

## Processing Lines and Alternative Preservation Techniques to Prolong the Shelf-life of Minimally Fresh Processed Leafy Vegetables

F. Artés and A. Allende

(Postharvest and Refrigeration Group, Department of Food Engineering, Technical University of Cartagena, Cartagena, Murcia, Spain)

### Summary

There is still a lack of information about the actual critical points of the industrial fresh-processing chain and on the current and feasible advances in the technologies to successfully preserve the minimally fresh processed (or fresh-cut) vegetable products. It is known that the minimal fresh-processing industry continuously needs to improve their technical support by renewing the processing lines as well as by introducing emerging and alternative preservation techniques, which reduce production losses and provide safer and higher quality products. In this work, an exhaustive review was done to put together information about the most critical points throughout all the stages of the production chain and storage conditions of minimally fresh processed leafy vegetables which determine their final microbial and sensory quality. The first part of the review includes an extensively discussion about the critical points of the production chain of fresh processed leafy vegetables such as cutting and washing. Recent improvements in washing and sanitizing agents such as chlorine, ozone, hydrogen peroxide, chlorine dioxide and antioxidant solutions, and their advantages and disadvantages are evaluated. The second part of this report is focused on the evaluation of feasible emerging preservation techniques such as superatmospheric O<sub>2</sub> atmospheres, hot water treatments and UV-C radiation, as alternatives to the conventional ones to improve the final microbial quality and to extend the sensorial quality of minimally fresh processed vegetables.

**Key words.** fresh-cutting – microbial and sensorial quality – ozone – hydrogen peroxide – chlorine dioxide – hot water treatments – superatmospheric oxygen – UV-C radiation – antioxidants – HACCP

### Zusammenfassung

#### **Bearbeitungsabläufe und alternative Konservierungstechniken zur Verlängerung der Haltbarkeit von minimal-bearbeiteten Blattgemüsen.**

Es bestehen immer noch Informationsdefizite hinsichtlich derzeit auftretender kritischer Punkte in der industriellen Verarbeitungskette sowie hinsichtlich aktueller und potentieller Fortschritte in den Methoden zur erfolgreichen Qualitätserhaltung von frischen minimal-bearbeiteten Blattgemüsen. Es ist bekannt, dass die minimal-bearbeitende Industrie ihre technische Ausstattung sowohl durch Erneuerung von Bearbeitungsabläufen als auch durch die Einführung neu entstehender und alternativer Qualitätserhaltungstechniken, die die Produktionsverluste verringern und sicherere und bessere Qualität liefern, ständig verbessern muss. In dieser Arbeit wurde ein ausführlicher Überblick an Informationen über die kritischsten Punkte in den einzelnen Stufen der Bearbeitungskette und in den Lagerbedingungen von minimal-bearbeiteten Blattgemüsen zusammengestellt, die letztendlich über ihre mikrobielle und sensorische Qualität entscheiden.

Der erste Teil des Überblicks beinhaltet eine umfassende Diskussion über kritische Punkte in der Bearbeitungskette wie dem Zerkleinern und Waschen. Aktuelle Verbesserungen bei den Wasch- und Desinfektionsagenzien wie Chlor, Ozon, Wasserstoffperoxid, Chlordioxid und Antioxidationslösungen sowie ihre Vor- und Nachteile werden beurteilt. Im zweiten Teil der Arbeit steht die Beurteilung von neu hinzugekommenen Konservierungstechniken wie der superatmosphärischen O<sub>2</sub>-Atmosphäre, der Heißwasserbehandlung und der UV-C-Bestrahlung, die als Alternativen zu den herkömmlichen Techniken die mikrobielle und sensorische Endqualität von minimal-bearbeiteten Blattgemüsen verbessern können.

