 to inactivate microorganisms inoculated onto fruit and vegetable surfaces have been focused on pathogens such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Typhimurium (Han et al., 2000b, 2004; Singh et al., 2002b; Du et al., 2002, 2003; Lee et al., 2004). Comparatively few studies have been devoted to the effect of ClO_2 gas on the spoilage microflora (see chapter 1, section 1.8.8.2) or the sensory properties of fruit and vegetables (see chapter 1, section 1.8.8.3).

Several authors have reported detrimental effects of gaseous ClO_2 on the sensory characteristics of fruits and vegetables, which depend on the treatment conditions. Apart from the reports revised in chapter 1, section 1.8.8.3, which is devoted to MPV, other reports have demonstrated the deleterious effects that gaseous ClO_2 can have on fruits and vegetables. Bleaching has been reported in potato skin (Tsai et al., 2001), green leaves of strawberry caps (Han et al., 2004), and strawberries (Sy et al., 2005a). Sapers et al. (2003) found darkening of apple lenticels, and Lee et al. (2006) observed the development of small black spots on the skin of whole apples during

storage trials. Effects on the appearance and colour of whole apples and peaches have been reported by Sy et al. (2005b).

MP carrots constitute one of the major minimally processed vegetables (MPV); the main problems that limit their shelf-life are white blush discoloration caused by tissue dehydration, and microbial spoilage (Emmambux and Minaar, 2003). Whitening was observed by Sy et al. (2005b) immediately after gaseous ClO_2 treatment of MP carrots, possibly due to the use of a comparatively low relative humidity in the treatment chamber. Therefore, a treatment that could inactivate their natural microflora keeping the tissue hydrated seems appropriate to prolong their shelf-life; hence ClO_2 might be a good alternative when used under conditions that achieve decontamination without impairing sensory attributes.

Although it was already mentioned in chapter 1, section 1.8.8.3, it is worth mentioning here again, the browning of MP lettuce and MP cabbage after ClO_2 treatment reported by Sy et al. (2005b), which was also observed in chapter 4, section 4.4.1. The discolouration resembled to that caused by polyphenol oxidase (PPO). The oxidation of phenols by ClO_2 has been reported (Fukuyama et al., 1986; Napolitano et al., 2005). Therefore, browning observed in those MPV possibly follows the same pattern than that characteristic of enzymatic browning, leading to formation of melanoidines. However, in this case the oxidant would be ClO_2 instead of O_2 and no PPO catalysis would be present. Consequently, browning could be suppressed by a compound used to arrest enzymatic browning not by acting on PPO but after quinone formation. L-cysteine was chosen because it has been reported to inhibit browning caused by PPO by trapping quinones as colourless adducts (Richard-Forget et al., 1992), which can not then polymerise to form melanoidines.

In this work, gaseous ClO_2 was evaluated for effectiveness in prolonging the shelf-life of MP carrots, MP lettuce and MP cabbage, the last two were previously immersed in a L-cysteine solution in order to inhibit browning. Three experiments are included in this article. In the first, the respiration rate of ClO_2 treated MPV were assessed compared to reference samples in order to determine possible differences in physiological activity and to be able to design the appropriate packaging configurations. The second experiment consisted in a shelf-life study where ClO_2

treated and reference MPV were packed in bags designed according to results of the first experiment. Finally, the effect of the immersion of MP lettuce and MP cabbage in a L-cysteine solution on the decontamination efficacy of gaseous ClO_2 was tested in order to explain the relatively low levels of microbial inactivation achieved in the shelf-life experiment.

5.3. Materials and methods

5.3.1 Vegetable processing

Carrots (*Daucus carota* L.) were purchased in a local produce market and stored at 4°C overnight before use. Carrot sticks were prepared according to the protocol described in chapter 3, section 3.3.3.1.

Iceberg lettuce (*Lactuca sativa* var. *capitata* L.) and white cabbage (*Brassica oleracea* var. *capitata* L.) were purchased in a local wholesale company (Van Landschoot, Gent, Belgium), stored at 7°C and processed within one day. Lettuce was shredded in 1 cm pieces by using a knife and white cabbage was shredded in 1 mm thick pieces using a Compacto Kitchen Cutter. The pieces were washed in tap water for 1 min and separated in two portions, one to be treated with ClO_2 gas and the other to remain untreated as reference samples. The batch to be treated with ClO_2 gas was immersed for 1 min in a solution of 0.5% HCl•L-cysteine monohydrate (Sigma-Aldrich, Steinheim, Germany), in which the pH was previously adjusted to 6.2 with NaOH. Both batches were dried for 1 min by means of a manual kitchen centrifuge.

5.3.2 ClO_2 gas treatment

The decontamination was performed essentially in the same way that the treatment described in chapter 4, section 4.3.2. Two kg of MP carrots, MP lettuce or MP cabbage were placed in a 48 litre closed cabinet with a glass window covered by aluminium foil. Then the relative humidity of the cabinet was adjusted at 90-91% by a flow of hot wet air; a thermohygrometer (Digitron 2020R, Devon, England) was used to measure relative humidity and temperature. A 1000 mg/l solution of ClO_2 was prepared by diluting a stock solution (Vernagene, UK) whose concentration had been

previously determined by the method described in chapter 4, section 4.3.4. ClO_2 was stripped from the diluted solution, previously warmed up to 48°C, by air bubbling (4 l/h) and led by the same air stream to the cabinet were perforated plastic pipes together with cabinet tumbling procured an homogeneous contact between the sanitizer gas and vegetable pieces. Treatment was performed at ambient temperature (22-28°C) up to 10 min, including 30 s of stripping, at maximal registered ClO_2 concentrations of 1.33, 1.74 and 1.29 mg/l for MP carrots, MP lettuce and MP cabbage respectively. ClO_2 concentration inside the cabinet was monitored by taking gas samples at regular intervals, and analysing samples by using the method described in chapter 4, section 4.3.5. When the pre-set treatment time was completed, the cabinet was opened and samples were taken for the respiration rate measurement or the shelf-life study.

5.3.3 Respiration rate measurements

The method described in chapter 3, section 3.3.4.1 was followed to measure the respiration rate of reference and ClO_2 treated MPV. In this case were however used different amounts of MPV, replicates and initial gas composition. One hundred grams of MP carrots, 150 grams of MP lettuce or 60 g of MP cabbage were placed in the airtight glass jars. Five replicates were used. The jars were closed under a gas mixture of 10-16 kPa O_2, 1-2 kPa CO_2, balanced with N_2.

The speed of O_2 consumption inside the jars is function of the amount of sample, temperature and the respiration rate of the MPV. In practice, the O_2 consumption can be easily followed by placing low amounts of fast respiring MPV or high amounts of slow respiring MPV. Additionally, in this case were used five replicates instead of three trying to improve the quality of the data.

5.3.4 Packaging of the MPV

Samples were packed under equilibrium modified atmosphere packaging (EMAP) where, by matching the film permeability to oxygen, fill weight (100 g) and bag dimensions with the respiration rate of the produce at a specific temperature, a constant oxygen composition is maintained inside the bags during the whole shelf-

life. The applied packages were supplied by (Hyplast N.V., Hoogstraten, Belgium). They were experimental films with known permeability for O_2 at 7°C and 90 kPa relative humidity. The packaging configuration was designed by using the method validated by Jacxsens et al. (1999a). MPV were packed in designed bags, then a gas mixture was injected into the bags as initial gas atmosphere by the gas packaging unit described in chapter 3, section 3.3.4.1.

5.3.5 Shelf-life study

Untreated and treated MPV were packed in the designed bags and stored at 7°C. During the shelf-life study, three bags per condition (treated and untreated) were taken at several days and independently analysed for O_2 and CO_2 headspace concentrations, microbiological and sensory quality, and pH. Additionally, samples were taken for microbiological analysis of the cut, unwashed MPV. The end of the shelf-life arrived when the population of a group of microorganisms reached an unacceptable level or when the sensory panel rejected the samples.

5.3.5.1 Headspace O_2 and CO_2 monitoring

O_2 and CO_2 concentrations in the headspace of the bags were determined by a Servomex gas analyser (Servomex 1450, Crowborough, England) before opening bags to take samples for the rest of the analyses. The gas analyser determines O_2 concentration by paramagnetism and CO_2 concentration by the infrared single beam-single wavelength technique.

5.3.5.2 Evaluation of microbological and sensorial quality

The methods described in chapter 3, section 3.3.4.3 were used for microbiological analysis of spoilage microorganisms, with the exception of the medium to enumerate yeasts. That was a prepared medium composed by 15 g agar (Agar N° 1, Oxoid, LP0011), 5 g yeast extract (Oxoid, L21), and 20 g dextrose (Sigma-Aldrich, Steinheim, Germany) per litre with 50 mg/l (Tournas et al., 2001) chlortetracycline (Difco, 233331).

Sensory evaluation was performed according to chapter 3, section 3.3.4.4.

5.3.5.3 pH measurement

A sample of 10 g of MPV was homogenized by using a mixer (Commercial blender 8010, Waring, Connecticut, USA) with 50 ml of demineralised water, and filtered. The pH of the filtrate was measured by using an electrode (PH 915600, Orion, Boston, USA) and measure unit (model 525A, Orion, Boston, USA).

5.3.6 Effect of L-cysteine on the decontamination efficiency of ClO_2 gas

To measure the effect of L-cysteine on the decontamination efficiency of ClO_2 gas, MPV immersed and not immersed in the cysteine solution were produced as indicated before. Both batches were treated with ClO_2 gas by putting 250 g of sample in the cabinet and stripping an 125 mg/l solution of ClO_2. Since the mass of sample treated in this experiment was smaller than that used in the experiment described in section 5.3.2, it was necessary to reduce the amount of applied ClO_2 to avoid exposing MPV to excessive gas concentrations. Other conditions were the same as described in section 5.3.2. The decontamination efficacy was determined by comparing APC before and after treatment for both batches. Experiments were run in triplicate.

5.3.7 Statistical analysis

Data were analysed for mean differences between treated and untreated MPV carrots with the t-test for independent samples and the Mann-Whitney test with $\alpha=0.05$. For MP lettuce and MP cabbage, respiration rate data were analysed by using the t-test for independent samples. Decontamination results were analysed for mean differences among different treatment conditions for each MPV with the t-test and test of Duncan. pH changes and results on the effect of L-cysteine were analysed by the Brown-Forsythe's test (for non homoscedastic groups). The used software was SPSS 12.0 (SPSS Inc., Chicago, USA), with $\alpha=0.05$.

5.4. Results and discussion

5.4.1 Respiration rates

In order to design appropriate packaging configurations to reach an equilibrated modified atmosphere it is necessary to know the respiration rate of the produce. Table 5.1 shows the respiration rates of reference and ClO_2 treated MPV, as well as the packaging configurations used for shelf-life studies. The values for the respiration rate of the untreated MP carrots and MP Iceberg lettuce found in this study agree with those reported by Jacxsens et al. (1999a), which were respectively 10.25±0.43 and 6.86±0.59 ml O_2/kg h at 7°C and 3 kPa. Since ClO_2 is an oxidant, the metabolism of the vegetable tissue could have resulted altered during treatment, leading to a respiration rate different than that of the untreated produce. ClO_2 gas treatment (after immersion in L-cysteine solution) increased the respiration rate of MP cabbage ($\alpha \leq 0.05$). On the other hand, no statistical differences ($\alpha \leq 0.05$) were found between the respiration rates of untreated and treated MP carrots, the average of both was taken to calculate the packaging configuration with regard to the shelf-life study. Moreover, the treatment did not influence the respiration rate of MP lettuce ($\alpha \leq 0.05$). The effect of ClO_2 on the respiration rate of MPV has not been studied before. Nevertheless washing with ozonated water, another powerful oxidizer, did not influence the respiration rate of MP Iceberg lettuce (Beltrán et al., 2005b).

Table 5.1. Package design for untreated and ClO_2 treated MPV. (Fill weight was 100 g).

MPV	Sample	Respiration rate[a]	Package area (cm^2)[b]	Film permeability[c]
Carrots	Reference	10.69 ± 3.63[d]	20 x 10.5	3529
	Treated	9.98 ± 3.47[d]	20 x 10.5	3529
Lettuce	Reference	6.19±1.47[d]	13 x 21.5	1482
	Treated	7.31±1.00[d]	15 x 18	1810
Cabbage	Reference	16.99±1.61[d]	16 x 16	4249
	Treated	20.76±1.80[e]	18 x 18	4249

[a] Mean ± SD (ml O_2/(kg.h)) at 7°C and 3 kPa O_2, n=5. [b] Length x width. [c]((ml $O_2/(m^2$.24h.atm)) at 7°C. [de] For the same MPV, means sharing a superscript are not statistically different ($\alpha \leq 0.05$) according to the t-test.

5.4.2 ClO_2 degradation

The highest registered ClO_2 concentrations were 1.33, 1.74 and 1.29 mg/l for MP carrots, MP lettuce and MP cabbage respectively, registered immediately after finishing the stripping. Because of the experimental set-up, where the sample remains in contact with ClO_2 for some time after the end of the stripping process, which results in degradation of ClO_2 by the vegetable tissue, it is not possible to report a single value of ClO_2 concentration but the actual changing concentration during time as it is shown in Figure 5.1. The concentration of ClO_2 inside the chamber increased throughout the 30 s of stripping, but it was impossible to monitor it during that period of time due to methodological limitations. Therefore, only the change of ClO_2 concentration after finishing the stripping process is presented in Figure 5.1. The concentration of ClO_2 during the treatment of MP carrots and MP lettuce felt to nil before 6 minutes, while still some ClO_2 remained after the treatment of MP cabbage (Figure 5.1a). Complete consumption of ClO_2 is desirable, otherwise it is necessary to evacuate and destroy it. The observed ClO_2 degradation can be only accounted to degradation by the MPV, as it was shown in chapter 4, section 4.4.1.

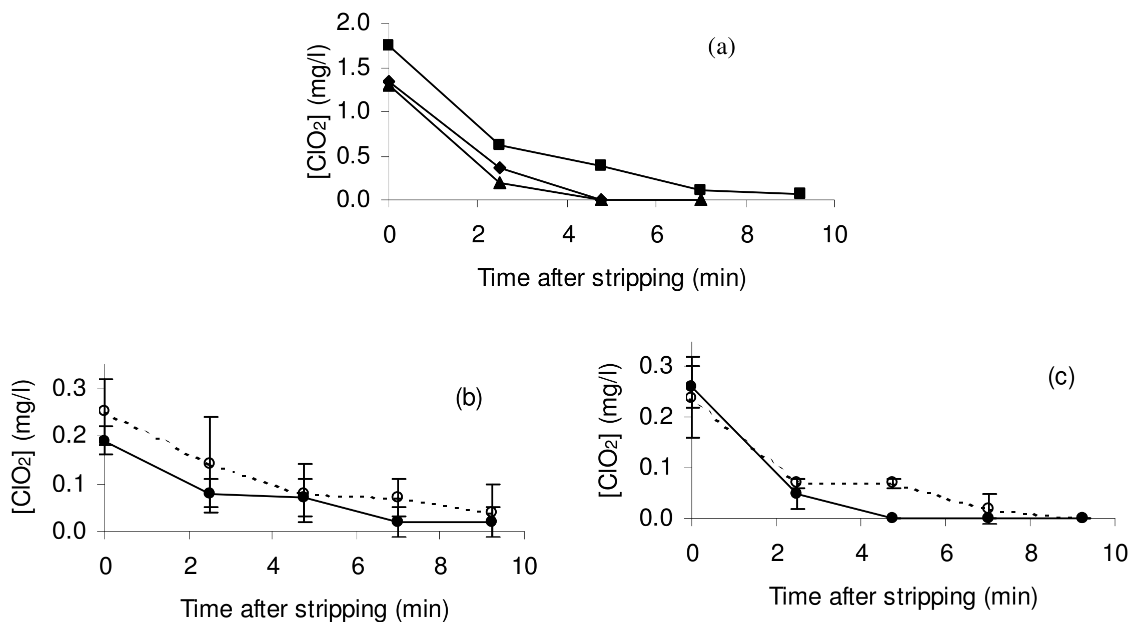

Figure 5.1. Change in concentration of ClO_2 gas during the treatment of MPV. (a) Treatment for shelf-life studies of: MP lettuce (–■–), MP carrots (–◆–), MP cabbage (–▲–). (b) Effect of L-cysteine on MP lettuce: without L-cysteine (--○--), with L-cysteine (–●–). (c) Effect of L-cysteine on MP cabbage: without L-cysteine (--○--), with L-cysteine (–●–). Error bars indicate ± SD.

