Part I Active and Atmospheric Packaging

1 Selected Techniques to Decontaminate Minimally Processed Vegetables

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Abstract: Production of minimally processed vegetables entails a big challenge. The product should be stable, fresh-looking and safe. Cutting and/or peeling accelerate physiological degradation and expose inner tissues to contamination, decreasing produce shelf life and increasing the risk of foodborne pathogen growth. Use of decontamination techniques to control microbial populations is limited because they must be mild enough to avoid impairing sensory characteristics of the product. As a consequence, novel decontamination techniques and decontaminating agents are being tested to cope with the challenge posed by this kind of product. This chapter addresses some novel developments and chemicals potentially useful to prolong the shelf life of minimally processed vegetables, namely continuous UV light, pulsed light, electrolysed oxidizing water, ozone and low-temperature blanching. It defines every technique or decontaminant, and then focuses on the effects on microbial population, produce physiology, sensory quality and nutritional consequences, if known.

Keywords: electrolysed oxidizing water; low-temperature blanching; minimal processing; novel decontamination agents; novel decontamination techniques; ozone; pulsed light; UV light

1.1 INTRODUCTION

During recent decades an intensive search for novel decontaminants and decontamination methods for minimally processed vegetables (MPV) has been pursued by researchers worldwide. The delicate nature of MPV and the absolute requirement of maintaining a fresh-looking product seriously limit the kind of techniques that can be used to obtain an innocuous and stable product.

A decontamination technique for MPV should reduce the risk of foodborne infections and intoxications, decrease microbial spoilage, preserve fresh attributes and nutritional quality, not leave hazardous residues or by-products (Gómez-López *et al.*, 2009) and be environmentally friendly.

This chapter presents selected decontamination techniques that vary greatly, and which have not been extensively reviewed in recent years. It includes two photonic methods: UV-C light and pulsed light; a chemical method: ozonation; and a thermal method: low-temperature blanching. It briefly defines each method and its microbial inactivation mechanism, and then its effect on foodborne pathogenic and spoilage microorganisms, MPV shelf life and the sensory and nutritional quality of the MPV.

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1.2 UV-C LIGHT

1.2.1 Definition

UV-C is the portion of the electromagnetic spectrum corresponding to the band between 200 and 280 nm. Inactivation of microorganisms with UV systems can be performed by means of mercury lamps, xenon pulsed lamps, or excimer lasers. This section deals only with disinfection using mercury lamps, called continuous-wave UV (CW UV). UV inactivation of microorganisms is frequently achieved by using low-pressure mercury lamps designed to emit light at 254 nm, called germicidal light (Bintsis *et al.*, 2000), or medium-pressure UV lamps that emit germicidal wavelengths from 200 to 300 nm (Bolton and Linden, 2003). Since UV light is a non-ionizing radiation, and irradiated products have had serious marketing problems, the term 'illumination' as proposed by Lagunas-Solar and Gómez-López (2007) will be used in place of 'UV radiation' or 'UV irradiated' to avoid misconception. UV-C illumination as a technique to preserve foods was discovered in the 1930s (Artés and Allende, 2005), and water disinfection by UV has been widely applied in Europe since the 1980s (Hijnen *et al.*, 2006). In food-related industries, applications include disinfection of water supplies, food contact surfaces, air in food-preparation areas (Bintsis *et al.*, 2000) and packaging materials (Mimouni, 2001).

UV illumination is characterized by being a relatively inexpensive and simple technique (although it is subject to certain safety precautions; Bintsis *et al.*, 2000), the lack of residual compounds (López-Rubira *et al.*, 2005) and the avoidance of chemicals that can cause ecological problems and/or are potentially harmful to humans (Allende and Artés, 2003b), although conventional mercury lamps generate ozone, which must be exhausted.

The technique is limited to decontaminating surfaces or transparent liquids due to the low penetrability of UV light. When illuminating a three-dimensional object, it is necessary to ensure that all surfaces receive adequate exposure to UV light, requiring equipment with radically new designs (Gardner and Shama, 2000).

The effect of UV light on microorganisms and foods depends on the energy incident on their surface, which is termed fluence (measured in Joules/meter^2, J/m^2).

1.2.2 Inactivation mechanism

The germicidal effect of UV-C light is primarily due to the formation of thymine dimers (cyclobutane dimers) which inhibit the formation of new DNA chains (Mitchell *et al.*, 1992; Giese and Darby, 2000). UV-C treatment of bacterial spores results mainly in formation of the 'spore photoproduct' 5-thyminyl-5,6-dihydrothymine (Slieman and Nicholson, 2000). Microbial cells can repair themselves from photochemical damage, mainly through photoreactivation, where the enzyme photolyase uses visible light energy to split cyclobutane dimers (Kao *et al.*, 2005). Photoreactivation has been studied in water bodies; however, as far as is known by this author, its effect on decontaminated produce has not been studied.