### Introduction

Minimally fresh processed (or fresh-cut) vegetables are constituted by 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 en-

sureing food safety. The processing operations eliminate every inedible part and reduce their several weeks or months shelf-life to a very short shelf-life (ROLLE and CHISM 1987; SHEWFELT 1987; BOLIN and HUXSOLL 1991a, b; BRACKETT 1992; NGUYEN-THE and CARLIN 1994; SCHLIMME 1995; AHVENAINEN 1996; ARTÉS 2000b; CANTWELL and TREVOR 2002).

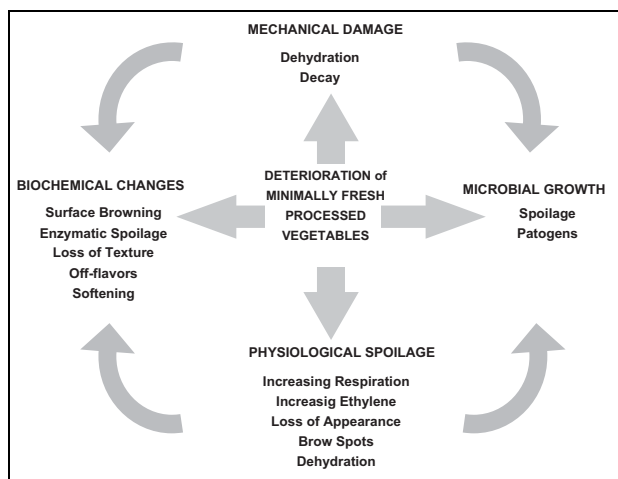


Fig. 1. Factors that affect deterioration and shelf-life of minimally fresh processed vegetables.

Deterioration of fresh processed vegetables is cumulative and it is mainly due to mechanical damage, biochemical changes, physiological aging, and microbial spoilage (BOGH-SORENSEN 1990; NGUYEN-THE and CARLIN 1994; ALLENDE et al. 2004a) (Fig. 1). In general, the extension of the shelf-life depends on a combination of Good Agricultural Practices, gentle transportation, chilling storage throughout the entire cold chain, good packaging conditions and manufacturing and handling practices. Currently, all steps of the process and distribution chain are accomplished using highly integrated systems, which follows a rigorous and systematic control of hygienic practices throughout the entire chain and where all stages are considered in conjunction with the others (OHLSSON 1994; BRACKETT 2000; DAY 2000; LADO and YOUSEF 2002). It implies effective sanitation programs that include the implementation and application of general preventive measures such as Good Hygienic Practices and Good Manufacturing Practices (REIJ and DEN AANTREKKER 2003), environmental sanitation systematised as Sanitation Standard Operating Procedures, environmental microbial testing programs and Hazard Analysis Critical Control Point Programs (HACCP) (APHA 1972; CAC 1997; IFPA 2001; JACXSSENS 2002).

The most frequently used techniques for keeping quality of minimally fresh processed vegetables are chilling, chemical preservation (e.g. antioxidants, chlorine, antimicrobial solutions, acidulants, etc.) and conventional modified atmosphere packaging (MAP) storage. Nevertheless some of them, like chlorine, have shown inconvenients for use and alternatives must be considered. In addition to this, considerable progress will be achieved with respect to product quality and shelf-life by advances in the technologies for minimal processing of leafy vegetables (ALLENDE 2003). Therefore, the food industry is aggressively seeking preservation technologies that deliver convenience products, which look fresh and palatable (VANKERSCHAUVER et al. 1996).

There is still a lack of information about the actual critical points of the processing chain of the industrial process and the available techniques to successfully preserve the fresh processed vegetable products. The

objective of this review is to compile all the current information related to the minimal processing lines of leafy vegetables as well as the new alternative techniques that can be successfully applied to preserve the quality and prolong their shelf-life. These techniques include different treatments in the processing chain such as washing and sanitizing agents (ozone, hydrogen peroxide, chlorine dioxide and antioxidant solutions) as well as preservation techniques that have been already tested in leafy vegetables (hot water treatments, super-atmospheric oxygen conditions and UV-C radiation).