When comparing studies on decontamination with ClO_2, it is important to consider the actual meaning of the reported ClO_2 concentrations. In the present work, the ClO_2 concentration during treatment increased, reached a peak and then decreased as it was explained above. This is different from the set-ups used by Lee et al. (2004) and Sy et al. (2005a,b) where the ClO_2 concentration increased during the whole treatment time. Moreover it is also different from the set-ups of Du et al. (2002, 2003) and Han (2000b, 2001a,b) where the dynamics of ClO_2 concentration was not followed, maybe because of the use of a large excess of cabinet volume with respect to the sample amount, resulting in a ClO_2 concentration that could have been considered constant. Therefore, comparisons between different studies should be taken with precaution.

5.4.3 Headspace O_2 and CO_2 concentrations

The goal of an EMAP is to establish constant O_2 and CO_2 concentrations during the entire shelf-life study. To check the appropriateness of the selected packaging configurations, a periodic monitoring of the concentration of these gases was carried out, the results for MP carrots are presented in Figure 5.2a. The mean O_2 concentration through the shelf-life of MP carrots was about 4 kPa, close to the desired 3 kPa, for both, treated and untreated samples. However, a high variability was observed, and some packages of untreated samples were at hypoxic level (<0.5 kPa) and had to be discarded. But for treated samples, the designed bags were able to maintain an acceptable O_2 level for the whole period of the shelf-life (5 days). The mean concentration of CO_2 through the shelf-life was around 6 kPa, CO_2 percentage dropped from their initial values, then stabilized or increased but without accumulation enough to reach the initial levels. Therefore, for ClO_2 treated samples, the designed MAP was able to keep O_2 and CO_2 concentrations at the desired levels (Jacxsens et al., 1999a) enough to retard respiration without causing fermentation (Beaudry, 1999).

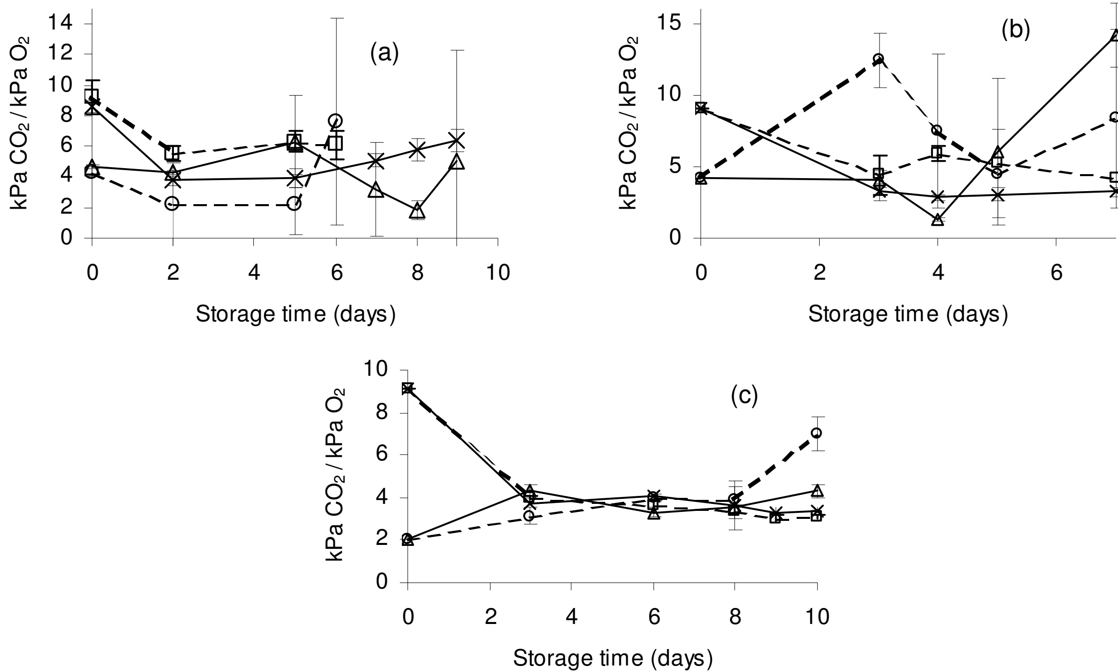

Figure 5.2. Headspace gas composition during storage of MPV under MAP at 7°C. (a) MP carrots, (b) MP lettuce, (c) MP cabbage. [O_2] of untreated MPV (---o---), [O_2] of ClO_2 treated MPV (—△—), [CO_2] of untreated MPV (---□---), [CO_2] of ClO_2 treated MPV (—x—). Error bars indicate ± SD.

The headspace gas concentrations of the bags were MP lettuce and MP cabbage were packed are presented in Figure 5.2b and Figure 5.2c respectively. The concentration of O_2 in the headspace of the bags of those MPV was above anoxic conditions. They became some days high in bags of MP cabbage but were always below the atmospheric concentration. The concentration of CO_2 dropped from 9 kPa to values between 2.6 and 6.3. This must have been consequence of the packaging configuration that allowed in all cases an outgoing flow of CO_2 not compensated by production of CO_2 due to respiration of the MPV. Therefore, the packaging configurations used in this work prevented CO_2 accumulation inside the bags, which could have had harmful effects on the physiology of the MPV (Kader et al., 1989).

5.4.4 Decontamination

The counts and levels of reduction for specific groups of microorganisms due ClO_2 gas treatment in MP carrots, MP lettuce and MP cabbage are reported in Table 5.2. The initial microbiological contamination was below the tolerance criteria proposed by

Debevere (1996) for fresh-cut produce on the production day (10^6 log CFU/g for psychrotrophs, and 10^4 log CFU/g for lactic acid bacteria, for yeasts and for moulds). The differences in counts between "initial" and "reference" samples reflect the joined effect of washing and spun drying. When compared with the counts found for the initial (after grating) microbial load, the counts of LAB in MP carrots increased after the washing-drying, likely due to contamination acquired in the spin dryer (centrifugation). For MP cabbage only the APC and yeast counts were affected by these processes, while no microbial population of MP lettuce suffered a significant change ($\alpha \leq 0.05$). Increment of contamination during the preparation of MPV has also been reported by Garg et al. (1990) for shredders used to prepare chopped lettuce and coleslaw, and by Allende et al. (2004) for the draining, rinsing, centrifugation and packaging steps required to prepare a MP Lollo Rosso lettuce.

Table 5.2. Counts (log CFU/g ± SD) of different groups of microorganisms in MP carrots, MP lettuce and MP cabbage and respective reduction after treatment with ClO_2 gas.

MPV and [ClO_2]	Sample	Aerobic plate count	Psychrotrophs	Lactic acid bacteria	Yeasts
	Initial	4.72 ± 0.17^a	4.82 ± 0.14^a	2.22 ± 0.18^b	2.23 ± 0.29^a
Carrots	Reference	5.07 ± 0.23^a	5.06 ± 0.19^a	2.93 ± 0.17^a	2.44 ± 0.09^a
(1.33 mg/l)	Treated	3.19 ± 0.16^b	3.35 ± 0.08^b	0.33 ± 0.58^c	1.78 ± 0.08^b
	Reduction[d]	1.88	1.71	2.60	0.66
	Initial	5.37 ± 0.25^a	5.77 ± 0.23^a	<1	1.1 ± 0.17^a
Lettuce	Reference	5.22 ± 0.47^a	5.30 ± 0.35^a	<1	0.97 ± 0.95^a
(1.74 mg/l)	Treated	4.38 ± 0.34^b	4.42 ± 0.33^b	<1	0.33 ± 0.58^a
	Reduction	0.84	0.88		0.64
	Initial	4.71 ± 0.10^a	4.72 ± 0.06^a	<1	1.90 ± 0.09^c
Cabbage	Reference	4.50 ± 0.10^b	4.55 ± 0.05^a	0.67±0.58	2.69 ± 0.11^a
(1.29 mg/l)	Treated	4.25 ± 0.09^c	4.28 ± 0.15^b	<1	2.23 ± 0.04^b
	Reduction	0.25	0.27	<1	0.46

[a-c] For the same MPV and microbial group, means sharing a superscript are not statistically different ($\alpha \leq 0.05$) according to the test of Duncan, except for LAB and yeasts in MP carrots, which were analysed according to the Mann-Whitney test. [d] Log count of reference – log count of treated.

For MP carrots, the reductions in comparison with the water washed reference samples were statistically significant (α≤0.05). Furthermore, they were enough to prolong the shelf-life of MP carrots for one day, as it is shown later on. Treatment with ClO_2 gas (after immersion in L-Cysteine solution) reduced APC and psychrotroph counts of MP lettuce and APC, psychrotroph counts and yeast counts of MP cabbage (α≤0.05). Results on yeast decontamination agrees with data published by Sy et al. (2005b), who reported reductions in yeasts and moulds between 0.41 and 1.09 log CFU/piece after treatment apples, tomatoes, onions and peaches with 1.4 mg/l ClO_2 gas for up to 5.4-6 min. Yet, they are far from the value of 4.16 log CFU/g obtained by Sy et al. (2005a) in the skin of strawberries treated with 8.0 mg/l ClO_2 up to 120 min. It is known that yeasts are more resistant than bacteria to ozone, another powerful oxidant and biocidal agent (Russell, 2003).

5.4.5 Effect of the decontamination treatment on sensory attributes

Treatment did not impair the sensory attributes of MP carrots significantly (α≤0.05) as it can be observed in Table 5.3 at day 0. This finding contrasts with the results reported by Sy et al. (2005b) for Julienne-style cut carrots, which showed a slight whitening after treatment with 1.4 mg/l of ClO_2 for 6.4 to 10.5 min at 79% to 84% relative humidity. There are two possibilities for carrot whitening, the so called “white blushing” or the carotene bleaching. White blushing is a white translucent appearance of cut carrots that has been attributed to tissue dehydration (Tatsumi et al., 1991) and lignification (Bolin and Huxsoll, 1991). It can not be assured that the whitening reported by Sy et al. (2005b) was either white blushing or carotene bleaching. When comparing their results with those reported hereby, the higher relative humidity used during treatment in this study seems to have avoided dehydration, and consequently white blushing, and no bleaching was observed. Nevertheless, it is not possible to completely rule out a role of ClO_2 in carrot whitening under other treatment conditions. In this regard Sy et al. (2005b) also reported that higher ClO_2 concentrations (2.7 and 4.1 mg/l) caused more whitening, once again these ClO_2 levels were also associated with relative humidity’s lower than 90% for even longer exposure times. Moreover, Sy et al. (2005b) concluded that adverse effects on sensory quality render the application of ClO_2 to carrots on a

commercial scale questionable, the results found in this thesis point towards a different conclusion.

Table 5.3. Sensory evaluation of untreated and treated with ClO_2 MP carrots stored at 7°C under EMAP.

Quality attributes		Time (days)					
		0	2	5	6	7	8
Odour[a]	Untreated	2.7±0.6[d]	3.7±0.7	4.5±1.5	4.7±1.9	-[c]	-
	Treated	2.3±0.5[d]	3.8±0.3	4.1±0.3	-	**5.1±0.2**	**5.8±0.2**
Flavour[a]	Untreated	1.3±0.1[e]	3.6±0.5	4.5±0.2	4.3±1.0	-	-
	Treated	1.4±0.3[e]	2.7±0.7	3.6±0.3	-	3.2±0.3	4.7±0.1
OVQ[a]	Untreated	1.2±0.2	3.1±0.5	3.6±0.2	3.9±0.3	-	-
	Treated	1.2±0.0	2.8±0.5	3.6±0.3	-	0.7±4.1	0.2±4.4
Texture[a]	Untreated	1.1±0.2	2.9±0.4	3.5±0.4	3.9±1.1	-	-
	Treated	1.1±0.1	2.8±0.4	3.0±0.3	-	3.1±0.7	4.3±0.2
White	Untreated	1.6±0.2[f]	2.4±0.3	2.9±0.2	2.9±0.3	-	-
blushing[b]	Treated	1.4±0.2[f]	2.4±0.3	2.6±0.4	-	3.0±0.3	**3.1**±0.3

Mean ± SD. Numbers in bold are scores above the acceptability limit. [a]Odour, flavour, OVQ (overall visual quality) and texture scores: 1=fresh, 9=spoiled. [b]White blushing: 1=none, 5=severe. [c]-Not determined. [d-f]Means with the same superscript are statistically equal ($\alpha \leq 0.05$) according to the t-test.

Tables 5.4 and 5.5 show the results of the sensory evaluation of MP lettuce and MP cabbage respectively. ClO_2 treatment (after immersion in L-Cysteine solution) did not cause any significant ($\alpha \leq 0.05$) alteration of the sensory attributes of these MPV, except for a weak off-odour in MP lettuce. Sy et al. (2005b) reported that treatment with 1.4 mg/l gaseous ClO_2 up to 10.5 min caused immediate significant decreases in hedonic scores for appearance, colour, aroma and overall sensory quality of MP lettuce and MP cabbage. Although time and concentrations are similar between both works, the dynamic of ClO_2 concentration was quite different. Treatment conditions seem to have been stronger in Sy et al. (2005b) than in the present work, which could account for the better results found in this thesis from the sensory point of view. In this study, ClO_2 concentrations decreased exponentially over time while in Sy et al. (2005b) it increased linearly. This means that the overall ClO_2 dosis was higher in Sy et al. (2005b) than in the present work.

Table 5.4. Sensory evaluation of reference and treated with ClO_2 gas MP lettuce stored at 7°C under MAP.

Quality attribute		Time (days) 0	3	4
Overall visual	Reference	1.17±0.17[a]	3.33±1.36	**6.00±0.17**
quality[c]	Treated	1.78±0.35[a]	4.67±1.20	**7.11±0.69**
Off-odour[d]	Reference	1.22±0.25[a]	1.61±0.63	1.61±0.54
	Treated	2.22±0.10[b]	2.17±0.88	**3.17±0.60**
Flavour[e]	Reference	1.50±0.29[a]	1.67±0.44	2.00±0.33
	Treated	1.89±0.25[a]	2.11±0.54	**3.22±0.42**
Leaf edge	Reference	1.11±0.10[a]	2.33±0.76	**3.78±0.42**
browning[f]	Treated	1.17±0.17[a]	2.78±0.63	**3.33±0.60**
Leaf surface	Reference	1.11±0.19[a]	1.67±0.44	2.78±0.25
browning[f]	Treated	1.00±0.00[a]	2.44±0.48	**3.22±0.35**
Wiltness[f]	Reference	1.11±0.19[a]	1.28±0.10	1.83±0.17
	Treated	1.44±0.19[a]	1.33±0.17	2.33±0.44
Dryness[g]	Reference	1.50±0.29[a]	1.33±0.17	1.94±0.35
	Treated	1.28±0.10[a]	1.67±0.17	1.89±0.35

Mean ± SD. Numbers in bold are scores above the acceptability limit. [ab]Within the same sensory parameter, means sharing a superscript are not statistically different ($\alpha \leq 0.05$) according to the t-test. Scores: [c]Overall visual quality: 1=excellent, 5=fair, 9=extremely poor. [d]Off-odour: 1=none, 3=acceptable, 5=severe. [e]Flavour: 1=fresh, 3=acceptable, 5=spoiled. [f]Browning, wiltness: 1=none, 3=acceptable, 5=severe. [g]Dryness: 1=fresh, 3=acceptable, 5=dry.

Very interestingly, no browning was observed in any sample after treatment with ClO_2, furthermore the scores for overall visual quality and browning were not affected ($\alpha \leq 0.05$). These findings contrast with the results published by Sy et al. (2005b) and with the results of triangle tests reported in chapter 4, section 4.4.1. It seems obvious that the immersion in the L-cysteine solution before ClO_2 treatment avoided the development of brown pigments in both MPV.

Table 5.5. Sensory evaluation of reference and treated with ClO_2 gas MP cabbage stored at 7°C under EMAP.