1.2.3 Effect on microbial populations

The efficacy of UV-C to inactivate a wide range of microorganisms has been demonstrated in viruses (Eischeid *et al.*, 2009), Gram-negative and Gram-positive bacteria (Chang *et al.*, 1985; Yaun *et al.*, 2003), bacterial spores (Mamane-Gravetz and Linden, 2004), conidia (Marquenie *et al.*, 2002) and parasites (Zimmer *et al.*, 2003; Hayes *et al.*, 2008) *in vitro* as well as when inoculated onto (Yaun *et al.*, 2004) and into vegetable matrixes (Hadjok *et al.*, 2008).

UV-C illumination is able to increase vegetable shelf life based on microbial spoilage counts by either inactivating microorganisms and/or decreasing their growth rate, and can even trigger a lag phase. This lag phase is more apparent in yeasts than in other microbial populations (Erkan *et al.*, 2001; Allende and Artés, 2003a, 2003b; Allende *et al.*, 2006) and can account for lower counts in illuminated compared with control samples at the end of the storage time. In contrast, UV-C may sometimes stimulate growth of lactic acid bacteria, possibly due to a higher resistance to UV-C and elimination of competing microflora (Allende and Artés, 2003a, 2003b).

Erkan *et al.* (2001) treated tissue slices of zucchini squash with UV-C up to 9.86 kJ/m^2, and Allende *et al.* (2006) did it to both sides of minimally processed (MP) Red Oak Leaf lettuce up to 7.11 kJ/m^2. In these cases, UV-C did not inactivate total bacterial and yeast populations, but slowed down their growth during storage. In the latter example, this meant a shelf-life extension of at least 2 days. On the other hand, illumination of MP Red Oak Leaf lettuce (Allende and Artés, 2003a) and MP Lollo Rosso lettuce (Allende and Artés, 2003b) both with UV-C up to 8.14 kJ/m^2 inactivated important microbial groups, bringing about a shelf-life prolongation of 2–3 days. In different cases, the published literature indicates that UV-C illumination of MPV can cause a shelf-life extension of at least 2 days from the microbial point of view when properly used.

1.2.4 Effect on sensory attributes

UV-C illumination has been reported to preserve or not affect the sensory quality of MPV. Erkan *et al.* (2001) stated that the decay of zucchini squash slices illuminated with UV-C at levels of 4.93 and 9.86 kJ/m^2 was significantly less than controls during storage at 5 and 10°C. However, after 12 days of storage at 10°C, a reddish-brown discoloration induced by UV-C was observed on the surface of illuminated tissues, which suggests the accumulation of phenolic compounds.

No effects were reported by Allende and Artés (2003a) on MP Red Oak Leaf lettuce treated with up to 8.14 kJ/m^2 UV-C on sensory quality evaluated in terms of overall visual quality, colour and browning, during storage in MAP at 5°C for 8 days. On the other hand, for MP Lollo Rosso lettuce stored at 5°C for 8 days, Allende and Artés (2003b) reported a beneficial effect of UV-C. No significant differences were found for aroma, texture, taste and colour, but samples treated with the highest fluences (2.44–8.14 kJ/m^2) had better-preserved 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), and a shelf-life prolongation may be estimated based on sensory scores. Lettuce tissue became shinier when the highest fluence was applied, which was attributed to a possible induction of lignification-like processes started by the lettuce tissue to protect itself against the UV-C stress.

Allende *et al.* (2006) illuminated two sides of MP Red Oak Leaf lettuce and reported no effect on overall visual quality of UV-C illumination at fluences up to 2.37 kJ/m^2, but 7.11 kJ/m^2 induced tissue softening and browning after 7 days of storage at 5°C. Polyphenol oxidase is the enzyme responsible for enzymatic browning of many types of produce. Manzocco *et al.* (2009) achieved complete inactivation of mushroom polyphenol oxidase *in vitro* by UV-C illumination.

1.2.5 Effects on the nutritional and phytochemical composition of MPV

It is very well known by post-harvest experts that UV 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. This change can be instantaneous or progressive and observed during produce storage. The biosynthesis of phenolic compounds is affected by UV illumination due to the increased activity of phenylalanine ammonia-lyase (Schreiner and Huyskens-Keil, 2006). Among the different controlled abiotic stresses useful to enhance the nutraceutical content of MPV, UV illumination is considered to be of high potential by the industry (Cisneros-Zevallos, 2003). There are no studies yet on the effect of UV-C on phytochemical composition of MPV. Nevertheless, increasing evidence of positive effects on whole produce and fresh-cut fruits suggests positive results, such as that reported by Cantos *et al.* (2001). Trans-resveratrol is a phytoalexin with health-promoting properties. Those authors demonstrated that UV-C can increase the concentration of phytoalexins in table grapes as much as 10-fold during storage. UV-C illuminated whole peppers (Vicente *et al.*, 2005) and broccoli heads (Costa *et al.*, 2006) showed increased antioxidant capacity with respect to controls during storage. Similarly, UV-C illuminated fresh-cut mango (González-Aguilar *et al.*, 2007) banana and guava, but not pineapple (Alothman *et al.*, 2009), showed increased antioxidant capacity but also vitamin C degradation.