### Processing Lines of Minimally Fresh Processed Vegetables

The industrial process typically begins with the raw product the most recently harvested as possible, transported in boxes or bins, pre-cooled and chilled stored in the factory before processing (GROSS et al. 2002). From this moment and up to consumer consumption, the time-temperature conditions of fresh-cut vegetables must be monitored to minimise the effects of wounding and microbial growth in fresh processed vegetables (BRECHT 1995; WATADA 1997; FRANCIS et al. 1999; WATADA and QI 1999 ARTÉS 2000b). Time-temperature conditions will determine the responsibility of the actual cold chain on the quality loss and shelf-life of these products and are essential Critical Control Points in a HACCP system (WILLOCX 1995; IFPA 2001; JACXSSENS 2002). Currently, there is a tendency in harmonizing temperatures and standardizing temperature control in the European Union (EU). However, there is not an international legislation concerning fresh fruit and vegetables (WILLOCX 1995). Additionally, temperature control varies from country to country. For example, in Spain and Belgium, there is no specific temperature control legislation for minimally fresh processed vegetables. However as they are included in the category of refrigerated products, they must be kept at 5 °C in Spain (BOE 3484 2000), and at 7 °C in Belgium, with a tolerance up to 10 °C in the warmest spot (JACXSSENS 2002). In France, minimally processed vegetables must be stored at maximum 4 °C and in the UK, under 8 °C (ANONYMOUS 1988; DAY 2001).

The schedule of a common production line is shown in Fig. 2 (HERY et al. 1998). The trimming zone is fitted with any kind of handling equipment for raw products as well as working tables designed for easy processing and quick cleanup. Trimming procedures as well as the rest of the processing chain are best carried out in segregated, hygienic, temperature controlled (5 to 10 °C) factory zones (ARTÉS 2000b; ARTÉS and ARTÉS-HERNÁNDEZ 2003). Removal of the inedible parts (e.g. outer or damaged leaves, tops, stalks, calyx and core) is the essential first stage in reducing the over-all contamination on fresh prepared vegetables before further processing (ADAMS et al. 1989; FRANCIS et al. 1999). Inedible produce parts should be immediately separated from the edible parts and disposed outside the factory in designated areas (DAY 2000).

Generally, cutting is the next stage. Several studies confirm that cutting and shredding must be performed with knives or blades made from stainless steel as sharp as possible (AHVENAINEN 1996; BARRY-RYAN and

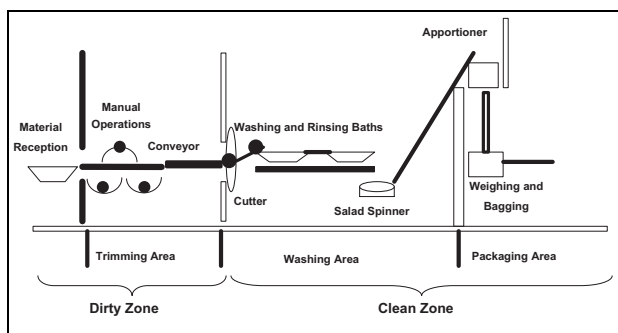


Fig. 2. Simplified scheme of the vegetable processing line (Adapted from HERY et al. 1998).

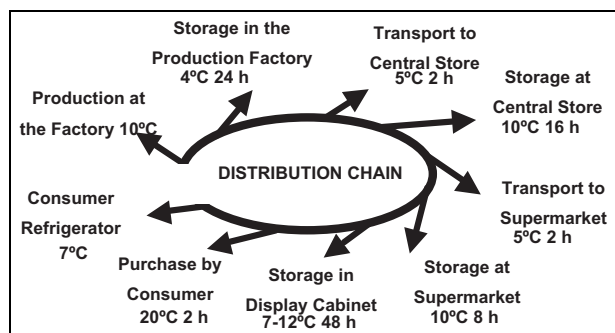


Fig. 3. Simulated distribution chain of fresh processed vegetables. (Adapted from ALLENDE et al. 2002).