Quality attribute[a]		Time (days)				
		0[b]	3	6	8	9
Overall visual quality	Reference	1.33±0.00	1.44±0.25	1.56±0.10	2.17±0.00	3.06±0.48
	Treated	1.33±0.17	1.39±0.19	2.17±0.00	3.17±0.17	4.44±0.54
Odour	Reference	1.39±0.10	2.78±0.77	2.61±0.25	2.44±0.51	3.22±0.42
	Treated	1.72±0.38	2.56±0.54	2.50±0.00	2.56±0.35	3.56±0.92
Flavour	Reference	1.22±0.10	1.56±0.10	2.33±0.67	2.39±0.25	4.00±0.17
	Treated	1.50±0.33	1.94±0.10	2.33±0.44	3.28±0.25	4.11±0.48
Texture	Reference	1.17±0.17	1.44±0.10	2.00±0.44	2.83±0.44	3.56±0.25
	Treated	1.28±0.10	1.50±0.17	2.56±0.19	2.83±0.17	3.11±0.67
Browning	Reference	1.06±0.10	1.00±0.00	1.67±0.17	2.28±0.25	2.56±0.48
	Treated	1.00±0.00	1.00±0.00	1.94±0.25	2.78±0.25	4.11±0.69

Mean ± SD. [a] Scores: 1=excellent, 5= fair, 9= extremely poor. [b] Within the same sensory parameter, no statistical differences ($\alpha \leq 0.05$) were observed in any of the evaluated attributes according to the t-test.

5.4.6 Microbial analysis during shelf-life

The changes in microbial populations of MP carrots during the shelf-life study can be observed in figs. 5.3 to 5.6. A lag phase of at least 2 days was observed for APC (Figure 5.3), psychrotrophs (Figure 5.4) and LAB (Figure 5.5), which is not common in this kind of studies and reveals a sublethal damage occasioned by ClO_2 to these groups of microorganisms. However, this effect was not observed to occur in yeasts (Figure 5.6). After the lag phase, LAB (Figure 5.5) on treated samples started to grow but neither them nor those on untreated samples reached high counts during shelf-life. Also after the lag phase, mesophilic (Figure 5.3) and psychrotroph (Figure 5.4) microorganisms on treated samples grew as fast as in untreated samples. The similitude observed in the growth curves of mesophilic and psychrotrophic bacteria suggests that the same microorganisms grew at both incubation temperatures. The psychrotroph counts reached unacceptable levels after 4 days on untreated samples but only after 8 days on the treated ones. This could have resulted in a shelf-life extension of four days, from the microbiological point of view, accounted to the

sublethal effect of ClO_2 causing a lag phase, together with the decontamination achieved. However, the yeast counts (Figure 5.6) of the treated samples, initially only minimally reduced with respect to the untreated ones, became the same at the fifth day and reached unacceptable levels (>10^5 CFU/g) almost on that day. When analysed together with the other results, it can be concluded that, from the microbiological point of view, only one extra day in shelf-life was achieved by applying gaseous ClO_2 to MP carrots, being yeasts the determinant for treated samples, and psychrotrophs for the untreated ones.

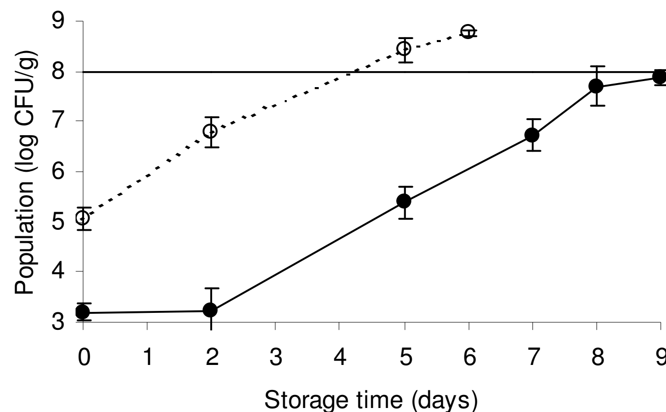

Figure 5.3. Aerobic plate count of untreated (---o---) and ClO_2 gas treated (—●—) MP carrots stored at 7°C under MAP. Horizontal line indicates the limit for shelf-life. Error bars indicate ± SD.

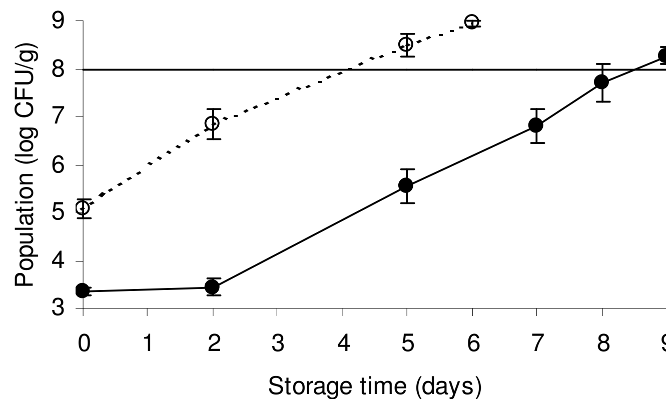

Figure 5.4. Psychrotrophs count of untreated (---o---) and ClO_2 gas treated (—●—) MP carrots stored at 7°C under MAP. Horizontal line indicates the limit for shelf-life. Error bars indicate ± SD.

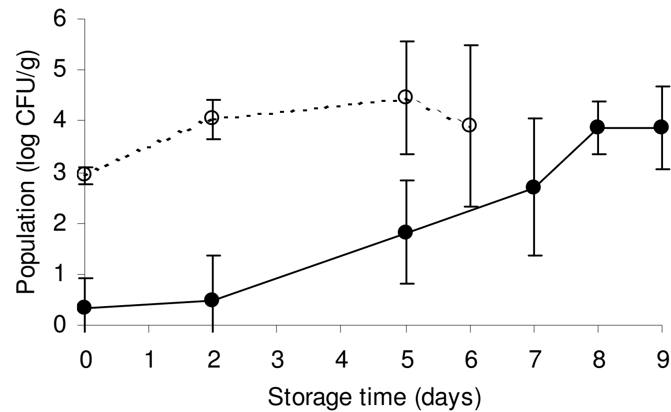

Figure 5.5. Lactic acid bacteria count of untreated (---o---) and ClO_2 gas treated (—●—) MP carrots stored at 7°C under MAP. Error bars indicate ± SD.

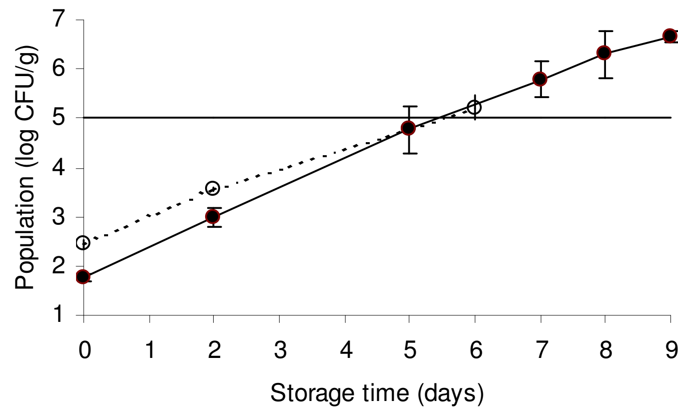

Figure 5.6. Yeasts count of untreated (---o---) and ClO_2 gas treated (—●—) MP carrots stored at 7°C under MAP. Horizontal line indicates the limit for shelf-life. Error bars indicate ± SD.

It is possible to speculate that the use of higher ClO_2 concentrations could have leaded to a longer shelf-life by reducing more the levels of the microbial populations. However, higher decontamination levels could increase the risk of pathogen proliferation, i.e. illness transmission, as it was explained in chapter 1, section 1.3.3. For example, it has been demonstrated that a treatment able to reduce the initial population of aerobic microorganisms of iceberg lettuce by 1.73-1.96 log CFU/g (Li et

al., 2001b) also enhances growth of *L. monocytogenes* during subsequent storage, which may result from reduction in numbers of competitive background microflora. However the specific case of MP carrots deserves a special consideration; studies summarized by Jacxsens et al. (1999b) and Sy et al. (2005b) have revealed a lack of growth and even a reduction in population of pathogens inoculated onto fresh-cut carrots. In spite of the possible presence in carrots of natural antimicrobial compounds, the safety of any MPV highly deprived from its natural microflora needs to be evaluated before looking for higher decontamination levels.

It has been generally considered that the shelf-life of MP carrots is limited by LAB and yeast growth (Nguyen-the and Carlin, 1994). However LAB counts were far to determine the shelf-life in this study, LAB growth in untreated samples stopped after 5 days and in the treated ones after 8 days (Figure 5.5), in both cases when the LAB population reached 4 log CFU/g. The same days APC and psychrotroph counts reached respectively unacceptable levels (Figures 5.3-5.4). This suggests that competition by non-LAB bacteria suppressed LAB growth. Also, Sinigaglia et al. (1999) and Klaiber et al. (2005) have found that LAB did not determine the shelf-life of this product.

The results of microbial growth during storage of MP lettuce and MP cabbage are shown in Figures 5.7 and 5.8 respectively. In both cases, mesophiles and psychrotrophs had the same counts, therefore only data for psychrotrophs are presented. Also Barriga et al. (1991) reported similarities in the levels and evolution of mesophilic aerobic and psychrotrophic microorganisms in MP Iceberg lettuce stored under controlled atmospheres at 4°C, and King et al. (1976) mentioned similar mesophilic and psychrotrophic counts in cabbage heads after storage at 6°C for 4 weeks. In spite of the reduction achieved after the decontamination treatment, mesophilic and psychrotrophic populations reached in treated samples higher levels than in the reference ones before the third day of the shelf-life study. Similar results were observed for the psychrotrophic count of MP cabbage treated with ILP (chapter 3, section 3.4.4), for MP cabbage treated with NEW (chapter 6, section 6.4.4), and it can be also found in the literature, as it was discussed in chapter 1, section 1.3.5.1. LAB did not grow in MP lettuce (detection limit 1 log CFU/g) and did not reach high levels in MP cabbage, the last is also valid for yeasts populations in both MPV.

Similar results were published by Barriga et al. (1991) and may be a consequence of competition with other microorganisms.

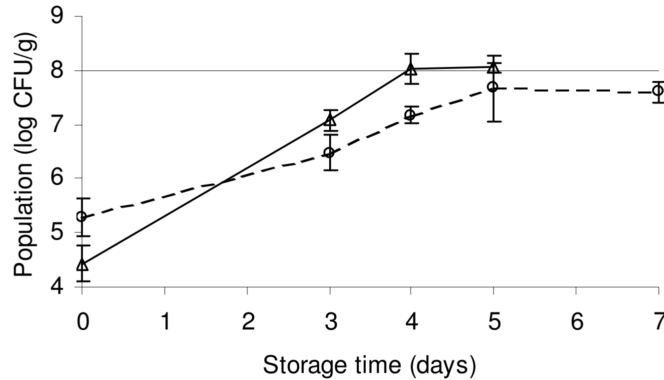

Figure 5.7. Microbial counts during storage of MP lettuce at 7°C under MAP. Psychrotrophs count of reference (---o---) and ClO_2 gas treated (—△—) samples. Horizontal line indicates the limit of psychrotroph counts for shelf-life. Error bars indicate ± SD.

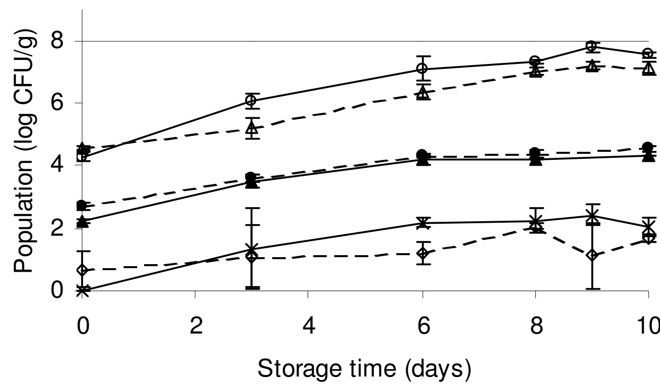

Figure 5.8. Microbial counts during storage of MP cabbage at 7°C under MAP. Psychrotrophs count of reference (---o---) and ClO_2 gas treated (—△—) samples. Yeast count of reference (---●---) and ClO_2 gas treated (—▲—) samples. LAB counts of reference (---◇---) and ClO_2 gas treated (—X—) samples. Horizontal line indicates the limit of psychrotroph counts for shelf-life. Error bars indicate ± SD.

Mesophiles and psychrotrophs did not reach the microbial limit after 10 days in MP cabbage. In MP lettuce, these populations reached the microbial limit in treated samples after 4 days, whilst in reference samples, they grew until the fifth day and then entered in stationary phase, without never reaching 8 log CFU/g. It seems that gaseous ClO_2 treatment facilitates the growth of microorganisms in these MPV. As

result of the faster growth of mesophilic and psychrotrophic populations in ClO_2 treated samples, no shelf-life prolongation was realized from the microbiological point of view.

5.4.7 Sensorial analysis during shelf-life

For MP carrots, the scores given by the panel to the different attributes were similar between untreated and treated samples along the shelf-life study (table 5.3). The only important attribute for the shelf-life from the sensory point of view was odour. After 6 days, untreated samples remained acceptable, the treated ones were unacceptable at the seventh day; however, since from the microbiological point of view both samples were not acceptable at earlier dates, no sensory parameter was important for determining shelf-life.

The shelf-life of MP lettuce from the sensorial point of view was limited to 4 days in both, reference and treated samples (Table 5.4). Reference samples suffered from poor overall visual quality and leaf edge browning, while treated samples suffered additionally from off-odour, bad flavour and leaf surface browning. In MP cabbage both kind of samples kept sensory acceptability during the 9 days of the sensory determinations (Table 5.5). In general, sensory parameters in treated MP lettuce and MP cabbage were poorer than in the reference samples. As consequence, no shelf-life prolongation was realized from the sensorial point of view. The worst scores for the sensory attributes were simultaneous with the highest values of APC and psychrotroph counts. For MP lettuce, a sharp increase in sensory scores (poorer quality) for off-odour, flavour and sogginess were observed in treated samples at the same time than APC and psychrotroph counts reached the tolerance limit of 8 log CFU/g at day 4. In contrast, that was not observed in reference samples. Whether the microbial growth deteriorated the product or a product deterioration facilitated microbial growth can not be determined by these kind of studies.

5.4.8 Change in pH during storage

A statistically significant ($\alpha \leq 0.05$) decrease of pH was detected after treatment of MP carrots (Figure 5.9a) and MP cabbage (Figure 5.9c), but not in MP lettuce (Figure

5.9b). The change might have been occurred due to formation of acid by the reaction between ClO_2 and organic matter. According to the review by Fukayama et al. (1986), ClO_2 can react with carbohydrates to form carboxylic acids and the progressive reduction of ClO_2 can also form acid. Since MP carrots and MP cabbage have a bigger cut surface than MP lettuce, it is possible that more ClO_2 could have been dissolved in the fluids of its tissue, resulting in a decrease of pH, which is not observed in the relatively less damaged MP lettuce. This finding is concomitant with the faster ClO_2 degradation that can be generally observed during treatment of MP carrots and MP cabbage (see Figure 5.1).

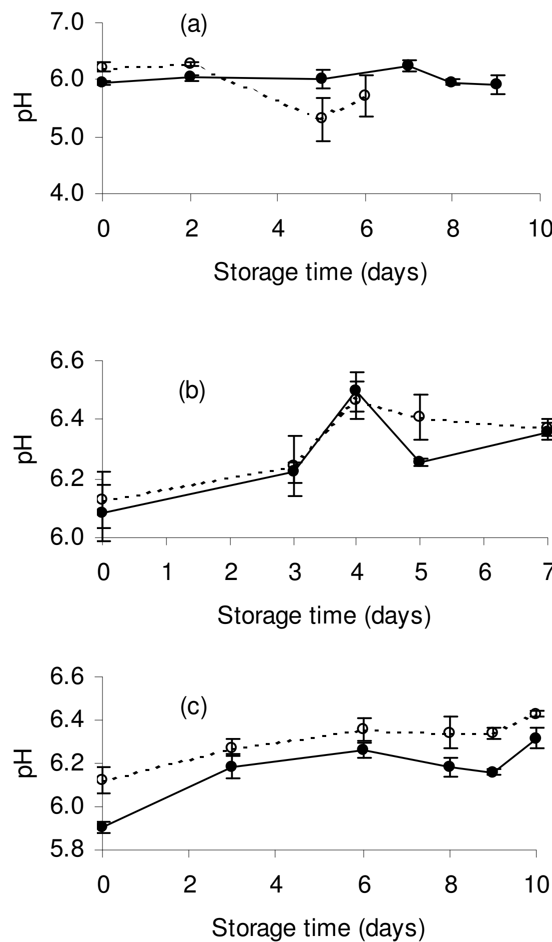

Figure 5.9. Changes in pH during storage of MPV at 7°C under MAP. (a) MP carrot, (b) MP lettuce, (c) MP cabbage. Untreated MPV (---o ---), ClO_2 gas treated MPV (—●—). Error bars indicate ± SD.