1.3 PULSED LIGHT

1.3.1 Definition

Pulsed light (PL) is a new method intended for decontamination of food surfaces by killing microorganisms using short-term, high-frequency pulses of an intense broad-spectrum illumination rich in UV-C light (Gómez-López *et al.*, 2005a). It derives from CW UV, with the main difference that PL delivers much higher fluence rates and uses xenon flash lamps instead of mercury lamps, and special hardware to produce a high peak power. Peak power is defined as the pulse energy divided by the pulse duration (Rice and Ewell, 2001). Very comprehensive reviews on the subject have been published by Elmnasser *et al.* (2007) and Gómez-López *et al.* (2007a); this section is an update, with a focus on MPV. It is common in the literature to find reports comparing the efficiency of PL versus low-pressure or medium-pressure UV lamps, where the biggest difficulty is comparing light sources which differ not only in peak power but also in emission spectrum. Recently, Bohrerova *et al.* (2008) compared the disinfection efficiency of PL and CW UV from low- and medium-pressure mercury lamps over *Escherichia coli* cells and phages T3 and T4, which were all inactivated more efficiently by PL at equivalent fluence levels.

PL has similar advantages and disadvantages to CW UV-C, but also some specific pros and cons. Perhaps the most important advantage of PL is the fast microbial inactivation. Specific disadvantages are high cost and that this process can increase produce temperature. When studying the inactivation of *Aspergillus niger* spores on corn meal, Jun *et al.* (2003) found that some experimental factor settings resulted in sample temperatures of 120°C.

1.3.2 Inactivation mechanism

Xenon flash lamps have an emission spectrum ranging from ultraviolet to infrared light, wherein the UV-C component is the most important for microbial inactivation. Rowan *et al.* (1999) reported that using a flash with high UV content the inactivation of seven micro-organisms was 5–6 log colony-forming units (CFU)/plate, but only 1–2 log CFU/plate with a

low-UV flash. The germicidal efficiency of xenon flash lamps determined against *E. coli* was found to be maximum around 270 nm, with a major contribution to inactivation in the 220–290 nm range (Wang *et al.*, 2005). Other wavelengths also contribute to microbial inactivation. Bohrerova *et al.* (2008) reported that a significant fraction of the enhanced PL inactivation efficiency compared to low- and medium-pressure mercury lamps was due to wavelengths greater than 295 nm, and even due to visible light in the case of viruses.

Different kinds of damage have been reported on viruses and microbial cells due to PL action. Lethal events are photochemical and/or photothermal. The primary lethal effect of PL, as with CW UV light, is the formation of cyclobutane pyrimidine dimers, as observed in cells of *Saccharomyces cerevisiae* (Takeshita *et al.*, 2003) and *E. coli* (Bohrerova *et al.*, 2008). Interestingly, the higher number of thymine dimers observed in flashed *E. coli* cells in comparison with CW UV-treated cells was attributed to an effect of light higher than 295 nm. Single-strand DNA breaks and cell-membrane damage have been reported for yeasts by Takeshita *et al.* (2003).

Cell rupture at fluences exceeding 0.5 J/cm^2 due to momentary overheating caused by absorbing all UV light from a flash lamp was revealed by Wekhof *et al.* (2001). Micrographs of flashed *A. niger* spores were presented showing severe deformation and rupture attributed to escape of the overheated contents of the spore. Bohrerova *et al.* (2008) have suggested that besides DNA damage, phage capsids might be ruptured by the visible portion of PL. As in CW UV treatments, photoreactivation can also occur after PL treatment (Otaki *et al.*, 2003).

In vitro studies performed by Gómez-López *et al.* (2005b) showed that proteins and oils decreased the decontamination effect of PL, while carbohydrates and water showed variable effects. For this reason MPV seem to be a suitable matrix for efficient PL decontamination.

1.3.3 Effect on microbial populations

PL has been proved to be effective against viruses (Roberts and Hope, 2003), Gram-negative bacteria (Sharma and Demirci, 2003), Gram-positive bacteria (Krishnamurthy *et al.*, 2004), bacterial spores (McDonald *et al.*, 2002), yeasts (Takeshita *et al.*, 2003), conidia (Marquenie *et al.*, 2003), fungal spores (Jun *et al.*, 2003) and parasites (Huffman *et al.*, 2000).

Research on the inactivation of human pathogens on vegetable surfaces is still pending. Regarding microorganisms naturally present on vegetables, Hoornstra *et al.* (2002) were first in demonstrating the potential of PL. The authors treated white cabbage, leek, paprika, carrots and kale with two pulses of PL amounting to 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 more than 2.6 log CFU/cm^2 for paprika. Gómez-López *et al.* (2005b) reported a reduction in mesophilic aerobic counts between 0.56 and 2.04 log CFU/g for spinach, radicchio, iceberg lettuce, white cabbage, carrots, green bell pepper and soybean sprouts. Later on, Kaack and Lyager (2007) used PL to inactivate *S. cerevisiae* cells inoculated onto carrot pieces.