O'BEIRNE 1999). In total, the initial preparation procedures lead to product losses of 20 to 70 % of the incoming raw materials weight (ARTÉS 2000b; DAY 2000). Cutting machinery constitutes a critical point, which needs to be cleaned and disinfected at regular intervals every working day to avoid build-up of organic residues (HEARD 2000). Shredding disrupts the plant tissues, breaking protective epidermal layers and releasing nutrient-rich vascular and cellular fluids (VAROQUAUX and WILEY 1994; SOLIVA-FORTUNY and MARTÍN-BELOSO 2003) causing physiological disorders. It appears to have a dramatic effect on nutritional value, overall quality, and shelf-life of the fresh processed vegetables (ADAMS et al. 1989; GARG et al. 1990; WATADA et al. 1990; KAYS 1991; MANGLES et al. 1993; BARRY-RYAN and O'BEIRNE 1999; AHVENAINEN 2000; ARTÉS 2000b; ROURA et al. 2000).

After the cutting process, leaves are conveyed to a washing zone with a restricted number of entrances (YILDIZ 1994; ARTÉS and ARTÉS-HERNÁNDEZ 2000). The washing procedure can consist of simply spraying with potable water or may involve attempts of disinfection by dipping in agitated, chilled (1 to 10 °C) potable water in a washing tank containing an antimicrobial washing solution (FRANCIS and O'BEIRNE 1997; ARTÉS 2000b; DAY 2000). There is a wide range of washing baths available for the fresh-processing industry (SAPERS 2001); however, the most common used washing bath is an open flume wash system with optional air agitation, which increases the surface contact, and hydro-chill conveyors, which utilise high flow water distribution systems. The turbulence generated by using the aeration allows eliminating almost all traces of earth and foreign matter without damaging the product (YILDIZ 1994; SIMONS and SANGUANSRI 1997). After disinfection the product must be rinsed. This stage is commonly done by using cold-water (1 to 2 °C) showers or dips. The final chlorine residue in the product must be lower than 5 ppm (ARTÉS 2000b), although the legal limit may vary from country to country. Contact times between the produce and the wash water are usually less than 5 minutes throughout all the washing stages (ALLENDE et al. 2004a). Dewatering systems include centrifugal spin dryers, vibrating racks, rotating conveyors, hydro-sieves, and spinless drying tunnels (SIMONS and SANGUANSRI 1997; SEYMOUR 1999). Alternative, continuous-flow conveyor systems using air removal can be used to avoid injury problems, but may also contribute to product warming. About 70 % of the veg-

etable processing industry in the UK used in 1999 spin drying systems (SEYMOUR 1999), that operate on-line, reducing manual labour, avoiding dewatering bottleneck and increasing through-put (JACXSENS 2002).

The product should be hygienically and quickly conveyed to the packaging area (HERY et al. 1998). Some vegetable products such as salads and soup-vegetables require mixing and assembling before packaging (YILDIZ 1994, JACXSENS 2002). Trumbles are the most common mixing machines applied for fresh processed vegetable products to avoid physical deterioration (YILDIZ 1994; AHVENAINEN 2000). In the packaging zone, the apportioning hopper automatically packs the fresh processed vegetables in bags. Selected polymeric films and MAP techniques must be applied to optimise this operation (AGUAYO et al. 2003; BRECHT et al. 2003). Once the product is sealed, bags pass by simple bag conveyors through weight control and metal detector. From this point some bags are taken at random for quality control and the rest are immediately put into boxes for consignment (ARTÉS and ARTÉS-HERNÁNDEZ 2003).

Finally, the product lots are directly chilled transported onto temperature-controlled trucks to different distribution places (e.g. local markets, distribution centres, logistic platforms), and then transported to supermarkets and retail display cabinets (Fig. 3).