The pH of MP carrots during storage was stable (Figure 5.9a), which agrees with the low counts of LAB observed during this experiment (Figure 5.5). For MP lettuce (Figure 5.9b) and MP cabbage (Figure 5.9c) in general, pH increased over time in all the samples but it seems not to be directly linked to the other tested parameters. pH increase during storage of MP lettuce has also been reported by King et al. (1991) and Jacxsens et al. (2003). The last authors stated that the pH augment is typical for vegetables in which Gram-negative microorganisms play an important role in the spoilage, and that can result from the breakdown of proteins with the release of basic compounds as result. That agrees with the low counts for lactic acid bacteria (Figures 5.7 and 5.8).

5.4.9 Effect of L-Cysteine

The decontamination effect of ClO_2 on the microflora of MP cabbage was very low. An experiment was run in order to test the possible effect of the immersion in the solution of cysteine on the decontamination efficacy of ClO_2. The results (Table 5.6) show that the application of cysteine reduced the decontamination efficacy of ClO_2 when applied to MP cabbage and MP lettuce. It has been reported that ClO_2 reacts with L-cysteine (Napolitano et al., 2005). Figures 5.1b and 5.1c show that the degradation of ClO_2 during the treatment of samples previously immersed in the L-cysteine solution was faster than in samples only water washed. Therefore, the low decontamination effect of gaseous ClO_2 on MPV might be assigned to the consumption of ClO_2 by L-cysteine before reaching the microorganisms.

Table 5.6. Effect of 1 min immersion in a 0.5% L-cysteine solution on the decontamination efficacy (log CFU/g ± SD) of MP lettuce and MP cabbage with ClO_2 gas.

MPV	Without L-Cys	With L-Cys
Iceberg lettuce	0.83 ± 0.08^{a}	0.33 ± 0.37^{a}
White cabbage	0.97 ± 0.17^{a}	0.30 ± 0.21^{b}

$^{a-b}$ For the same MPV, means sharing a superscript are not statistically different ($\alpha \leq 0.05$) according to the Brown-Forsythe's test.

Although L-cysteine was able to suppress browning after ClO_2 treatment, its effect on the decontamination efficacy of gaseous ClO_2 can impair its practical application.

Nevertheless, this thesis proves that it is possible to inhibit the browning caused by ClO_2. Searching for another inhibitory compound, or for other treatment conditions that overcome the effect of L-cysteine on the decontamination efficacy of ClO_2 could open the practical implementation of this disinfectant in MPV. Sy et al. (2005b) considered ClO_2 as a promising sanitizer to inactivate pathogens in fresh-cut cabbage but not for fresh-cut lettuce, having brown discoloration as main drawback. Since the present study failed to prolong the shelf-life of MP cabbage, gaseous ClO_2 might have a practical implementation in producing a safer MP cabbage with the same shelf-life of an untreated MP cabbage. Used together with an antibrowning agent, the product could also have a better visual quality, therefore, a higher potential for practical implementation.

5.5. Conclusions

Gaseous ClO_2 is a promising alternative to prolong the shelf-life of MP carrots. Under the conditions used in this work, a treatment with gaseous ClO_2 does not affect the respiration rate nor the sensory attributes of MP carrots, decontaminate them and prolonged their shelf-life for one day. Yeast growth limited the shelf-life of treated samples. Also under the conditions used in this study, gaseous ClO_2 failed to prolong the shelf-life of MP lettuce and MP cabbage. Immersion of these MPV in a browning inhibitor solution before ClO_2 treatment is effective in preventing darkening caused by ClO_2. A solution of 0.5% L-cysteine used as pre-treatment browning inhibitor decreases the decontamination efficacy of gaseous ClO_2. Searching for other antibrowning agents with no effect on the ClO_2 decontamination efficacy, or optimisation of treatment conditions can be useful to reduce populations of pathogens in MP cabbage without causing browning.

Chapter 6 is the next part of this thesis. It has a similar structure than chapter 5 but using neutral electrolysed oxidising water as disinfectant instead of ClO_2. It is also a continuation of chapter 4 because it tests the possibility of prolonging the shelf-life of a MPV by using a decontamination method whose efficacy was tested in that chapter.

CHAPTER 6

Shelf-life of minimally processed cabbage treated with neutral electrolysed oxidising water and stored under equilibrium modified atmosphere[6]

[6] *Redrafted from: Gómez-López, V. M., Ragaert, P., Ryckeboer, J., Jeyachchandran, V., Debevere, J., Devlieghere, F.*

Chapter 6. Shelf-life of minimally processed cabbage treated with neutral electrolysed oxidising water and stored under equilibrium modified atmosphere

6.1 Abstract

Minimally processed (MP) vegetables have a short shelf-life. Neutral electrolysed oxidising water (NEW) is a novel decontamination method. The objective of this study was to test the potential of NEW to extend the shelf-life of a MP vegetable, namely MP cabbage. Samples of MP cabbage were immersed in NEW containing 45 mg/l of free chlorine or tap water (control) up to 5 minutes, and then stored under equilibrium modified atmosphere at 4°C and 7°C. Proliferation of aerobic mesophilic bacteria, psychrotrophic bacteria, lactic acid bacteria and yeasts were studied during the shelf-life. Also pH and sensorial quality of the samples as well as O_2 and CO_2 composition of the headspace of the bags was evaluated. From the microbial groups, only psychrotrophic counts decreased significantly ($\alpha \leq 0.05$) due to the effect of NEW, but the counts in treated samples and controls were similar after 3 days of storage at 4°C and 7°C. Packaging configurations kept O_2 concentration around 5 kPa and prevented CO_2 accumulation. pH increased from 6.1-6.2 to 6.4 during the shelf-life. No microbial parameter reached unacceptable counts after 14 days at 4°C and 8 days of storage at 7°C. The shelf-life of controls stored at 4°C was limited to 9 days by overall visual quality (OVQ), while samples treated with NEW remained acceptable during the 14 days of the experiment. The shelf-life of controls stored at 7°C was limited to 6 days by OVQ and browning, while that of samples treated with NEW were limited to 9 days by OVQ, browning and dryness. According to these results, a shelf-life extension of at least 5 days and 3 days in samples stored respectively at 4°C and 7°C can be achieved by treating MP cabbage with NEW. NEW seems to be a promising method to prolong the shelf-life of MP cabbage.

Keywords: Electrolysed oxidising water, minimally processing, fresh-cut, modified atmosphere, decontamination, shelf-life, white cabbage.

6.2 Introduction

Minimally processed vegetables (MPV) are fresh, raw vegetables processed in order to supply a ready-to-eat or ready-to-use product. The vegetables are usually trimmed, peeled, or cut if necessary, washed, and sometimes disinfected. The products are packaged in sealed pouches, or in plastic trays sealed with polymeric films. A shelf-life of several days after refrigeration is necessary for feasible transport and retail of final products (Nguyen-the and Carlin, 1994). While in chapter 5, the feasibility of the use of gaseous ClO_2 to prolong the shelf-life of MPV was tested, in the present chapter the same objectives were pursued but using another decontaminant, namely electrolysed oxidising water (EOW).

EOW is one of the potential alternatives to prolong the shelf-life of MPV. It has an increased level of free chlorine and some newly formed compounds with antimicrobial effects. As it was explained in chapter 1, section 1.7.1, most of the literature related to the application of EOW in vegetables deals with inactivation of pathogens with the acidic type (Kim et al., 2000). The present chapter however refers to the effects of the neutral type on the natural microflora and sensory quality of a MPV during storage, in which the literature is scarce. To discuss whether or not AcEW and NEW are essentially the same was not the goal of this research, an experimental comparison of the effect of both types should be necessary to clarify the issue. Therefore, in this chapter they will be considered different when discussing the literature. Reports on AcEW will be used when those on NEW are not available.

The literature on the use of NEW to inactivate spoilage microflora of MPV was revised in chapter 1, section 1.7.5. As example of the use of NEW to decontaminate MPV, Izumi (1999) found that NEW containing 20 ppm available chlorine reduced by 0.6 to 2.6 logs CFU/g total microbial counts of several MPV without affecting tissue pH, surface colour, or general appearance. Alternatively, several studies have been reported on the effect of AcEW on the shelf-life of MPV, which are summarized in chapter 1, sections 1.6.5 and 1.6.6.

Decontamination treatments and low temperature storage can be combined with modified atmosphere packaging as a multiapproach strategy to prolong the shelf-life of MPV. Jacxsens et al. (1999a) validated a systematic approach to design equilibrium modified atmosphere packages (EMAP) for fresh-cut produce. In this kind of modified atmosphere packaging, the air around the commodity is altered to a gas combination of 1 to 5 kPa O_2 and 3 to 10 kPa CO_2 (balance N_2). This atmosphere is for most produce optimally in that range (Kader et al., 1989). By knowing the respiration rate of the produce at the desired storage temperature, a packaging configuration can be designed to keep the gas concentrations constant during the storage time of the produce. To this, packages are designed by selecting a suitable film permeability at the storage temperature, amount of MPV in the package, and the package dimensions.

As it was revised in chapter 1, section 1.3.5.3; it seems that storage temperatures can be succesfully used to avoid that microbial counts of decontaminated samples reach the same levels than the controls.

Consequently, the present study had the goal to evaluate the potential of NEW to prolong the shelf-life of a MPV stored under EMAP combined with low temperatures, including a storage temperature of 4°C.

6.3 Materials and methods

6.3.1 Vegetable processing

White cabbage was purchased and processed as indicated in chapter 3, section 3.3.3.1. Then, cabbage pieces were decontaminated as indicated below. Samples were also taken for microbiological analysis of the non washed product.

6.3.2 NEW generation

NEW with 45 mg/l of free chlorine and pH 8.3 was produced with the device and protocol described in chapter 4, section 4.3.7, then stored in plastic drums to be used next day. Free chlorine concentration was again measured and did not change

overnight. Tap water used to treat controls contained 0.02 mg/l of free chlorine. Free chlorine concentration was determined according to the DPD method (see chapter 4, section 4.3.8).

6.3.3 Experimental layout

Two experiments are included in this chapter. In the first, the respiration rate of control and NEW treated MP cabbage were determined in order to design appropriate packaging configurations to achieve EMAP. The second consisted in a shelf-life study, where control and NEW treated MP cabbage were packed under EMAP and stored at 4 and 7°C. During storage, three bags containing control produce and three bags containing NEW treated produce were taken from each storage temperature and analysed in the following sequence. Firstly, the headspace gas composition of each bag was determined. Secondly, the bags were opened under sterile conditions and samples were taken from each bag for microbiological analysis. Thirdly, samples were taken from each bag for pH determination and sensorial analysis. Results on day 0 were separately analysed to assess the effect of NEW treatment as decontamination treatment.

6.3.4 Dipping treatments

The washing treatment of MP cabbage was performed by immersing 80 g of sample in 1.6 l of NEW or tap water (control) in a bowl with gentle, continuous agitation using a shaker (OS10, Ika-Werke, Staufen, Germany) at 120 rpm for 5 min at 25°C. At the end of the contact time, the treatment liquid was drained off, and the samples were spun dried for 15 s by means of a manual kitchen centrifuge (Zyliss, Bern, Switzerland).

6.3.5 Respiration rate measurements

The method described in chapter 3, section 3.3.4.1 was followed to measure the respiration rate of reference and NEW treated MPV. In this case were however used different amounts of MPV, initial gas composition, incubation temperature and desired percentage of O_2 at the equilibrium. Forty grams of untreated sample and 30 g NEW treated samples were placed in the airtight glass jars. Triplicates were used.

The jars were closed under a gas mixture of 9.9 kPa O_2, 1.6 kPa CO_2, balanced with N_2; and stored at 7 as well as 4°C. Gas concentrations were measured by injecting 3 ml of gas sample in a gas chromatograph Finnigan Trace GC ultra (Thermo Electron Corp., Waltham, USA). Oxygen concentration is determined in this device using two Restek (Bellefont, Pennsylvania, USA) columns, RT-Msieve 5A and Rt-Uplot, and a thermal conductivity detector. Carbon dioxide concentration is determined using a Varian (Palo Alto, California, USA) CP-ParaBOND Q column and a flame ionisation detector. The injector temperature was 30°C, and the detector temperature was 35°C. Helium was used as a carrier gas. Data were processed according to Jacxsens et al. (1999a) to estimate O_2 consumption at 4 °C and 7°C, and 5 kPa O_2.

Although packages can be designed to attain a specific oxygen concentration inside bags, there is variability that makes the gas concentration fluctuate around the desired value. Designs with the goal to attain relatively low oxygen concentrations can yield anoxic bags due to this variability. Therefore, the target oxygen concentration at equilibrium was changed from 3 kPa (see chapter 3, section 3.3.4.1, and chapter 5, section 5.3.3) to 5 kPa to avoid anoxic conditions such as those observed previously in some bags (see chapter 5, section 5.4.3). This oxygen concentration is in the range recommended by Kader et al. (1989). The storage temperature of 4°C was included to design packages that reach EMAP at 4°C.

6.3.6 Packaging for shelf-life studies

Control and NEW treated MP cabbage were packaged in designed bags with appropriate film permeabilities (Amcor Flexibles, Gloucester, UK). The packaging configuration was designed by using the method validated by Jacxsens et al. (1999a). MPV were packed in designed bags, then a gas mixture was injected into the bags as initial gas atmosphere by the gas packaging unit described in chapter 3, section 3.3.4.1. Finally, bags were stored at 4°C and 7°C.

6.3.7 Determination of changes during storage

Headspace O_2 and CO_2 concentrations were determined according to chapter 5, section 5.3.6.1. The analysis of spoilage microorganisms was performed as

described in chapter 3, section 3.3.4.3; including the modification stated in chapter 5, section 5.3.6.2. In this chapter however, LAB were incubated for 6 days instead of 3 days in order to increase the recovery. The sensory evaluation was performed according to the description given in chapter 3, section 3.3.4.4; and pH measurements according to chapter 5, section 5.3.6.4.

6.3.8 Statistical analysis

A t-test was used for paired comparisons by using the software SPSS 12.0 (SPSS Inc., Chicago, USA), with $\alpha=0.05$. Samples to assess the effect of decontamination and to evaluate shelf-life were taken in triplicate.

6.4 Results and discussion

6.4.1 Respiration rates

The packaging configurations used in the present study to achieve EMAP at 4°C and 7°C are presented in table 6.1, including the respiration rates of control and NEW treated MP cabbage at both temperatures. The respiration rate of MP cabbage at 7°C obtained in this part of the thesis agrees with that reported in chapter 3, section 3.4.3. The respiration rate of control and treated products were statistically equal ($\alpha \leq 0.05$) when comparing results obtained at the same temperature. Therefore, under the conditions of this study, it seems that treating MP cabbage with NEW is a decontamination method mild enough to avoid changes in the respiration rate of MP cabbage. In this regard, Koseki and Itoh (2002) reported that MP lettuce and MP cabbage treated with AcEW showed greater CO_2 production than did water washed controls, as it was mentioned in chapter 1, section 1.7.7. Those findings correspond to a storage temperature of 10°C, however, no significant differences in CO_2 production between treated and non-treated produce were observed at 1°C or 5°C. Some authors such as Cliffe-Byrnes and O'Beirne (2005b) have considered a reduction of respiration rate after a decontamination treatment as being beneficial. Although a lower respiration rate generally implies a slower rate of deterioration and loss of quality (Cliffe-Byrnes and O'Beirne, 2005a), the alteration of the metabolism of the MPV might in some cases indicate tissue damage. Wounding accelerates the

respiration rate of vegetables (Brecht, 1995), while the latter decreases with senescence. Low temperature storage and an appropriate MAP decrease the respiration rate of MPV. Decontamination of MP Iceberg lettuce with calcium lactate solutions decreases its respiration rate due to perhaps a protective effect of calcium in maintaining the cell wall structure (Rico et al., 2006). However, it would be risky to state that the decrease in respiration rate of a MPV as consequence of whichever process is beneficial for the shelf-life of MPV.