In order to prove whether microbial inactivation brings about shelf-life extension of MPV, Gómez-López *et al.* (2005b) flashed shredded iceberg lettuce and shredded white cabbage, and stored them under modified-atmosphere packaging at 7°C. For iceberg lettuce, even though a 0.46 log reduction was achieved in psychrotroph counts, control and flashed samples did not last 3 days of storage due to excessively high psychrotroph counts and bad sensory quality. For white cabbage, a 0.54 log decrease in psychrotroph counts was achieved, but after 2 days of storage control and flashed samples had similar psychrotroph counts. It is worth noting that this kind of results is common for MPV shelf-life studies, and might be avoided using storage temperatures not higher than 4°C (Gómez-López *et al.*, 2008a).

1.3.4 Effect on sensory attributes

Little is known on the effect of PL on MPV. Except for some discoloration of iceberg lettuce, no adverse effects of PL on sensorial quality were reported by Hoornstra *et al.* (2002) after 7 days of storage at 7 or 20°C of five illuminated vegetables. Heating was a concern for Gómez-López *et al.* (2005b), who were unable to efficiently treat grated carrots without avoiding its dehydration. The same authors reported the appearance of off-odour in MP cabbage immediately after treatment, which disappeared overnight. MP cabbage stored at 7°C was sensorially acceptable for up to 9 days, while untreated controls were rejected after 7 days, which means a shelf-life extension of 2 days from the sensorial point of view. For MP lettuce, however, no shelf-life extension was achieved by PL treatment.

1.3.5 Effects on the nutritional and phytochemical composition of MPV

There is a lack of studies about the nutritional and phytochemical consequences of the application of PL to fruit and vegetables, but encouraging results for phytochemical synthesis enhancement by CW UV makes PL a potential fast method for phytochemical content improvement. Additionally, PL did not affect levels of riboflavin and vitamin C in beef, chicken and fish (Dunn *et al.*, 1995).

1.4 ELECTROLYSED OXIDIZING WATER

1.4.1 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. The most common type of electrolytic cell is a two-cell chamber. The generation of EOW using the two-cell chamber involves reactions in cells containing positively charged and negatively charged electrodes, respectively, separated by a membrane, and through which a much 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 is characterized by a pH of less than 3, a high oxidation-reduction potential (ORP), about 1150 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).

A single stream of neutral EOW (NEW), also named mixed-oxidant solution (or MIOX), with enhanced levels of HOCl, is produced by the single-cell system, where no membrane is present. Therefore, three kinds of EOW can be produced: AcEW, AlEW and NEW (MIOX). Very comprehensive reviews on EOW has been published by Al-Haq *et al.* (2005) and Huang *et al.* (2008).

EOW 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 the raw materials, water and sodium chloride, are found virtually everywhere (Venczel *et al.*, 1997). EOW can be generated on site (Len *et al.*, 2000), and therefore transportation and storage of potentially hazardous chemicals are not needed (Nakagawara *et al.*, 1998). EOW is not only a

decontaminant but can also prevent enzymatic browning during storage of MPV (Koseki and Itoh, 2002).

AcEW could be more effective in inactivating microorganisms than chlorinated solutions, having the same concentration of available chlorine (Koseki *et al.*, 2001). Consequently, there should be lower formation of chloramines and trihalomethanes. NEW has also the advantage of its neutral pH, so 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).

1.4.2 Inactivation mechanism

It has been observed that AcEW produces blebs and breaks in the outer membrane of *Pseudomonas aeruginosa*, inactivates nitrate reductase and degrades chromosomal DNA (Kiura *et al.*, 2002). There is no agreement about which is the primary factor contributing to the microbicidal activity of EOW. Factors involved are concentration of free chlorine, redox potential (ORP) and pH. Kim *et al.* (2000a, 2000b) concluded that ORP may be the primary factor affecting microbial inactivation of AcEW, while Nakagawara *et al.* (1998), Len *et al.* (2000), Koseki *et al.* (2001) and Kiura *et al.* (2002) have concluded that HOCl concentration is the main contributor.

Other antimicrobial compounds besides HOCl are hypothetically present in EOW, which should explain the higher effectiveness of EOW over conventional HOCl solutions to inactivate microorganisms *in vitro* (Venczel *et al.*, 1997). But those compounds, such as hydroxyl radicals, have not been detected (Stan *et al.*, 2005), and disinfection experiments on lettuce under controlled conditions of chlorine concentration, pH and ORP do not agree with this hypothesis (Park *et al.*, 2001). Perhaps additional compounds become quickly degraded by organic matter, and are not relevant for decontamination of MPV.

Chlorine is certainly involved and pH determines the most important species: Cl_2 below pH 3 (typical of AcEW), and HOCl and ClO^- above pH 4 (typical of NEW) (Nakagawara *et al.*, 1998). The maximum *in vitro* antibacterial activity of AcEW occurs around pH 4 (Nakagawara *et al.*, 1998; Len *et al.*, 2000; Park *et al.*, 2004). However, results from *in vivo* tests do not confirm the applicability of *in vitro* results. Yang *et al.* (2003) dipped MP lettuce in EOW at different pHs, and found no pH effect for the inactivation of *Salmonella Typhimurium* and *Listeria monocytogenes*, and two inactivation peaks for *E. coli* O157: H7 at pH 4 and 8.