### Chemical Surface Decontamination

Washing and disinfection are the only stages in the production chain where a reduction in the microbial load can be obtained, thus minimising populations of potential pathogens (NGUYEN-THE and CARLIN 1994; WILEY 1994; BEUCHAT et al. 1998; FRANCIS et al. 1999; DAY 2001), as well as elimination of unwanted dirt, soil, insects and foreign matter, and retardation of enzymatic discoloration reactions (SAPERS 2001; SEYMOUR 1999). It is important that microbiological and sensory quality of the washing water must be good and frequently checked and its temperature low to avoid cross-contamination of the product (NGUYEN-THE and PRUNIER 1989; AHVENAINEN 2000). Nevertheless, disinfection of salad leaves (using chlorine and organic acids) does not usually reduce microbial contamination by more than 2–3 log units (ADAMS et al. 1989; NGUYEN-THE and CARLIN 1994).

In general, more disinfectants and disinfection treatments are allowed in USA as in EU (BEUCHAT 1998; JACXSENS 2002). For example, chlorine may not be used as a disinfectant in Belgium, Netherlands, and Germany (ANONYMOUS 1989), while chlorinated water is permitted to disinfect vegetable products in some other countries (e.g. France, Italy, Spain and UK). In the USA and Spain, it is usually recommended to use cold water (less than 5 °C) containing between 100 and 200 ppm of chlorine solution, and acidified with about 150 to 200 ppm of citric acid to manage optimum pH values (between 6.5 and 7.5), where the chlorine is more effective against microorganisms (ARTÉS 2000b).

### Chlorine

Probably, the most widely used antimicrobial agents are sodium hypochlorite and several chlorine-based compounds, due to the effective action of chlorine against many foodborne microorganisms, (BRACKETT 1987; ADAMS et al. 1989; BRACKETT 1992; FRANCIS and O'BEIRNE 1997; KIM and YOUSEF 1998; KIM et al. 1999a).

However, the use of chlorinated solutions has disadvantages. The reaction of chlorine with organic residues constantly decreases the amount of free available chlorine during washing of vegetables. Additionally, there are increasing concerns over the fact that some food constituents may react with chlorine to form potentially toxic products (mutagenic or carcinogenic reaction products), denominated disinfectant by-products. The most notable of these products are the trihalomethanes, some of them are reported as mutagens (RICHARDSON 1994; RICHARDSON et al. 1998; JACXSENS 2002; ARTÉS 2004). Some restrictions in the use of chlorine are being implemented by regulatory agencies (SAPERS 2001).

Therefore, new disinfection agents have been tested such as chlorine dioxide, ozone, organic acids, hydrogen peroxide, quaternary ammonium compounds, trisodium phosphate, sucrose esters, iodine compounds, alcohols, anionic and non-ionic surface-active agents, aldehydes, phosphoric acids, cysteine, methyl jasmonate and bioflavonoids (PAPPALARDO et al. 1990; VAN DE WEYER et al. 1993; ZHUANG and BEUCHAT 1996; BEUCHAT et al. 1998; SAPERS and SIMMONS 1998; SEYMOUR 1999; DAY 2001).

### Ozone

Ozone is a strong sanitizer agent that may meet expectations of the industry, approval of the regulatory agencies, and acceptance of the consumers (KHADRE et al. 2001). This disinfectant has been commonly used as a sanitizer in many European water treatment plants, swimming pools, sewage plants (GOMELLA 1972; RICE et al. 1982), cold room air treatment, equipment sterilization, as well as for the preservation and shelf-life extension of fruit and vegetables (GRAHAM 1997; KIM et al. 1999a, b; XU 1999; ZANG et al. 2004).

In 1997, an expert panel decreed that O<sub>3</sub> was a generally recognized as safe (GRAS) substance for use as a disinfectant or sanitizer for foods compatible with good manufacturing practices in the USA (SUSLOW 2003) and it was approved as a disinfectant or sanitizer in foods and food processing in the USA (USDA 1997, 1998).