6.4.2 Effect of the decontamination treatment on microbial populations and sensory attributes

Table 6.2 shows the effect of water washing (control) and NEW washing on different microbial populations. In comparison with the initial populations, NEW washing decreased significantly ($\alpha \leq 0.05$) APC, psychrotroph counts and yeasts counts, but not those for LAB. Only the psychrotroph population was significantly ($\alpha \leq 0.05$) lower in NEW washed samples with respect to water washed ones, although the decontamination level was low. Since MPV are stored at chilling temperatures, the psychrotrophs is the most important group of microorganisms in determining the shelf-life of MPV. The results showed that NEW is more effective than simple water washing to decontaminate MP cabbage. These results are in the range reported by Izumi (1999), namely 0.3-1.6 log reduction in total microbial count, after rinsing several MPV with NEW containing 50 ppm of free chlorine for 4 minutes.

Table 6.1. Package design for untreated and NEW treated MP cabbage at 4℃ and 7℃.

	4℃		7℃	
	Control	Treated	Control	Treated
Respiration rate[a]	10.87±0.94[b]	9.60±0.01	15.36±0.04	14.44±0.33
Fill weight (Kg)	0.100	0.100	0.100	0.100
Package area (cm^2)[c]	13 x 21.5	18 x 15	16 x 16	18 x 18
Film permeability[d]	[e]	[e]	4600	4600

[a] Mean ± SD (ml O_2/(Kg.h)) at 7℃ and 5kPa O_2, n=3. [b] Within the same temperature, means were not statistically different ($\alpha \leq 0.05$) according to the t-test. [c] Length x width. [d] (ml O_2/m^2.24h.atm) at 7℃. [e] Supplier confidential data.

Table 6.2. Counts (log CFU/g ± SD) of different groups of microorganisms in MP cabbage and respective reduction after treatment with NEW.

	APC^{x}	Psychrotrophs	LAB^{y}	Yeasts
Non-washed	5.56±0.36^{a}	5.67±0.28^{a}	1.83±0.22^{b}	2.40±0.17^{a}
Control	4.44±0.10^{b}	4.67±0.16^{b}	2.52±0.29^{a}	2.08±0.18^{ab}
Treated	4.24±0.09^{b}	4.27±0.03^{c}	1.98±0.33^{ab}	1.80±0.28^{b}
Reduction^{z}	0.21	0.40	0.53	0.28

^{x} Aerobic plate count. ^{y} Lactic acid bacteria. ^{z} Count of control – count of treated. $^{a-c}$ For the same microbial group, means sharing a superscript are not statistically different ($\alpha \leq 0.05$) according to the test of Duncan.

In order for a decontamination treatment to be useful for treating MPV, it has not only to decontaminate but also not modify their sensory quality. Tables 6.3 and 6.4 show the results of the sensory evaluation. Treatment did not significantly affect ($\alpha \leq 0.05$) the sensory attributes of the MP cabbage compared to water washed samples (see data on day 0). Therefore, NEW is mild enough to be applied in MP cabbage treatment.

6.4.3 Headspace O_2 and CO_2 concentrations during storage

The change in concentrations of oxygen and carbon dioxide in bags containing control and NEW treated MP cabbage stored at 4°C and 7°C can be observed in Figures 6.1a and 6.1b respectively. The oxygen concentration in the headspace of all the bags was about 5 kPa throughout the study, revealing that the designed packaging configurations were appropriate to maintain an EMAP. Carbon dioxide concentrations dropped from 10 kPa to about 5 kPa during the study. Therefore, the packaging configurations were also suitable to avoid the accumulation of CO_2, which would have been harmful for the vegetable tissue (Kader et al., 1989).

Table 6.3. Sensory evaluation of untreated and treated with NEW MP cabbage stored at 4°C under EMAP.

Quality attribute		Time (days)					
		0[e]	3	6	9	13	14
OVQ[a]	Control	2.33±0.17	2.11±0.10	2.44±0.35	**4.06±0.67**[f]	3.61±0.10	5.39±0.10
	Treated	2.50±0.17	2.06±0.51	2.61±0.10	3.83±0.44	3.28±0.10	3.72±0.10
Off-odour[b]	Control	2.67±1.14	1.72±0.58	1.78±0.25	[g]	2.44±0.25	3.50±0.17
	Treated	1.53±0.42	1.83±0.75	2.00±0.33	[g]	2.50±0.50	2.28±0.42
Flavour[c]	Control	2.50±0.67	1.94±0.10	2.61±0.19	[g]	2.94±0.10	**4.33±0.44**
	Treated	2.17±0.73	1.89±0.10	2.78±0.51	[g]	2.94±0.10	2.61±0.25
Dryness[d]	Control	2.11±0.42	1.39±0.38	2.11±0.42	3.78±0.42	3.22±0.10	3.83±0.29
	Treated	2.50±0.17	1.83±0.60	2.50±0.17	3.72±0.42	3.67±0.50	3.61±0.25
Browning[b]	Control	1.89±0.25	1.44±0.19	1.83±0.17	4.00±0.44	3.50±0.00	**5.56±0.25**
	Treated	1.94±0.51	1.44±0.35	1.72±0.25	3.33±0.60	3.39±0.10	3.89±0.19

Average ± SD. [a] OVQ (overall visual quality): 1=excellent, 4=acceptable, 7=extremely poor. [b] Off-odour, browning: 1=none, 4=acceptable, 7=severe. [c] Flavour: 1=fresh, 4=acceptable, 7=spoiled. [d] Dryness: 1=none, 4=acceptable, 7=dried. [e] Within the same sensory parameter, no statistical differences (α≤0.05) were observed at day 0 in any of the evaluated attributes according to the t-test. [f] Numbers in bold indicate the end of the shelf-life from the sensorial point of view. [g] Not determined.

Table 6.4. Sensory evaluation of untreated and treated with NEW MP cabbage stored at 7°C under EMAP.

Quality attribute		Time (days)					
		0[e]	3	6	7	8	9
OVQ[a]	Control	2.33±0.17	2.39±0.19	**4.50±1.26**[f]	3.56±1.00	4.33±0.58	5.72±0.79
	Treated	2.50±0.17	2.00±0.29	3.06±0.25	3.22±0.19	3.83±0.17	**4.78±0.75**
Off-odour[b]	Control	2.67±1.14	1.78±0.19	2.67±1.14	2.06±0.19	2.72±0.10	[g]
	Treated	1.53±0.42	1.72±0.35	1.53±0.42	2.11±0.19	2.22±0.19	[g]
Flavour[c]	Control	2.50±0.67	2.33±0.44	3.11±0.63	3.06±0.69	2.89±0.38	[g]
	Treated	2.17±0.73	2.17±0.29	2.89±0.19	3.22±0.48	2.39±0.10	[g]
Dryness[d]	Control	2.11±0.42	1.39±0.38	3.89±0.54	3.39±0.48	**4.06±0.54**	4.22±0.25
	Treated	2.50±0.17	2.06±0.38	3.33±0.29	3.83±0.73	3.56±0.35	**5.06±0.51**
Browning[b]	Control	1.89±0.25	1.89±0.25	**4.50±1.33**	3.67±1.09	4.44±0.82	5.61±0.69
	Treated	1.94±0.51	1.56±0.25	2.89±0.19	3.28±0.25	3.89±0.25	**4.50±0.44**

Average ± SD. [a] OVQ (overall visual quality): 1=excellent, 4=acceptable, 7=extremely poor. [b] Off-odour, browning: 1=none, 4=acceptable, 7=severe. [c] Flavour: 1=fresh, 4=acceptable, 7=spoiled. [d] Dryness: 1=none, 4=acceptable, 7=dried. [e] Within the same sensory parameter, no statistical differences (α≤0.05) were observed at day 0 in any of the evaluated attributes according to the t-test. [f] Numbers in bold indicate the end of the shelf-life from the sensorial point of view. [g] Not determined.

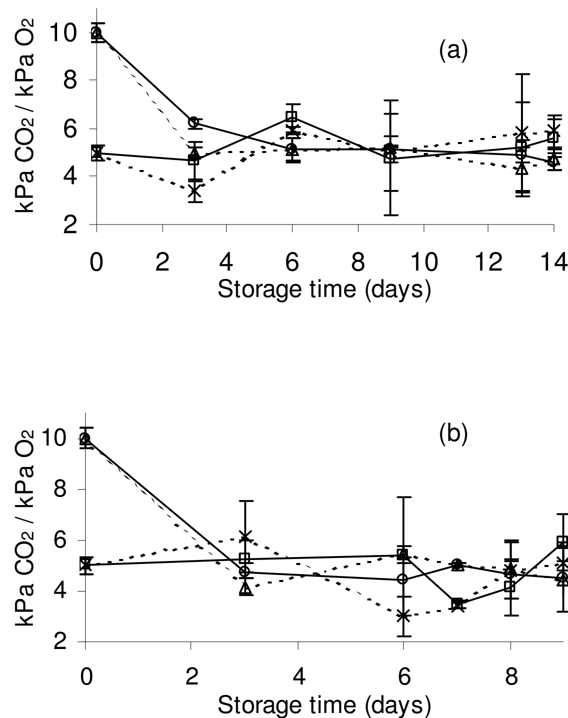

Figure 6.1. Headspace gas composition inside bags during storage of MP cabbage under EMAP at different storage temperatures. (a) 4°C, (b) 7°C. O_2 control (---X---), O_2 NEW treated (—□—), CO_2 control (---△---), CO_2 NEW treated (—○—). Error bars show SD, n=3.

6.4.4 Microbial analysis during storage

Results of the microbial counts during storage at 4°C and 7°C are shown in Figure 6.2. Independently of the decontamination treatment and storage conditions, control and treated samples did not reach unacceptable levels after 14 days of storage at 4°C and 8 days at 7°C. Moreover, total aerobic plate counts, psychrotrophs and yeasts reached stationary phase after 6 days of storage at 4°C and did not increase anymore after 13 days at 7°C. Therefore, it seems that counts would have never reached the microbial limit even with longer storage times. LAB counts were between 1.9 and 3.5 throughout the study. As expected, the growth of microorganisms at 4°C was slower than at 7°C.

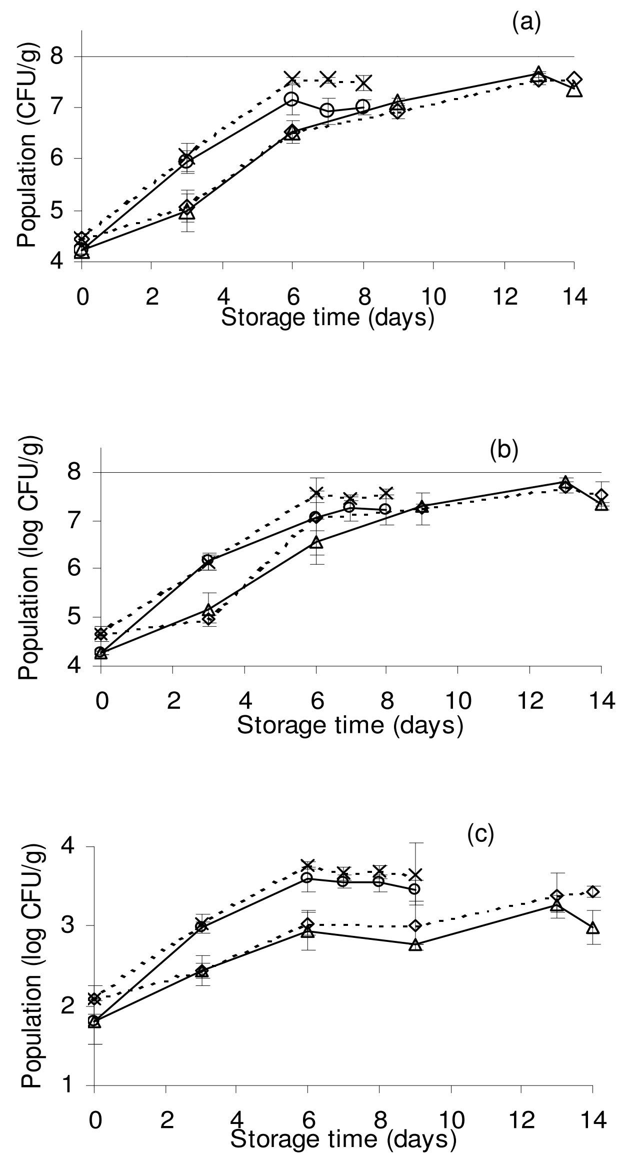

Figure 6.2. Microbial counts during storage of MP cabbage under EMAP at different storage temperatures. (a) Psychrotrophs, (b) aerobic plate counts, (c) yeasts counts. Control at 7°C (---X---), NEW treated at 7°C (—○—), control at 4°C (---◇---), NEW treated at 4°C (—△—). Error bars show SD, n=3. Horizontal lines indicate the limit of counts for shelf-life.

A parallelism in the growth of mesophilic (Figure 6.2a) and psychrotrophic (Figure 6.2b) microorganisms was observed. Although some similitude can be observed in the results published for MP cabbage by several studies, it seems that no generalization is possible. King et al. (1976) reported comparable counts of those populations in cabbage heads after storage at 6°C for 4 weeks. Nicholl et al. (2004)

studied the growth dynamics of indigenous microbial populations on MP cabbage washed with tap water or 100 ppm free chlorine (from sodium dichloroisocyanurate), reporting similar initial levels of mesophilic and psychrotrophic populations. Psychrotrophic counts were however over 1 log cycle higher than mesophilic counts after 3 and 5 days of storage at 5℃. Fantuzzi et al. (2004) reported stable mesophilic and psychrotrophic counts after storing MP cabbage at 1℃ and 5℃ for 20 days.

In the present study, after 3 days of storage, mesophilic aerobes (Figure 6.2a), psychrotrophs (Figure 6.2b) and yeasts (Figure 6.2c) populations in NEW treated produce reached those of the controls at both temperatures. The results are in line with those of Nicholl et al. (2004) who stated that the effect of treating MP cabbage did not last more than 5 days. Moreover, similar results are reported in sections 3.3.4 and 5.4.6, discussed in a general way in section 1.3.5.1. This phenomenon has been explained by Koseki and Itoh (2001) stating that the microorganisms in decontaminated samples would have “plenty of room for microbial growth because of the reduction in initial populations” and by Nicholl et al. (2004) expressing that survivors can grow more rapidly because of fewer bacterial competitors for nutrients. It is also possible that some decontamination treatments cause some kind of damage to the tissue integrity of the produce that provides easier access to nutrients, allowing in consequence faster growth. The use of a storage temperature of 4℃, as suggested in chapter 1, section 1.3.5.3 resulted unsuccessful in keeping the effect of the decontamination, the evidence compiled in that section however indicates that it is worth testing a storage temperature of 4℃ again.

6.4.5 Sensory analysis during storage

Several sensory attributes commonly used in the evaluation of the quality of MPV were assessed in this study. Additionally, dryness was also evaluated. MP cabbage has a large cut surface. Cutting or peeling a fruit or vegetable drastically increases the water evaporation rate (Brecht, 1995). Since the films used to pack MP cabbage were selected only according to their oxygen transmission rate, it was possible that their permeability to water would have been not low enough to prevent product dehydration. This happened in preliminary experiments of this thesis. This concern is more related to the choice of appropriate films according to an overall criterion than

to the goal of this study, but it had the potential to affect the results of the experiment. Therefore, the assessment of dryness was included in the study.

OVQ determined the end of the shelf-life from the sensorial point of view in controls at day 9 under storage at 4℃ (table 6.3), whilst NEW treated products were acceptable until the end of the study (14 days). Since no microbial population reached the microbial limit neither at 4 nor 7℃, it can be said that NEW extended the shelf-life of MP cabbage stored at 4℃ by at least 5 days. In view of the fact that no evaluations were the 7th and 8th days of storage, the shelf-life of control MP cabbage can only be deduced (see chapter 1, section 1.3.4.3).