1.4.3 Effect on microbial populations

Different studies have proved the efficacy of EOW to inactivate human pathogens both *in vitro* (Venczel *et al.*, 1997; Venkitanarayanan *et al.*1999; Kim *et al.*, 2000a, 2000b; Nakajima *et al.*, 2004; Park *et al.*, 2004) and inoculated onto vegetable surfaces (see Table 1.1) (Deza *et al.*, 2003; Abadías *et al.*, 2008; Park *et al.*, 2008).

As for spoilage microorganisms, Izumi (1999) studied the effect of rinsing five MPV with NEW (20 ppm available chlorine, pH 6.8, 4 min) on mesophilic aerobic microorganisms, finding a 1.8 log reduction for trimmed spinach leaves to no significant effect for carrot slices, Japanese radish shreds or diced potatoes. The author concluded that the effect of NEW was influenced by the surface area, anatomy and microstructure of the tissues, which differ among vegetables, as well as the type of cut, which would affect the extent of contact of EOW with microorganisms. Koide *et al.* (2009) reported 1.5 and 1.3 log reductions in total aerobic

Table 1.1 Studies on the effect of electrolysed water on pathogenic microorganisms inoculated onto fresh or minimally processed vegetables.

Produce	Microorganism	Free chlorine concentration (mg/l)	Time (min)	pH	Log reduction	Reference
Tomato	*E. coli* O157:H7	89	1	8.1	4.92	Deza *et al.* (2003)
	Salmonella Enteriditis				4.30	
	L. monocytogenes				4.74	
Lettuce	*E. coli* O157:H7	89	3	8.6	1.2	Abadias *et al.* (2008)
	Salmonella				1.7	
Spinach	*E. coli* O157:H7	20	10	6.3–6.5	1.25	Guentzel *et al.* (2008)
	S. Typhimurium				2.14	
	L. monocytogenes				2.94	
Iceberg lettuce	*E. coli* O157:H7				0.14	
	S. Typhimurium				1.41	
	L. monocytogenes				2.99	
Green onion	*E. coli* O157:H7	37.5	1	2.1	4.45	Park *et al.* (2008b)
	S. Typhimurium				4.24	
	L. monocytogenes				4.82	
Tomato	*E. coli* O157:H7				>5.86	
	S. Typhimurium				4.27	
	L. monocytogenes				>5.91	
Iceberg lettuce	*E. coli* O157:H7	50	2	2.6	0.72	Keskinen *et al.* (2009)
Romaine lettuce					0.77	

bacteria, yeast and mould counts after dipping MP cabbage in NEW (20 mg/l, 10 min). As for shelf-life prolongation, Rico *et al.* (2008) achieved 1.4–1.6 log reduction in aerobic mesophilic counts on lettuce compared to water washing, a difference that persisted after 7 days of storage at 4°C.

Regarding AcEW, it reduced total aerobic plate count of MP cilantro leaves by 0.66 log compared to water washing (Wang *et al.*, 2004), by more than 1 log in whole chinjon, leafy cabbage, spinach, cucumber and snap beans, and just 0.9 log in green peppers. With AlEW alone, a no more than 0.5 log reduction was observed (Lin *et al.*, 2005). Monitoring the effect of AcEW during the storage of MP cilantro at 0°C, Wang *et al.* (2004) observed that the total aerobic plate count and total Enterobacteriaceae count exhibited a 4-day lag phase. AcEW (50 ppm free chlorine, pH 2.8) reduced *E. coli* O157:H7 counts by 0.72 and 0.77 logarithmic cycles in MP iceberg and Romaine lettuce respectively (Keskinen *et al.*, 2009).

AlEW lacks antimicrobial properties, but it is considered to act like a diluted sodium hydroxide solution. Thus, it would work like a surface-active agent on vegetable surfaces. Consequently, the microorganisms on the surface would be reached more easily by AcEW during a sequential process, which explains higher decontamination found in lettuce (Koseki *et al.*, 2001), whole cucumber (Koseki *et al.*, 2004) and MP lettuce and MP cabbage (Koseki and Itoh, 2001, 2002). The shelf life of the latter MPV was estimated to be extended

by at least 1 day in spite of bacteria growing faster in samples treated with AcEW than in water-washed samples.

1.4.4 Effect on sensory quality

EOW washing does not change sensory properties of vegetables immediately after treatment. It does not affect the surface colour of carrot slices, trimmed spinach leaves or cucumber slices (Izumi, 1999); the taste, appearance or smell of whole tomatoes (Deza *et al.*, 2003); or the appearance of whole cucumbers (Lin *et al.*, 2005), MP lettuce, carrot and endive (Abadías *et al.*, 2008). Panellists of triangle tests were unable to differentiate MP iceberg lettuce, white cabbage and carrots treated with NEW (40 mg/l free chlorine, 5 min) from water-washed samples (Gómez-López *et al.*, 2008b). A residual chlorine odour after washing vegetables with AcEW (50 ppm active chlorine) can be eliminated by soaking with AlEW (Lin *et al.*, 2005). Furthermore, Park *et al.* (2001) found no effects of AcEW on visual quality, stem discoloration, wilting and colour of whole lettuce leaves stored for 14 days at 4°C.