O<sub>3</sub> is useful in decreasing the microbial load, the level of toxic organic compounds, the chemical and biological O<sub>2</sub> demands and it is able to convert many non-biodegradable organic materials into biodegradable forms (KIM et al. 1999a; KHADRE et al. 2001). Additionally, the application of O<sub>3</sub> has been reported to increase the shelf-life of some vegetable products, as well as to decrease chemoluminescence, O<sub>2</sub> uptake, catalase and peroxidase activities (KIM et al. 1999b).

The bactericidal action of O<sub>3</sub> have been documented on a wide variety of organisms, including those that are resistant to chlorine, being useful for extending the shelf-life of a number of fruits and vegetables (RICE et al. 1982; BUSTA 1991; Foegeding and RESTAINO et al. 1995; BEUCHAT 1998; RICHARDSON et al. 1998). Inactivation of microorganisms by O<sub>3</sub> is a complex process, which seems to be more effective against bacteria cells than bacterial and fungal spores. O<sub>3</sub> is able to react to numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycan in spore coats and virus capsids (EPRI 1997; KHADRE et al. 2001). L'HERAULT and CHUNG (1984) reported that O<sub>3</sub> can damage the cellular DNA provoking the death of the cell. Some authors regard the molecular O<sub>3</sub> as the main inactivator of microorganisms, while for others the antimicrobial activity is due to their reactive by-products of decomposition such as .OH, O<sub>2</sub><sup>-</sup>, and HO<sub>3</sub> (CHANG 1971; HARAKEH and BUTLER 1995; HUM and MARINAS 1997). The process implicates the destruction of microorganisms by the progressive oxidation of vital cellular components (KIM et al. 2003) but the complete mode of action is still unclear.

GOMELLA (1972) reported that O<sub>3</sub>, compared to chlorine, showed stronger and more rapid antimicrobial action against spores, faecal and pathogenic microorganisms, and viruses, mainly in an environment with high organic-matter content. In clean, potable water free of organic debris and soil particulates, O<sub>3</sub> is a highly effective sanitizer at relatively low concentrations (0.5 to 2 ppm) and short exposition times (3–5 min) (BELTRÁN 1995; KIM et al. 2003). In addition, the molecule decomposes spontaneously to O<sub>2</sub>, thus, using O<sub>3</sub> minimises the accumulation of inorganic waste in the environment (HORVATH et al. 1985).

Nevertheless, the use of O<sub>3</sub> in the storage of plant products can have detrimental effects oxidising the fruit, as happened in some berries with very thin skin (NORTON et al. 1968; RICE et al. 1982). It is known that O<sub>3</sub> can oxidise several organic compound (e.g. vitamin C), particularly those with phenolic rings (RAZUMOVSKI and ZAIKOV 1984) and double bonds. Additionally, it was found that the O<sub>3</sub> effectiveness reducing growth of different pathogenic microorganisms and decay is not equal in all the tested fruit and vegetables (SPOTTS and CERVANTES 1992; SAPERS 2001).

One point to take into account is that the O<sub>3</sub> solubility in water increases at lower temperatures. However, the low temperatures reduce the speed reaction. Additionally it is difficult to maintain a constant dose of O<sub>3</sub> during the treatment of fresh processed vegetables and it must be generated in place, since O<sub>3</sub> cannot be stored. Finally, an important problem for the process-

ing industry is the toxicity of the O<sub>3</sub> at certain concentration. Therefore, the Occupational Safety and Health Administration of the USA has fixed the permissible limits in 0.1 ppm for the time weighted average and 0.3 ppm for short-term exposure limit (GIL et al. 2003).

#### *Hydrogen peroxide*

The antimicrobial properties of H<sub>2</sub>O<sub>2</sub> have been long recognised. It is classified as GRAS for use in food products as a bleaching agent, an oxidising, reducing and antimicrobial agent (SAPERS and SIMMONS 1998), but has not yet been approved as an antimicrobial for washing produce (SAPERS 2001). Various experimental antimicrobial applications of H<sub>2</sub>O<sub>2</sub> for foods have been described, including preservation of vegetable salads (HAGENMAIER and BAKER 1997; SOLIVA-FORTUNY and MARTÍN-BELLOSO 2003).