OVQ together with browning determined the shelf-life of control samples stored at 7℃, which was no longer than 6 days. Interpolation was used once more to deduce the shelf-life of control samples, since no evaluation were performed on days 4 and 5 of the storage study. Interpolation reduces the accuracy of the shelf-life determinations (see chapter 1, section 1.3.4.3). Also OVQ and browning, but this time together with dryness determined the shelf-life of NEW treated samples at day 9. Therefore, NEW treatment extended the shelf-life of MP cabbage stored at 7℃ by at least 3 days. In general, the most important attribute in determining shelf-life was OVQ.

Browning is a very well known problem in cut cabbage. NEW arrested browning at both temperatures. This is in agreement with findings by Koseki and Itoh (2002) where AcEW treated MP cabbage presented less browning than water washed controls after 5 days of storage at 5℃ and 10℃. It is remarkable how an oxidant such as EOW can prevent an oxidative process such as enzymatic browning. As it was mentioned in chapter 1, section 1.7.6; Koseki and Itoh (2002) postulated that since AcEW has a strong ORP, the enzymes may have been oxidized and weakened by EOW. However, it is easier to oxidize a phenol than an enzyme such as polyphenol oxidase (PPO), so that browning should occur before the enzyme is inactivated. It is possible that ions derived from NaCl used to produce NEW could have played some role in PPO inhibition. The inhibition of PPO by NaCl has been reported (Gómez-López, 2002). It is believed that the inhibition of PPO by NaCl is due to formation of a complex between the halide ion and copper in the enzyme

(Zawistowski et al., 1991). Also Brecht (1995) reported the inhibition of browning of snap beans by rinsing in 175 ppm NaOCl. Also Fukumoto et al. (2002) and Beltrán et al. (2005b) observed browning inhibition by using chlorinated water (see chapter 1, section 1.6.3). Furthermore, a more complex reason is foreseeable, more related to the physiology of the vegetable than to a simple enzymatic oxidation.

Samples stored at 4°C did not show dehydration as evaluated by the dryness sensory attribute by the panel. At 7°C, dryness was detected by the panel at day 8 in control samples, two days after those samples have already been rejected due to other sensorial defects. For NEW treated samples stored at 7°C however, dryness was a shelf-life determinant but together with OVQ and browning. Therefore, it can be concluded that in general the packaging configurations used in the present study were suitable to preserve the humidity of the produce. However, tests controlling the relative humidity outside the package would be necessary to perform in order to establish definitive conclusions.

No problems of off-odour and bad flavour were detected by the panel, except for bad flavour after 14 days of storage control samples at 4°C, when they had already been rejected 5 days before due to bad OVQ. These results can be related to the low microbiological counts, which never reached the microbial limit.

6.4.6 Changes in pH during storage

Samples treated with NEW had initially a lower pH than water washed ($\alpha \leq 0.05$). It is possible that NEW reacted with some compounds of the cabbage tissue producing acids. However, Izumi (1999) reported that NEW did not decrease the pH of five different MPV (carrots, spinach, bell peppers, Japanese radish and potatoes) in comparison with water washed samples.

pH raised in all samples during storage (Figure 6.3). Also Fantuzzi et al. (2004) reported a pH increase from 5.6 to 6.5 in MP cabbage after 20 days of storage at 1°C and 5°C. King et al. (1991) and Jacxsens et al. (2003) found the same change for MP lettuce. The last authors stated that the pH augment is typical for vegetables in which Gram-negative microorganisms play an important role in the spoilage, and that can

result from the breakdown of proteins with the release of basic compounds as result. Consequently, the slower increase in pH at 4℃ in comparison with 7℃ could be consequence of the slower microbiological growth at 4℃. It is also known that the pH of cabbage can decrease due to LAB action. No LAB growth was however detected during storage, which is consistent with the observed pH changes.

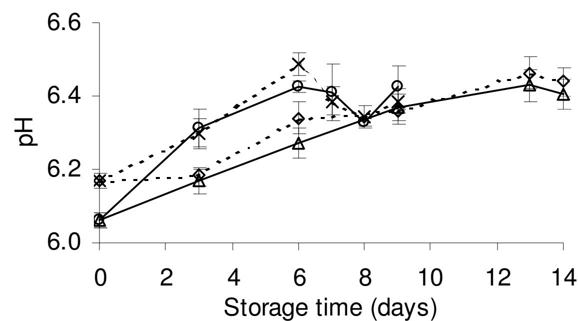

Figure 6.3. Changes in pH of during storage of MP cabbage under EMAP at different storage temperatures. Control at 7℃ (---X---), NEW treated at 7℃ (—○—), control at 4℃ (---◇---), NEW treated at 4℃ (—△—). Error bars show SD, n=3.

6.5 Conclusions

In conclusion, a shelf-life extension of at least 5 days and 3 days in samples stored respectively at 4℃ and 7℃ can be achieved by treating MP cabbage with NEW. This benefit is related to a slow down in sensory deterioration. OVQ was the most important parameter in determining shelf-life. The reproducibility of this result should be confirmed according to the idea expressed in chapter 1, section 1.3.4.2. However, these results together to those obtained by Koseki and Itoh (2002) on the use of AcEW give a good evidence that EOW can inhibit browning. Therefore, NEW seems to be a promising method to prolong the shelf-life of MP cabbage.

Although NEW was used as a decontamination method, its preservative effect was limited by the sensory quality of a product, which never reached high microbial populations. It should be interesting to test NEW (or AcEW) in a MPV where microorganisms play a more direct role in determining the shelf-life, in order to establish a more general conclusion encompasing its effects on a bigger variety of MPV.

General discussion, conclusions and perspectives

General discussion, conclusions and perspectives

General comment

This research was pursued with the aim of recommending to the food industry one or several decontamination treatments to prolong the shelf-life of minimally processed vegetables (MPV), based on scientific facts.

Classical methods in food science and technology, such as APC and sensory evaluation, are used to evaluate the effect of decontamination methods on the shelf-life of MPV. MPV are however relatively new products. The decontamination of MPV has been mainly focused on pathogen inactivation, and it is also relatively new. Searching for novel decontamination methods for MPV started when food technologists realized that, due to several reasons, they could not rely on chlorinated water for complete pathogen inactivation. Research on the application of decontamination methods to prolong the shelf-life of MPV is very recent, as most of the articles on this subject have been published during the time span of the development of this PhD. It is a new research area that applies old tools to measure the effect of novel preservation alternatives, but having some methodological weaknesses that should be addressed before accomplishing the aforementioned goal.

The present research started assuming that in a general way, the decontamination of a MPV would yield a prolongation of its shelf-life. Results have showed that that was not true. The growth rate in decontaminated samples can even be faster than in control samples. Therefore, after some time under storage, microbial counts from both sources equal, and the benefit of the decontamination is lost. Hence, it is not only necessary to decontaminate, but also to keep microbial counts lower in treated than in control samples.

A successful prolongation of the shelf-life of MP cabbage and MP carrots was found in this research. The research performed in this thesis as well as most of this kind of research was not replicated. A successful result in one experiment, although encouraging, has never been conclusive in scientific research. In the same way, an

unsuccessful result is not enough to discard a new decontamination method. Replication of experiments should enable to quantify the variability associated with processing different batches.

This research tried to test the effect of decontamination methods on MPV as such, in order to evaluate their advantages and limitations. During the development of this work, own as well as others´ results showed the drawbacks of a decontamination method, namely, the induction of browning in MP cabbage by gaseous chlorine dioxide. This research showed that it was possible to suppress that browning through L-cysteine treatment.

Factors affecting the inactivation of microorganisms by intense light pulses

Despite of some research that has been done on the factors affecting the inactivation of microorganisms by intense light pulses (ILP), some basic study was still necessary to serve as a bridge between basic and applied research, chapter 2 tried to cover this gap. Previous work has demonstrated that microorganisms differ in their susceptibility to ILP, however, they were performed with a limited number of microorganisms. Chapter 2 of this thesis listed the susceptibility of 26 different species of microorganisms, however, no pattern was observed among different groups. The assessment of different enumeration methods to evaluate the efficacy of microorganism inactivation by ILP showed that results reported in the literature using the incubation method likely overestimated the inactivation efficacy of ILP. Moreover, the strike method is recommended to measure the lethality of ILP towards microorganisms illuminated in Petri dishes. Early literature coming from industrial sources claimed that ILP inactivates the photoreactivation mechanism of illuminated cells, in contrast with continuous UV. This thesis shows that photoreactivation is possible in flashed cells, and consequently Petri dishes in experimental protocols should be protected from visible light after flashing. Chapter 2 also proved the importance of the appropriate placement of the samples in order to achieve an optimal treatment, and also the inefficacy of ILP to decontaminate samples possessing microbial counts in excess of a certain threshold. The development of resistant microflora is not expected to develop according to the results of this thesis. Heating of the agar surface was observed during flashing, which could cause

problems for the practical implementation of ILP decontamination of heat-sensitive products such as MPV. The heating might however be beneficial or even act synergistically for other kind of commodities.

Intense light pulses decontamination of minimally processed vegetables and their shelf-life

Chapter 3 continues the exploration of the basics of the application of ILP for inactivation of microorganisms present on food surfaces started in chapter 2, showing, by using *in vitro* conditions, its suitability to inactivate bacteria on vegetable surfaces. Next to it, the efficacy of ILP on vegetable surfaces was proven. The results of chapter 2 regarding the appropriate placement of the samples in the treatment chamber were used to set the disinfection protocol for MPV. The application of decontamination treatments on MPV and the shelf-life studies started in chapter 3. ILP was shown to affect the respiration rate of MP lettuce but not that of MP cabbage, revealing interspecific susceptibility differences. ILP was unsuccessful to prolong the shelf-life of two MPV. However, the considerations stated in chapter 1 should be taken into account before drawing definitive conclusions, specially the importance of the storage conditions such as temperature. From the microbial point of view, one extra storage day at 7°C was achieved for MP Iceberg lettuce, however, samples were rejected due to bad overall visual quality. It is therefore possible that the application of an antibrowning treatment in combination with ILP could increase the shelf-life of MP iceberg lettuce.

The crossing of the growth curves of microorganisms present in treated and untreated samples was observed in this thesis for the first time in chapter 3. Psychrotroph counts determined the shelf-life from the microbiological point of view in both, MP lettuce and MP cabbage, a result that repeated itself during the rest of the experiments of this thesis. Consistently with chapter 3, sample heating posed a problem for treatment of MPV, and limited the treatment time.

Decontamination of minimally processed vegetables by gaseous and liquid chlorine dioxide and neutral electrolysed oxidising water

Chapter 4 opens the study of chemical disinfection methods. Since different time-concentration combinations were used to test the efficacy of liquid disinfectants, more general conclusions can be derived from this chapter. Aqueous ClO_2 resulted effective to decontaminate MP carrots without affecting sensory quality, but not MP lettuce or MP cabbage. NEW resulted effective to decontaminate MP carrots, MP lettuce, and MP cabbage without affecting sensory quality. Gaseous ClO_2 was effective to decontaminate several types of MP lettuce, MP carrots, and MP cabbage; however, it browned MP Iceberg lettuce and MP cabbage. Furthermore, gaseous ClO_2 was more effective than liquid ClO_2 to decontaminate MP Iceberg lettuce, MP white cabbage, and MP carrots. These results formed the basis to perform chapter 5 and chapter 6, which are shelf-life studies corresponding to some of the results of these decontamination treatments. Browning caused by gaseous ClO_2 to MP lettuce and MP cabbage suggested the necessity of the use of an antibrowning agent, which was used in chapter 5.

Shelf-life of minimally processed carrots, lettuce, and cabbage treated with gaseous ClO_2

Chapter 5 of this thesis showed that gaseous ClO_2 can prolong the shelf-life of MP carrots. The shelf-life extension was limited by microbial growth. Three aspects of the evolution of microbial populations are remarkable. 1) A lag phase was observed in mesophile, psychrotroph, and LAB populations of treated samples, which is uncommon in this kind of studies and suggests a sub-lethal damage to the microbial cells, which did not occur with the possibly more ClO_2 resistant yeasts. 2) The growth rate of the microbial populations after the lag phase was similar, therefore, the counts of the ClO_2 treated product did not equal those of the untreated product. 3) The shelf-life of control and treated samples was limited by different microbial populations. This kind of shelf-life extension might be more difficult to replicate because it will depend on initial levels and grow characteristics of two different groups of microorganisms. Since yeast growth constituted the main problem here for shelf-life extension, it could be suggested to combine ClO_2 treatment with other disinfectant targeting the yeast population. The off-odour detected in the treated samples after 7 days of storage might have been related to yeast action, since at that time only yeasts were present at relatively high levels. This possibility reinforces the idea that suppression of yeast

growth could lead to a longer and/or consistent shelf-life than the one achieved in this study.

Chapter 5 also proved that the browning occurring during treatment of MP lettuce and MP cabbage with gaseous ClO_2, which was observed in chapter 4 and reported at that time by Sy et al. (2005b), can be inhibited by using an antibrowning compound, L-cysteine in this case. This substance, however, decreased the antimicrobial efficacy of gaseous ClO_2 on MPV. No shelf-life prolongation was however achieved. Although significant reductions were attained immediately after treatment in most of the microbial groups, the microbial populations in treated samples became higher than controls and reached the microbial limit. Since gaseous ClO_2 has been previously recommended to produce safer MP cabbage, its use together with a browning inhibitor could be feasible for practical implementation. The outcome would be a safer MP cabbage with the same shelf-life of an untreated MP cabbage.

Shelf-life of minimally processed cabbage treated with neutral electrolysed water and stored under equilibrium modified atmosphere

The decontamination efficacy of NEW on several MPV was proven in chapter 4. Chapter 6 tested and proved that NEW can prolong the shelf-life of a MPV, namely MP cabbage. Washing with NEW significantly reduced psychrotroph counts but not APC, LAB, and yeast counts; decreased the pH of MP cabbage but did not affect its respiration rate or sensory quality. Therefore, NEW treatment showed to be a decontamination method mild enough to decrease microbial counts without affecting negatively the vegetable matrix. Once more the effect of the decontamination was readily lost during storage at 7℃. Different from chapters 3 and 5, a storage temperature of 4℃ was also used in this work. The purpose was to avoid that microbial counts of treated samples reached fast the same levels than those of controls, according to reasons explained in chapter 1. In that chapter, the literature review showed that the decontamination effect is kept when using storage temperatures between 0 and 4℃. The attempt was unsuccessful. Yet, this was one isolated case, therefore the assertion of chapter 1 is worth to be tested again. Faster

growth rates in MP white cabbage and MP Iceberg lettuce treated with AcEW were reported by Koseki and Itoh (2001).

No microbial population reached the microbial limit during the storage time. This thesis proved, however, that NEW arrests the visual deterioration of MP cabbage, which accounted for the observed shelf-life extension. Browning inhibition has been reported before for MP white cabbage treated with acidic electrolysed water (Koseki and Itoh, 2002), but not for treatment with NEW.

Although NEW was used as a decontamination method, its preservative effect was limited by the sensory quality of a product, which never reached high microbial populations. It should be interesting to test NEW (or AcEW) in a MPV where microorganisms play a more direct role in determining the shelf-life, in order to establish a more general conclusion encompasing its effects on a bigger variety of MPV.

Perspectives

Photoreactivation of ILP treated cells on Petri dishes was shown in this thesis. Consequently, the assessment of the importance of photoreactivation in cells present on the surface of vegetables should be of paramount importance for an industrial application. This evaluation could lead to two possibilities. The first one is that photoreactivation is not important from the practical point of view. The second is that it is relevant. In the latter case, studies should be performed to find out how long a MPV has to be stored in darkness to avoid photoreactivation, and how feasible this dark storage is from the industrial point of view. Alternatively, the use of more stringent treatment regimes could be an alternative as long as the MPV do not result damaged. The possible existence of the dark repair mechanism in flashed cells should be also evaluated.

The combination of intense light pulses with photosensitization could be tested as a way to produce a more efficient decontamination process. This process would combine the effect of the UV part of the flash light with the effect of the effect of the visible part of the flash light on the photosensitizer.

The effect of population size and growth stage of microorganisms on the microbicidal efficacy of ILP should be evaluated in separate. Flashing cells in the same growth stage at different levels of inoculation on Petri dishes should enable to determine the effect of population size. Additionally, flashing cells at different growth stages but the same level of inoculation on Petri dishes should enable to determine the effect of the growth stage.