As for shelf-life prolongation, Gómez-López *et al.* (2007b) extended the shelf life of shredded cabbage stored at 4°C by 5 days due to treatment with NEW. Rico *et al.* (2008) also showed beneficial effects of increasing concentrations of free chlorine (12–120 ppm) in NEW to decrease browning potential of lettuce and polyphenol oxidase activity and improving sensory quality evaluated in terms of fresh appearance, photosynthetic and vascular browning, and general acceptability; although high free-chlorine content NEW produced changes associated to blanching and loss of crispness.

AcEW can have beneficial effects in the sensorial stability of some MPV during refrigerated storage, such as decreasing the progress of browning in MP lettuce and MP cabbage (Koseki and Itoh, 2002). The authors suggested the enzymes responsible for browning may have been oxidized and weakened by the strong ORP of AcEW. But AcEW can also have deleterious effects, such as loss of aroma of cilantro leaves during storage, possibly correlated, according to the authors, to a higher tissue electrolyte leakage caused by oxidation of the cilantro cell membrane by AcEW (Wang *et al.*, 2004).

1.4.5 Effects on the nutritional and phytochemical composition of MPV

Treating MP leek with NEW up to 30 mg/l free chlorine compared to water-wash treatment did not influence vitamin C, α-tocopherol and total phenol content, the antioxidant capacity measured by the ferric reducing antioxidant power (FRAP) method, and the concentration of the carotenoids all-*trans*-β-carotene and 9-*cis*-β-carotene; but decreased violaxanthin and lutein concentrations, as well as γ-tocopherol content (Vandekinderen *et al.*, 2009).

1.5 OZONE

1.5.1 Definition

Ozone (O_3) is a 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. Production of ozone is generally accomplished by

using the corona discharge method (Güzel-Seydim *et al.*, 2004). Ozone is a strong oxidant active against all types of microorganism. Excess ozone auto-decomposes rapidly to produce oxygen, and thus it leaves no residues in food. It can be applied as a gas, or in aqueous solution where the gas does not appreciably react with water; therefore it forms a true physical solution (Khadre *et al.*, 2001).

The use of this disinfectant has some limitations. Because of its instability, ozone must be generated at the usage site. Metal and other types of surfaces with which it comes into contact are subject to corrosion or other deterioration because of its strong oxidizing power (Beuchat, 1998). Several food components interfere with its microbicidal efficacy (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; therefore microorganisms embedded in product surfaces are more resistant to ozone than those readily exposed to sanitizer (Mahapatra *et al.*, 2005). Ozone-detection and -destruction systems and respirators are needed for the safety of workers in food-processing facilities (Kim *et al.*, 1999b).

1.5.2 Inactivation mechanism

Ozone is a strong oxidant and disinfectant. It can react with contaminants directly as molecular ozone or indirectly as ozone-derived free radicals such as •OH and $\bullet H_2O$ (Koseki and Itoh, 2001). Ozone decomposes in solution in a stepwise fashion, producing in turn hydroperoxyl ($\bullet HO_2$), hydroxyl (•OH) and superoxide ($\bullet O_2^-$) 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.*, 1999b). Different targets have been proposed for its bactericidal action: double bonds of unsaturated lipids in the cell envelope, interference with the respiratory system and damage to the genetic material, and a general oxidation of protoplasm (Kim *et al.*, 1999b). Young and Setlow (2004) proposed 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.

1.5.3 Ozonated water

1.5.3.1 Effect on microbial populations

The effect of ozonated water on pathogen populations of vegetable surfaces is very variable. Singh *et al.* (2002) reported 1.42 and 1.80 log reductions respectively in *E. coli* O157:H7 after treating MP lettuce and baby carrots with ozonated water (16.5 mg/l, 10 min). In contrast, counts of *L. monocytogenes* and *E. coli* O157:H7 in shredded lettuce were reduced from 6.0 log CFU/g to less than 1.0 log CFU/g by washing with ozonated water (3 ppm, 5 min) (Rodgers *et al.*, 2004). Finally, counts of *Shigella sonnei* inoculated on shredded lettuce decreased 1.7 log units by ozonated water (5 ppm, 5 min) (Selma *et al.*, 2007); and counts of *L. monocytogenes* in green leaf lettuce were reduced by more than 2 log units (Ölmez and Akbas, 2009).

Ozonated water has also shown mixed effectiveness to reduce microorganisms naturally present in MPV. Kim *et al.* (1999a) observed that bubbling ozone while stirring at high velocity (300 rpm) was the best of different alternatives tested to decontaminate lettuce, with log reductions of 1.9 log CFU/g for both counts of mesophilic and psychrotrophic bacteria. Thereafter, a number of articles have reported from non-significant effects to almost 2-log reductions (Table 1.2).

Table 1.2 Studies on the effect of ozonated water on natural microflora of fresh or minimally processed vegetables.