Use of H<sub>2</sub>O<sub>2</sub> as an alternative to chlorine for disinfecting minimally fresh processed vegetables appear to reduce microbial populations and extend shelf-life without leaving significant residues, since it is rapidly decomposed by catalase to water and O<sub>2</sub>, or causing loss of quality. H<sub>2</sub>O<sub>2</sub> could be applied by using vapour or solution treatments. The vapour treatments should be given as a surface disinfection treatment to reduce the population of potential spoilage organisms and pathogens (SAPERS and SIMMONS 1998). However, such treatments require lengthy application times (15–60 min) and can cause injury to some commodities (SAPERS 2001).

The application of a liquid solution of H<sub>2</sub>O<sub>2</sub> (5–10 %) was efficient to extend shelf-life of different commodities (SAPERS and SIMMONS 1998) due in part to the lethal effect of H<sub>2</sub>O<sub>2</sub> on bacteria in the washing medium that were removed from product surfaces during treatment. However, the use of diluted H<sub>2</sub>O<sub>2</sub> solutions can be also injurious to some commodities (SAPERS 2001).

#### *Chlorine dioxide*

The chlorine dioxide is a strong disinfectant agent (about 2.5 times the oxidative capacity of chlorine) with a broad biocide efficacy. Many authors already tested the chlorine dioxide efficacy in reducing the microbial population and pathogenic bacteria growth in fresh processed leafy vegetables (ZHANG and FARBER 1996; SINGH et al. 2002). However, to date, the Food and Drug Administration of the USA (USDA 1998) has only allowed the use of aqueous ClO<sub>2</sub> in washing of uncut and unpeeled fruits and vegetables. Additionally, ClO<sub>2</sub> is unstable, and it must be generated on-site and can be explosive when concentrated (JACXSENS 2002).

#### *Antioxidants*

Antioxidants can be used after washing to avoid browning, but only few chemicals are currently allowed in the EU (only the compounds included in a positive list) (JACXSENS 2002). Control of enzymatic browning in minimally processed vegetables has received a great deal of attention by researchers because of its importance in the acceptance of the consumer. The enzymatic browning is catalysed by the polyphenol

oxydase (PPO) enzyme and many strategies has been already described to inactivate this reaction: by heat inactivation of the enzyme (LOAIZA-VELARDE and SALTVEIT 2001); exclusion, removal or excess of one or both substrates: O<sub>2</sub> (by applying MAP or superatmospheric O<sub>2</sub>) (DAY 2001; JACXSENS et al. 2001; ALLENDE et al. 2001, 2002, 2003a, b, 2004b) and phenols (BARRET 1996; DAY 1996; ARTÉS et al. 1998; KADER and BEN-YEHOSHUA 2000); lowering the pH to 2 or more units below the optimum pH (MCEVILY et al. 1992); reaction-inactivation of the enzyme, and addition of PPO inhibitors (ARTÉS et al. 1998). However, the most common method for controlling oxidative (enzymatic) browning is the addition of reducing agents such as sulphites and ascorbic acid to the dipping solution, which prevent browning by reducing the enzymatically formed quinones back to their parent o-diphenols. They act as PPO inhibitors, and antimicrobial agents.