The decontamination methods used in this work should be tested with other MPV in order to evaluate more extensively their possibilities. Also, optimisation of the use of antibrowning compounds to suppress darkening of some MPV due to ClO_2 should be studied, especially to avoid the decrease of the antimicrobial efficacy caused by the use of L-cysteine.

Since ours results show that ClO_2 can promote colour changes in some vegetables, one can speculate some potential problems when this decontaminant is tested with other MPV. Green vegetables such as several types of lettuce, spinach, green pepper might suffer from bleaching due to chlorophyll degradation. Purple vegetables such as Lollo Rosso lettuce might suffer of anthocyanin degradation. White vegetables or vegetables with some extension of white surfaces such as white cabbage, Chinese cabbage, Iceberg lettuce, celeriac, and white asparagus can suffer from browning due to oxidation of phenolic compounds by chlorine dioxide. These changes might be dose-dependent. The careful choice of appropriate time-concentration conditions seems to be a delicate task that researchers should assume when testing the application of gaseous chlorine dioxide to other vegetables and MPV.

Since ours results show that NEW can protect a MPV from darkening during storage, one can speculate that NEW might be useful for treatment of vegetables susceptible to enzymatic browning such as white cabbage, chicory endive, celeriac, and Iceberg lettuce. However, we could not prove its efficacy to prolong shelf-life from the microbiological point of view. Since NEW is a technique also useful to decontaminate washing water, it has potential for use in the washing of MPV in order to avoid cross-contamination due to the pollution of water during the washing step. The resultant wastewater can be recycled towards the electrolytic cell of the NEW unit to become

decontaminated. Using another technique, the decontamination itself of the produce could be performed, unless NEW is proved to extend the shelf-life from the microbiological point of view as well. In such a case, it would be also useful for overall shelf-life extension by itself.

The five approaches commented in chapter 1 should be integrated for completeness of the evaluation of a novel decontamination method, namely reducing and assessing the risk of foodborne infections and intoxications, decreasing microbial spoilage, preserving fresh attributes, preserving nutritional and phytochemical quality, and evaluating the possible toxicity of treated products. The last two aspects deserve to be better evaluated, specially regarding antioxidative capacity and other nutritionally important substances.

Literature shows that if a pathogen is present in a MPV, decontamination will enhance its growth rate, probably because of a lack of competition and/or a decrease of the resistance of the vegetable matrix to microbial attack caused by the decontamination method. Hence, challenge tests should be performed in order to evaluate how much the risk of a foodborne infection or intoxication is increased as a function of different levels of inactivation of the natural microflora.

Research should be performed to look for the possibility to predict the extension of shelf-life of a specific MPV as a function of the level of decontamination.

It is of foremost importance for an industrial application to assess the variability in shelf-life extension and expiration date among batches treated under the same conditions. This thesis showed that only some groups of microorganisms are relevant for the commodities and experimental conditions used. For example, LAB growth never determined shelf-life. Replication of experiments have to show if that result is consistent in order to further determine only the microbial groups that are indeed relevant.

Equations such as that proposed by Corbo et al. (2006) should be used to estimate the variability within batches, and to assess the effect of decontamination methods. The rejection of samples due to a mean reaching certain microbial limit should be

substituted by criteria taken into account variability. An option might be to use attribute plans such as those used to approve or reject lots according to the counts of certain pathogens. However, before that, a better understanding of the relationship between microbial growth and product spoilage should be achieved, which should be the basis for assigning product specific microbial limits.

Future studies should eliminate the risk of finding the expiration date by interpolation. An effort should be made to evaluate the quality of MPV daily within the expected expiration date. An alternative approach could be the use of predictive microbiology to better interpolate results.

An effort should be made to try to keep microbial counts lower in treated than in untreated samples. Refrigeration temperatures of 4°C or less seem to be suitable to accomplish this goal. A real understanding of the enhancement of growth rates after decontamination would help to better design the most appropriate strategies.

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SUMMARY

SUMMARY

Chapter 1 consists in a critical literature review on decontamination methods for minimally processed vegetables (MPV). The structure of what should be a complete assessment of the effect of decontamination methods to prolong the shelf-life of MPV is proposed. Thereafter, the problems derived from the accessibility of sanitizers to microorganisms are discussed in its different aspects.

A critical analysis of the methodological problems inherent to the current protocols used to evaluate the efficacy of decontamination treatments to prolong the shelf-life of MPV is exposed in this chapter. This is the most important part of the literature review, since the overall conclusions derived from this thesis are formulated according to the limits stated thereby.

Different decontamination methods are then exposed. The literature about the methods tested in this thesis is reviewed, namely intense light pulses, electrolysed oxidizing water and chlorine dioxide in its aqueous and gaseous phases. Some other treatments were also included because of different reasons: continuous UV treatment is very closely related to intense light pulses, chlorine is the most used sanitizer, ozone is gaining importance as disinfectant of MPV and it can compete with ClO_2 as substitute of chlorine. The review of these methods comprehended a critical revision of the mechanism of action. Furthermore, their effects on MPV are compiled according to the five approaches mentioned at the beginning of the literature review: 1) human pathogens, 2) spoilage microflora, 3) sensory attributes, 4) MPV physiology, nutrients and phytochemicals; and 5) presence of toxic compounds.

In **chapter 2**, the results of some basic research necessary to apply intense light pulses (ILP) to food decontamination is exposed. The susceptibility of different groups of microorganism was tried to set, but a clear pattern was not observed. A critical assessment of the methods used to measure the inactivation of microorganisms on Petri dishes was performed, and also its relation with the shading effect and the photoreactivation phenomenon. The incubation method, commonly used in this kind of research, was found to overestimate the decontamination by ILP,

and the strike method is recommended as the most suitable. Inactivation of microorganisms by ILP was related to both the photochemical and the photothermal effect, and an equation of level of inactivation as function of the distance between the light source and the illuminated body was proposed (R^2=0.96). Photoreactivation was proved to occur in flashed microorganisms. The effect of the placement of the sample relative to the lamp was established as well. The decontamination efficacy of ILP as function of the cell density was also studied, as well as the increase in temperature as function of the number of flashes and the depth of the illuminated body. Finally, the possible development of resistant microflora was tested.

Chapter 3 demonstrated that proteinacieous or fatty foods are not suitable for decontamination with ILP, while vegetables could be suitable matrixes for ILP treatment. This was demonstrated by reaching as high as 2 log CFU/g reductions on aerobic plate counts (APC) of minimally processed (MP) Iceberg lettuce, as well as decontamination of seven other MPV with varied efficacy. Subsequently, MP white cabbage and MP Iceberg lettuce were flashed and stored under modified atmosphere at 7°C, in order to assess the effect of ILP on the shelf-life of MPV. The respiration rate of MP Iceberg lettuce was increased by ILP, while that of MP white cabbage was not, revealing differences in susceptibility between vegetables. No shelf-life prolongation was realized. An unacceptable overall visual quality (OVQ) was the common shelf-life limiting factor of both untreated and ILP treated MP Iceberg lettuce, reaching the acceptability limit at the third day of storage. Aerobic psychrotroph count limited the shelf-life of MP white cabbage after its levels surpassed those of the untreated samples. Although ILP did not yield promising results, other assay conditions or commodities might produce successful results.

Chapter 4 opens the study on the use of chemical decontaminants in this thesis. The effectiveness of gaseous and liquid ClO_2, as well as neutral electrolysed oxidizing water (NEW) to decontaminate MPV, and their effects on the sensory quality are presented thereby. APC was used as a microbial indicator and a triangle test was used to assess possible differences between the sensory attributes of untreated and treated samples. Samples washed with water were used as controls. Gaseous ClO_2 yielded more than 1 log reduction in MP carrots, MP Lollo Bionda lettuce, MP Lollo Rosso lettuce, MP Iceberg lettuce and MP white cabbage. Browning of the last two

commodities was however observed, which is in line with results reported by Sy et al. (2005b). The reduction in MP green bell peppers was merely 0.75 log units. Therefore, gaseous ClO_2 was found to be an efficient decontaminant for a number of MPV. Liquid ClO_2 reduced significantly the APC of MP carrots but not those of MP white cabbage and MP Iceberg lettuce; moreover this treatment produced differences in the sensorial attributes on the last commodity. For MP carrots, the longer the contact time and the higher the ClO_2 concentration, the higher the decontaminant effect ($\alpha \leq 0.05$). The interaction between both independent factors was however not statistically significant ($\alpha \leq 0.05$). NEW yielded more than 1 log reduction for MP carrots, MP Iceberg lettuce, and MP white cabbage without affecting their sensorial properties.

The potential of gaseous ClO_2 to prolong the shelf-life of MPV was tested on MP carrots, MP Iceberg lettuce and MP white cabbage; the respective results are presented in **chapter 5**. Chapter 4 had already shown the decontaminant efficacy of gaseous ClO_2 on these commodities as well as the induction of browning in the last two. Those results formed the basis to develop this part of the thesis. With regard to MP carrots, white blushing is a defect of shredded carrots, which is consequence of dehydration occurring when being in dry environment. Moreover, ClO_2 seems to be more effective at high relative humidities. Therefore, the combination of high relative humidity and gaseous ClO_2 seemed to be suitable for the decontamination of this product. The decontamination levels achieved after ClO_2 treatment were 1.88, 1.71, 2.60, and 0.66 log units for APC, psychrotrophs, lactic acid bacteria and yeasts respectively without affecting the sensory quality. A lag phase of 2 days was observed in APC, psychrotrophs, and LAB of treated samples but not in those of untreated samples. This is an uncommon result in this kind of experiments. During storage under modified atmosphere packaging (MAP) at 7°C, psychrotroph growth limited the shelf-life of ClO_2 treated samples, while yeast growth limited the shelf-life of untreated samples. Odour was the only sensory attribute evaluated negatively by the sensory panel, but it was not a shelf-life limiting factor. The shelf-life of MP carrots was essentially extended by one day due to gaseous ClO_2. The low inactivation level attained for yeasts, together with the apparent absence of a lag phase in the growth curve of yeasts accounted for the limited shelf-life prolongation.

With regards to MP Iceberg lettuce and MP white cabbage, since the change in colour of these commodities during gaseous ClO_2 treatment was similar to that typical of enzymatic browning, it was hypothesized that an antibrowning compound that does not act on polyphenol oxidase could suppress it, as for example, L-cysteine. The immersion of samples in a L-cysteine solution was successful in suppressing the browning induced by ClO_2. L-cysteine however decreased significantly the decontaminant efficacy of gaseous ClO_2 on MPV. Gaseous ClO_2 failed to prolong the shelf-life of both commodities. It was observed that mesophilic and psychrotroph populations of treated MP lettuce reached those of the untreated samples before the third day of storage under MAP at 7°C. Therefore the benefit of the decontamination was readily lost. The shelf-life of untreated and ClO_2 treated MP lettuce was essentially limited to 4 days. OVQ and leaf edge browning determined the shelf-life of the untreated product, while the ClO_2 treated product suffered additionally from off-odour, bad flavour, leaf surface browning, and psychrotroph growth. Therefore, gaseous ClO_2 seems to have pernicious effects on the sensorial stability of the product and facilitates the growth of some groups of microorganisms.

With regard to MP white cabbage, it was shown in chapter 5 that neither the microbial counts nor the sensory attributes of both, untreated and ClO_2 treated MP cabbage reached unacceptable values after 9 days of storage under MAP at 7°C. It is remarkable however that the microbial counts of ClO_2 treated samples were higher than those of untreated samples. Although no shelf-life prolongation was realized, the literature shows that gaseous ClO_2 is an effective disinfectant of MP cabbage for human pathogens. Therefore, its use together with immersion in L-cysteine solution might be useful to produce a microbiologically safe MP cabbage, without ClO_2-induced browning, and the same shelf-life of the untreated counterpart.

In **chapter 6**, the effect of NEW on the shelf-life of MP cabbage was tested. It was demonstrated in chapter 4 that NEW can decontaminate MP cabbage without affecting its sensory attributes. Chapter 6 tested if NEW can prolong the shelf-life of a MPV stored under EMAP at 4 and 7°C. The benefit of the decontamination was lost after 3 days at both temperatures, but no microbial population reached the microbial limits. Moreover, they reached the stationary phase, indicating that they would have never reached the limits even in the case of longer storage times. Therefore

microbial growth was not a shelf-life limiter. A shelf-life extension was however achieved due to preservation of the sensory quality. The shelf-life at 4℃ of controls was limited to 9 days due to bad OVQ, while MP cabbage treated with NEW remained acceptable during the 14 days of the storage experiment. At 7℃, the shelf-life of controls was limited to 6 days by OVQ and browning, while that of samples treated with NEW were limited to 9 days by OVQ, browning, and dryness. Therefore, NEW prolonged the shelf-life of MP cabbage by at least 5 days at 4℃, and by 3 days at 7℃. The chlorine ions present in NEW might have inhibited polyphenol oxidase, accounting for the browning inhibition.

SAMENVATTING

SAMENVATTING

Hoofdstuk 1 omvat een kritische literatuurstudie over decontaminatie-technieken voor minimaal behandelde groenten (MBG). Er wordt een structuur voorgesteld van een volledige beoordeling over de effecten van decontaminatie-technieken om de houdbaarheid van MBG te verlengen. Problemen betreffende de bereikbaarheid van decontaminatie-producten tegenover de micro-organismen, worden daarna in hun verschillende aspecten besproken.

In dit hoofdstuk wordt een kritische analyse weergegeven van de methodologische problemen die eigen zijn aan de gebruikte protocols om de efficiëntie van de decontaminatie-technieken in het verlengen van de houdbaarheid te bepalen. Dit is het belangrijkste deel van de literatuurstudie aangezien de algemene conclusies, afgeleid van de resultaten in deze thesis, geformuleerd werden rekening houdend met de vermelde beperkingen.

Verschillende decontaminatie-technieken worden vervolgens uiteengezet. Er werd een literatuur overzicht gemaakt van de methoden die in deze thesis gebruikt werden, meer bepaald intense lichtflitsen, elektrolyse water en chloordioxide in vloeibare en in gasvorm. Enkele andere technieken werden eveneens besproken om verschillende redenen: continu UV licht aangezien dit sterk gerelateerd is met intense lichtflitsen, chloor aangezien dit het meest gebruikte decontaminatieproduct is en ozon omdat dit steeds belangrijker wordt als decontaminatietechniek voor MBG en omdat het in competitie kan gaan met ClO_2 as substituut voor chloor. Dit overzicht bevat eveneens een kritisch overzicht van de inactivatiemechanismen en de effecten op MBG volgens de vijf benaderingen die vermeld werden in het begin van de literatuurstudie verwijzend naar het effect van de geselecteerde decontaminatiebehandelingen op 1) pathogenen voor de mens, 2) bederfflora, 3) sensorische kenmerken, 4) fysiologie, nutriënten en fytochemicaliën; en 5) aanwezigheid van toxische bestanddelen.

In **hoofdstuk 2** worden de resultaten voorgesteld van enkele basis onderzoeken, nodig om de behandeling met intense lichtflitsen (ILF) toe te passen voor

decontaminatie van voeding. Er werd getracht om de gevoeligheid van verschillende micro-organismen te bepalen, maar er werd geen duidelijk patroon waargenomen. Er werd een kritische evaluatie uitgevoerd van de methoden om de inactivatiegraad van micro-organismen in petriplaten te bepalen, en de relatie met de verschijnselen 'schaduweffect' en 'fotoreactivatie' werden eveneens onderzocht. De incubatiemethode, meestal gebruikt in dit soort onderzoek, toonde een overschatting aan van de decontaminatie met ILF, en de strijkplaat methode werd als de meest geschikte bevonden. Een verband werd gevonden tussen inactivatie van micro-organismen door ILF en zowel fotochemische als fotothermische effecten en er werd een vergelijking opgesteld van inactivatiegraad in functie van de afstand tussen de lichtbron en het geflitste object (R^2=0,96). Er werd aangetoond dat fotoreactivatie optrad in de geflitste micro-organismen. Eveneens werd de invloed van de plaats van het behandelde object in de flitskamer bepaald. De decontaminatie efficiëntie van ILF in functie van de celdensiteit werd bestudeerd, evenals de stijging van temperatuur in functie van het aantal flitsen en de diepte van het geflitste object. Er werd geen immune microflora gedetecteerd.