Produce	Microbial group	Ozone concentration (mg/l)	Log reduction	Reference
Lettuce	Mesophilic aerobes	5	1.5	Koseki *et al.* (2001)
	Yeasts and moulds		1.0	
Lettuce	Mesophilic aerobes	2.5–7.5	0.6–0.8	Garcia *et al.* (2003)
	Psychrotrophs	7.5	0.5	
Cucumber	Mesophilic aerobes	5	0.7	Koseki *et al.* (2004).
Celery	Mesophilic aerobes	0.18	1.69	Zhang *et al.* (2005)
Potato strips	Mesophilic aerobes, psychrophiles, lactic acid bacteria, yeasts, coliforms, anaerobic microorganisms	20	NS	Beltrán *et al.* (2005a)
Lettuce	Mesophilic aerobes	20	1.6	Beltrán *et al.* (2005b)
Green pepper	Mesophilic aerobes	3.95	NS	Ketteringham *et al.* (2006)
Rocket leaves	Mesophilic aerobes	10	1	Martínez-Sánchez *et al.* (2006)
	Psycrophilics		>1	
	Coliforms, yeasts and moulds		NS	
Green leaf lettuce	Mesophilic aerobes	2	1.5	Ölmez and Akbas (2009)
	Psychrotrophs		1.1	
	Enterobacteriaceae		1.5	
Asparagus	Mesophilic aerobes	0.1	1.91	Sothornvit and Kiatchanapaibul (2009)
Carrot	Mesophilic aerobes	1	0.4	Alegria *et al.* (2009)
	Yeasts and moulds		0.7	

NS, non-significant effect.

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).

1.5.3.2 Effect on sensory attributes

It has been shown that ozonated water maintains the sensory quality of MPV, among which MP lettuce is the most studied. According to microscopy observations, lettuce surface is not affected by ozone (Koseki *et al.*, 2001). Ozonated water had positive effects on sensory quality of MP lettuce stored at 4°C for 9 days (Ölmez and Akbas, 2009), the shelf life of which can be 4 days longer than chlorinated samples (Garcia *et al.*, 2003). 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, water-washed controls decreased in visual quality and browned sharply after 5 days. Moreover, ozone did not affect its texture (Beltrán *et al.*, 2005b).

Fresh-cut cilantro leaves washed with ozonated water and stored at 0°C for up to 14 days had the same colour as 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). Ozonated water (0.18 ppm) was beneficial in maintaining colour, visible structural integrity and general appearance of MP celery during storage at 4°C (Zhang *et al.*, 2005). As for MP rocket leaves, ozone (10 mg/l) affected neither colour nor visual quality (Martínez-Sánchez *et al.*, 2006).

Potato strips treated with ozonated water (20 mg/l) stored under vacuum at 4°C during 14 days showed no evidence of browning, and maintained the full typical aroma and a very firm and turgid texture (Beltrán *et al.*, 2005a). Treatment of MP green asparagus with 1 mg/l aqueous ozone for 30 min delays the synthesis of the structural cell-wall constituents that lead to asparagus-toughening lignin, cellulose and hemicelluse during storage at 3°C for 25 days (An *et al.*, 2007).

Shredded carrots were affected by immersion in ozonated water (1 ppm, 5 min), suffering from loss of colour due to leaching, softening, losses of perceived aroma and low general acceptance, although the highly exposed cut area could account for the observed losses (Alegria *et al.*, 2009).

1.5.4 Gaseous ozone

1.5.4.1 Factors affecting the efficacy of ozone gas

The factors affecting the decontaminant efficacy of ozone gas were studied by Han *et al.* (2002) in *E. coli* O157:H7 inoculated onto green bell pepper surface. The order of significance and 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. A synergism was found between gas concentration and relative humidity. Li and Wang (2003) also found that germicidal efficiencies over four microorganisms increased as relative humidity increased, which could be related to the higher number of radicals from the ozone reaction with more water vapour at higher relative humidity.

1.5.4.2 Effect on pathogenic microorganisms and sensory quality

There are few studies about the application of ozone in the gas phase to decontaminate MPV. Singh *et al.* (2002) reported 1.79 and 2.64 log reductions in *E. coli* O157:H7 populations after treating respectively MP lettuce and baby carrots with gaseous ozone (7.6 mg/l, 15 min). Decoloration of lettuce leaves was observed after treatment with 5.2 or 7.6 mg/l O_3 for 10 and 15 min. Han *et al.* (2002) reported 7.35 log reduction (log CFU/5 g) 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. Klockow and Keener (2009) developed an ozone-generation system where bags of vegetables, spinach in this case, were subjected to an electric field to generate ozone, and then stored. *E. coli* O157:H7 populations decreased by 5.8 and more than 6.4 log CFU/leaf after 24 h of storage at 5 or 22°C

respectively, but leaf colour changed from green to white, especially at the stem and outer edges of the leaf.

1.5.5 Effects on the nutritional and phytochemical composition of MPV

As with other disinfectants, the effect of ozonated water has been primarily studied in MP iceberg lettuce, while the effect of gaseous ozone is still pending for tests. It seems that in general ozone has neutral nutritional consequences for MPV. Ozonated water at 5 ppm, 5 min (Koseki and Isobe, 2006) and at 2–4 ppm, 2 min (Akbas and Ölmez, 2007; Ölmez and Akbas, 2009) did not affect the ascorbic acid content of MP lettuce; although harder treatments (10 or 20 mg/l O_3) did reduce vitamin C content (Beltrán *et al.*, 2005b). In contrast, 0.03 and 0.08 ppm of ozone in washing water delayed the oxidation of vitamin C in MP celery during storage at 4°C up to 9 days (Zhang *et al.*, 2005). According to the authors, inhibition of polyphenol oxidase and delay of tissue metabolism by ozonated water could account for this effect.

Ozone (10 mg/l) had no effect on the total polyphenol content of MP lettuce (Beltrán *et al.*, 2005b) or rocket leaves (Martínez-Sánchez *et al.*, 2006), and ozonated water (2–4 ppm, 2 min) did not affect β-carotene content of MP lettuce (Akbas and Ölmez, 2007; Ölmez and Akbas, 2009).

1.6 LOW-TEMPERATURE BLANCHING

1.6.1 Definition

Low-temperature blanching consists of immersion of MPV in hot water for up to 50°C and 120 s, high enough to provoke beneficial physiological changes and microbial reduction without cooking. Most of the literature on low-temperature blanching has been published in articles dealing with the effect of warm chlorinated water. However, because of the concerns on the use of chlorine for decontamination of fruits and vegetables, only the results of blanching in pure water will be summarized in this section.

1.6.2 Effect on microbial populations

The first results on the effect of mild heat treatment on the stability of MPV were discouraging from the microbiological point of view. Compared to samples washed at 20°C, immersion of MP lettuce in warm water (50°C, 90 s) decreased psychrotrophic counts by 0.56 log, but did not significantly affect counts of mesophilic microorganisms, Enterobacteriaceae, and yeasts and moulds. After less than 4 days of storage at 5 or 15°C the effect on psychrotroph counts was lost (Li *et al.*, 2001). Experiments under identical conditions showed that mild heat treatment enhances growth of *L. monocytogenes* during subsequent storage (Li *et al.*, 2002). The enhanced growth of microorganisms after a decontamination process is also frequent for other decontamination techniques as revised by Gómez-López *et al.* (2008a).

Compared to washing at 20°C for respective times, washing lettuce with warm water (50°C, 1 or 5 min) reduced counts of *E. coli* O157:H7 by 1.68 and 1.98 logs respectively, and *Salmonella* counts by 1.51 and 2.03 logs (Koseki *et al.*, 2004). A more than 1.0 log reduction was reported for total bacteria, pseudomonades and Enterobacteriaceae after pre-washing of iceberg lettuce with warm water (50°C, 60 s) after trimming and coring but before shredding

in comparison with washing with cold water (4°C, 90 s) after shredding. The sanitizing effect persisted throughout storage (4°C, 9 days) (Baur *et al.*, 2005). Similarly results were reported by Klaiber *et al.* (2005) for aerobic mesophilic bacteria, lactic acid bacteria and enterobacteria of shredded carrots pre-washed in warm water (50°C, 120 s) after peeling and topping but before shredding, in comparison with non-washed samples.

1.6.3 Effects on sensory quality

The main beneficial effect of low-temperature blanching is suppression of lettuce browning. Immersion in warm water (50°C, 90 s) retarded browning for at least 7 days at 5°C, and 2 days at 15°C (Li *et al.*, 2001). Fukumoto *et al.* (2002) studied the effect of warm water (47°C, 3 min) compared with cold water (4°C) on the photosynthetic and vascular tissues of iceberg lettuce stored for 7 days at 5°C. Washing at 47°C strongly inhibited edge browning, phenylalanine ammonia-lyase activity and peroxidase activity. Baur *et al.* (2005) reported that pre-washing of MP iceberg lettuce with warm water (50°C, 60 s) decreased cut-edge vascular tissue browning and phenylalanine ammonia-lyase during storage (4°C, 9 days).

Low-temperature blanching has been also applied to other MPV. Colour and odour of low temperature blanched MP endives stored for 2 days at 4°C were negatively affected when blanching conditions were 50°C/10 min. When 52.5°C/10 min and 55°C/5 min conditions were used, appearance, taste and texture were additionally damaged (Mayer-Miebach *et al.*, 2003). Pre-washing of MP carrots with warm water (50°C, 120 s) did not affect overall visual quality, colour, odour, texture, sweetness and flavour during storage (4°C, 9 days) as determined by sensory panel; it also did not affect whiteness index, and contents of glucose, fructose, sucrose, as well as total sugar. However, longer blanching times (60°C, 40 min) decreased carrot hardness due to pectin demethoxylation (Lemmens *et al.*, 2009).

1.6.4 Effects on the nutritional and phytochemical composition of MPV

Low-temperature blanching of detached whole endive leaves at conditions 50°C/10 min, 52.5°C/10 min and 55°C/5 min reduced polyphenol contents and antioxidant capacity (Mayer-Miebach *et al.*, 2003). In contrast, blanching carrots at 60°C for 40 min did not modify β-carotene levels of carrots (Lemmens *et al.*, 2009).

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