Hoofdstuk 3 toonde aan dat proteïn – of vetrijke voeding niet geschikt zijn voor decontaminatie met ILF, in tegenstelling tot groenten die wel geschikt bevonden waren voor ILF behandeling. Dit laatste werd aangetoond doordat er tot 2 log reductie in aerobe plaattellingen (APT) van minimaal behandelde (MB) ijsbergsla werd bekomen, alsook een decontaminatie van zeven andere minimaal bewerkte groenten met variërende efficiëntie. MB witte kool en MB ijsbergsla werden geflitst en bewaard onder beschermde atmosfeer bij 7°C, om het effect van ILF op de houdbaarheid van MBG te beoordelen. De respiratie snelheid van MB ijsbergsla was toegenomen na het flitsen terwijl dit niet het geval was MB witte kool, wat verschillen in gevoeligheid tussen de groenten aantoont. Er werd geen verlenging van de houdbaarheid geconstateerd. Een algemeen onaanvaardbare visuele kwaliteit (OVK) was de gemeenschappelijke limiterende factor betreffende houdbaarheid van zowel onbehandelde als met intense lichtflitsen behandelde MB ijsbergsla en waarbij de aanvaardbaarheidslimiet op de derde dag van bewaring werd bereikt. De houdbaarheid van MB witte kool werd beperkt door aerobe psychrotrofe aantallen nadat hun aantal in behandelde stalen deze van onbehandelde stalen overschreed.

Alhoewel de bekomen resultaten met ILF niet veelbelovend zijn, zouden andere testcondities of producten betere resultaten kunnen opleveren.

Hoofdstuk 4 bespreekt de studie van het gebruik van chemische decontaminatietechnieken in deze thesis. De effectiviteit van gasvormig en vloeibaar ClO_2 enerzijds en dit van neutraal geëlectrolyseerd oxiderend water (NGW) anderzijds voor de decontaminatie van MBG en hun effecten op de sensorische kwaliteit werden onderzocht. APT werd gebruikt als een microbiologische indicator en triangel testen werden gebuikt om de mogelijke verschillen in sensorische eigenschappen tussen behandelde en niet-behandelde stalen vast te stellen. Stalen gewassen met water werden gebruikt als controle. Gasvormig ClO_2 leidde tot meer dan 1 log reductie in MB wortelen, MB groene krulsla, MB rode krulsla, MB ijsbergsla en MB witte kool. Bruinkleuring werd geobserveerd voor de laatste twee, wat overeenstemt met de resultaten van Sy et al. (2005b). De reductie in MB groen paprika bedroeg 0,75 log eenheden. Bijgevolg kan gasvormig ClO_2 beschouwd worden als een efficiënte decontaminatietechniek voor een verscheidenheid aan bladgroenten. Vloeibaar ClO_2 verminderde significant de APT bij MB wortelen maar niet deze bij MB witte kool en MB ijsbergsla. Bovendien gaf deze behandelingen voor deze laatste verschillen in sensorische eigenschappen. Voor MB wortelen, nam het decontaminatie effect toe ($\alpha \leq 0.05$) bij een langere contacttijd en een hogere ClO_2 concentratie. De interactie tussen beide onafhankelijke factoren was echter niet statistisch significant ($\alpha \leq 0.05$). NGW leidde tot meer dan 1 log reductie voor MB wortelen, MB ijsbergsla en MB witte kool zonder de sensorische eigenschappen te beïnvloeden.

De mogelijkheid van gasvormig ClO_2 om de houdbaarheid van MBG te verlengen, werd getest op minimaal behandelde wortelen, ijsbergsla en witte kool; de overeenkomstige resultaten zijn weergeven in **hoofdstuk 5**. In hoofdstuk 4 werd het decontaminerend effect van gasvormig ClO_2 op deze producten reeds aangetoond alsook het optreden van bruinverkleuring in de laatste twee producten. Deze resultaten vormden de basis voor de ontwikkeling van de dit deel van de thesis. Wat de minimaal behandelde wortelen betreft, witte blozen is een verschijnsel bij geraspte wortelen dat ontstaat door dehydratie wanneer ze zich in een droge omgeving bevinden. Bovendien blijkt ClO_2 meer effectief te zijn bij hoge relatieve vochtigheid. Daarom bleek de combinatie van een hoge relatieve vochtigheid en

gasvormig ClO_2 geschikt te zijn voor de decontaminatie van dit product. De decontaminatie niveaus die bereikt werden na ClO_2-behandeling waren 1,88, 1,71, 2,60 en 0,66 log-eenheden voor het totaal aëroob kiemgetal, psychrotroof kiemgetal, melkzuurbacteriën en gisten, respectievelijk en dit zonder de sensorische kwaliteit te beïnvloeden. Een lagfase van twee dagen werd waargenomen voor wat betreft het totaal aëroob psychrotroof kiemgetal, het aantal melkzuurbacteriën en het aantal gisten in de behandelde stalen maar niet in de onbehandelde stalen. Dit is een ongewoon resultaat voor dergelijke experimenten. Tijdens bewaring onder gemodificeerde atmosfeer bij 7°C werd de houdbaarheid van de ClO_2-behandelde stalen gelimiteerd door de groei van psychrotrofen, terwijl de houdbaarheid van de onbehandelde stalen beperkt werd door groei van gisten. De geur was het enige sensorische attribuut dat negatief beoordeeld werd door het sensorisch panel, maar het was niet houdbaarheidsbepalend. De houdbaarheid van minimaal behandelde wortelen werd verlengd met één dag door het gasvorming ClO_2. De lage inactivatie niveaus voor de gisten, samen met de afwezigheid van een lagfase in de groeicurve van de gisten, verklaart de beperkte houdbaarheidsverlenging.

De bruinkleuring waargenomen gedurende de ClO_2 behandeling van MB Iceberg sla en MB witte kool was gelijkaardig aan de typische enzymatische bruinkleuring. De hypothese werd gesteld dat een antibruiningscomponent, niet gebaseerd op polyphenol oxidase, de kleurverandering kan tegengaan. Hoewel de intensie er niet was om deze hypothese te valideren werd er aangetoond dat het onderdompelen van de stalen in een L-cysteïne oplossing suucesvol was in het tegengaan van de ClO_2 geïnduceerde bruinkleuring. L-cysteïne veroorzaakte een significante vermindering van het decontaminerende effect van gasvormig ClO_2 op MBG. Er werd geobserveerd dat mesofiele en psychrotrofe populaties van behandelde MB sla deze van de niet-behandelde stalen bereikten vóór de derde dag van bewaring onder gemodificeerde atmosfeer bij 7°C. Hierdoor ging het voordeel van de decontaminatie snel verloren. Bijgevolg faalde gasvormig ClO_2 in het verlengen van de houdbaarheid van beide groenten. De houdbaarheid van ClO_2 behandelde en onbehandelde MB sla was gelimiteerd tot 4 dagen. Verwerpingscriteria voor onbehandelde stalen waren OVK en bruinkleuring aan de bladrand. De ClO_2 behandelde stalen werden verworpen door slechte geur, slechte smaak, bladrandverkleuring en psychotrofe groei. Hieruit blijkt dat gasvormig ClO_2 een schadelijk effect heeft op sensorische

stabiliteit van het product en het vergemakkelijkt de groei van sommige groepen micro-organismen.

Wat betreft MB witte kool, werd in hoofdstuk 5 aangetoond dat noch de microbiële tellingen, noch de sensorische eigenschappen van zowel onbehandelde als met ClO_2 behandelde MB kool, onaanvaardbare waarden bereikte na 9 dagen van opslag onder gemodificeerde-atmosfeerverpakkingen bij 7°C. Nochtans waren de microbiële tellingen van ClO_2-behandelde stalen hoger dan die van de onbehandelde stalen. Hoewel geen verlenging van de houdbaarheid werd gerealiseerd, werd wel aangetoond dat ClO_2-gas een effectieve desinfectant is tegen humane pathogenen op MB kool. Om die reden zou het gebruik van ClO_2, in combinatie met een onderdompeling in een L-cysteïne oplossing om de ClO_2 geïnduceerde bruining tegen te gaan, nuttig kunnen zijn bij de productie van microbieel veilige MB kolen, met dezelfde houdbaarheid als de onbehandelde kolen.

In **hoofdstuk 6** werd het effect van NGW op de houdbaarheidsperiode van MB kool getest. In hoofdstuk 4 werd immers aangetoond dat NGW MB kool kan decontamineren zonder de sensoriele eigenschappen te beïnvloeden. In hoofdstuk 6 werd getest of NGW de houdbaarheidsperiode kan verlengen van MBG bewaard onder evenwichtsgemodificeerde-atmosfeerverpakkingen op 4 en 7 °C. Het effect van de decontaminatie was verdwenen na drie dagen bij beide temperaturen, maar geen enkele microbiële populatie bereikte de microbiële limieten. Bovendien hadden de populaties de stationaire fase bereikt, wat aantoont dat de limieten niet meer zouden orden bereiken, zelfs niet bij een langere bewaarperiode. Bijgevolg beïnvloedde de microbiële groei de houdbaarheidsperiode niet langer. Anderzijds zorgde het behoud van de sensoriele kwaliteit voor een verlenging van de houdbaarheidsperiode. De houdbaarheidsperiode van de controlegroep bij 4°C was beperkt tot negen dagen wegens een slechte OVK, terwijl MB kool behandeld met NGW van aanvaardbare kwaliteit bleef gedurende de 14 dagen van de bewaarproef. Bij 7°C was de houdbaarheidsperiode van de controlegroep beperkt tot zes dagen omwille van OVK en bruinkleuring, terwijl de periode bij de met NGW behandelde stalen beperkt was tot negen dagen wegens OVK, bruinkleuring en uitdroging. Samengevat, NGW verlengde de houdbaarheidsperiode van MB kool met minstens

vijf dagen bij 4℃, en met drie dagen bij 7℃. De chloorionen aanwezig in NGW remmen mogelijks het polyphenol oxidase, wat leidt tot bruinkleuring.

CURRICULUM VITAE

A) PERSONAL DATA

1. Name
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B) EDUCATION

1. University.
   Universidad Simón Bolívar, Caracas, Venezuela. 1988.
   Licentiate in Biology.

2. Post-graduate courses.
   Universidad Simón Bolívar, Caracas, Venezuela. 1991.
   Magíster Scientiarum in Food Science.
   Awarded "with honours". Average mark of all exams: 5.00 (out of 5.00).
   Thesis title: Stability Studies of Intermediate Moisture Tropical Fruit Leathers.
   Distinction "outstanding". Promoter: Prof. Valentín Roa.

   The Hebrew University of Jerusalem, Faculty of Agricultural, Food and Environmental Quality Sciences. 1998.
   Course: "Second International Post-graduate Course on Food Technology". Rehovot, Israel. Special project mark: 90-95 (out of 100).

C) SCHOLARSHIPS

CDCH, UCV, Venezuela. 2002-2006. To pursue doctoral studies.
MOSHAV, Ministry of Foreign Affairs, Israel. 1998.
Fundayacucho, Venezuela. 1991. To pursue doctoral studies. (Not accepted).

D) CONTESTS AND DISTINCTIONS

System for Researcher Promotion. Level: candidate. 1996-1999. Level I: 2003-2004.

Meritorious Professor Level III. National Commission for Academic Benefits. 1997-1998, 1999-2000.

Research Stimulus Program. Active Researcher. Vicerrectorado Académico CDCH-UCV. 1997-98, 1999-2000.

Contest to the position "Assistant Professor", Escuela de Biología, Facultad de Ciencias, UCV. 1996.

Bi-annual award of the Professors Union of the Universidad Central de Venezuela to the best technology book, as collaborator. 1995.

National contest to the charge "Instructor", Escuela de Biología, Facultad de Ciencias, UCV. 09-02-1993.

"Excellence Acknowledgment". Fundación Gran Mariscal de Ayacucho, 1991.

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From July 1996 until September 2000. Assistant Professor.

Universidad Central de Venezuela, Facultad de Ciencias, Instituto de Ciencia y Tecnología de Alimentos. From February 1993 until July 1996.
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F) PUBLICATIONS IN JOURNALS PART OF SCI EXPANDED

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I) INTERNATIONAL CONGRESSES

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(Effect of blanching on the vacuum impregnation of ascorbic acid into apples and papayas).
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(Development of a functional food incorporating calcium and zinc into melon).
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(Effect of blanching and vacuum impregnation on the integrity of the cellular membrane of apple, papaya, guava and plantain).
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J) NATIONAL CONGRESSES

Devlieghere, F.; Ragaert, P.; **Gómez-López, V.M.**; Debevere, J.
Nieuwe decontaminatietechniken voor groenten en fruit.
Facultaire Thema Namidag, Faculteit Bio-ingenieurswetenschappen, Universiteit Gent. January 13, 2006, Gent, Belgium.

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11th Symposium on Applied Biological Sciences, October 6, 2005, Leuven, Belgium.

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Workshop on Antioxidant Phytochemicals and Nutrition. September 29, 2004, Gent, Belgium.

Gómez, V.; Carreño, R.
Relación entre polifenoles, carotenoides, actividad polifenol oxidasa y el oscurecimiento del mesocarpo de dos variedades de aguacate (*Persea americana* Mill.).
(Effect of polyphenols, carotenoids and polyphenol oxidase activity with the enzymatic browning of the mesocarp of two avocado varieties).
I Venezuelan Congress on Food Science and Technology "Dr. Nikita Czyhrinciw", 1996, Caracas, Venezuela.

Roa, V.; Rivero, O.; **Gómez, V.**
Caracterización del proceso de secado en bandejas de un alimento a base de pulpa de frutas tropicales.
(Characterization of a tray drying process of a food based on tropical fruit pulps).

XLV Convención Anual de AsoVAC, Asociación Venezolana para el Avance de la Ciencia, 1995, Caracas, Venezuela.

Gómez, V.; Carreño, R.; Granados, A.; Avilán, L.
Características físico-químicas de algunas variedades de aguacate cultivadas en el CENIAP.
(Physic-chemical characteristics of several avocado varieties grown at CENIAP).
XLV Convención Anual de AsoVAC, Asociación Venezolana para el Avance de la Ciencia, 1995, Caracas, Venezuela.

Carreño, R.; **Gómez, V.**; Granados, A.; Avilán, L.
Evaluación de la calidad de aguacates (*Persea americana* MILL.) procedentes del banco de germoplasma del CENIAP-FONAIAP.
(Quality evaluation of avocados from the germplasm bank of CENIAP-FONAIAP).
XLIII Convención Anual de AsoVAC, Asociación Venezolana para el Avance de la Ciencia, 1993, Mérida, Venezuela.

Gómez, V.; Brito, O.
Estudio de sustitución de avena por amaranto y enriquecimiento de amaranto crudo con tiamina.
(Substitution of oat by amaranth and enrichment of raw amaranth with thiamine).
XXXVIII Convención Anual de AsoVAC, Asociación Venezolana para el Avance de la Ciencia, 1988, Maracay, Venezuela.

K) INVITATED SPEAKER

Gómez-López, V.M.; Devlieghere, F.; Ragaert, P.; Debevere, J. 2005. Intense light pulses for decontamination of fruit and vegetables. Cost action 924 working group meeting, The Use of UV as a Postharvest Treatment: status and prospects. November 9-11, Antalya, Turkey.

Gómez, V.
Round table "Functional Foods". Jornadas Técnicas de Investigación del Instituto de Química y Tecnología, Facultad de Agronomía, UCV. 2001.

Gómez, V.
Stabilization of passion fruit pulp by combined methods.
I Simposio de Ingeniería y Alimentos.
Universidad Nacional Experimental de los Llanos Occidentales Ezequiel Zamora. 2000. San Carlos, Venezuela.

L) REFEREE

Journal of Food Processing and Preservation. 2006.
Instituto Nacional de Investigaciones Agrícolas. 2002.
Revista de la Facultad de Agronomía de la Universidad del Zulia. 2001.
Revista Agronomía Tropical. 1997-98, 2002.

M) REPRESENTATIONS

Professors delegate before the Council of Escuela de Biología, UCV, 1998-2000.

N) EXTENSION ACTIVITIES

Member of the Organizing Committee of the III Venezuelan Congress on Food Science and Technology and II Panamerican Congress on Sanitary Quality of Foods. 2002, Caracas, Venezuela.