Sulphites have long been used as food additives to inhibit enzymatic and non-enzymatic discolorations, to control the growth of microorganisms and to act as bleaching agents and antioxidant (MCEVILY et al. 1992; SAPERS 1993; ARTÉS et al. 1998; LAURILA et al. 1998; AHVENAINEN 2000). The most frequently used sulphating agents for fresh prepared produces are sulphurous acid, sulphur dioxide, sodium and potassium bisulphites, and metabisulphites. However, the Food & Drug Administration of the USA has restricted the use of sulphating agents as inhibitors of enzymatic browning in foods. Since the exposure to SO<sub>2</sub> may cause health problems of persons with respiratory problems and may impart an objectionable taste in some products (ANONYMOUS 1991; SAPERS 1993). Due to these increased regulatory restrictions there is an urgent need for safe, convenient and economically viable alternatives (AHVENAINEN 1996; MARTINEZ and WHITAKER 1995; DUNCAN 1999; DAY 2001). The current alternatives include: ascorbic acid and its salts, citric acid and its salts, benzoic acid, sodium benzoate, sodium chloride, calcium chloride, zinc chloride, cinnamic acid, sodium cinnamate, cysteine, glutathione, β-cyclodextrins, glycine betaine solutions, commercial polyphosphate and their various combinations (SAPERS and ZIOLKOWSKI 1987; HSU et al. 1988; DUDLEY and HOTCHKISS 1989; FRIEDMAN and MOLNAR-PERL 1990; LOZANO-DE-GONZÁLEZ et al. 1993; MARTINEZ and WHITAKER 1995; HURME et al. 1999; AHVENAINEN 2000; DAY 2001).

Among all these candidates, ascorbic acid could be probably the most suitable alternative to sulphite (GIL et al. 1998). The most common dipping times are usually in the range of 2–5 minutes to permit sufficient absorption without provoke detrimental effect on the appearance of the product due to excessive water absorption (AHVENAINEN 2000; DAY 2000; JACXSENS 2002). A final potable water rinse-off stage is typically required after anti-browning dipping of minimally fresh processed produce items.

### **Modified Atmosphere Packaging (MAP)**

The combination of chilling with MAP is the most used preservation technique for fresh processed leafy

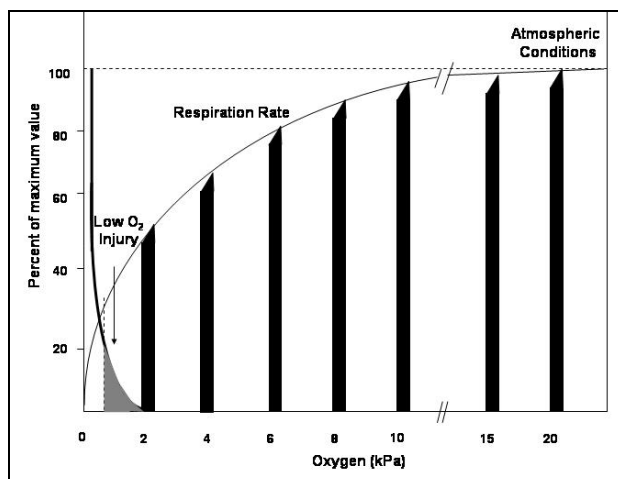


Fig. 4. Respiration of lettuce in varying oxygen atmospheres (Redraw from SALTVEIT 2003).

vegetables (BRECHT 1995; BEAUDRY 1999; ARTÉS 2000a). A well designed MAP is able to generate the optimal gas conditions within packages to extend shelf-life, improve safety and maintain sensory attributes of fresh processed vegetables by inhibiting metabolic activity, decay, and especially  $C_2H_4$  biosynthesis and action (KADER 1986; BRECHT 1995; VERMEIREN et al. 1999; ARTÉS 2000a).

#### Low oxygen MA

A raised level of  $CO_2$ , as well as a low  $O_2$  concentration, as the most common gas concentrations, may lower product respiration (Fig. 4) and enzymatic browning rates, reducing sensorial and microbial deterioration and extending the shelf-life of the fresh-cut product (THEOLOGIS and LATIES 1978; KADER 1986; VANKERSCHAUER et al. 1996; ARTÉS 2000a; 2002a, b; THOMAS and O'BEIRNE 2000). Therefore, it is widely used in the prevention of browning of minimally fresh processed lettuce (MATEOS et al. 1993, LEE et al. 1996; ARTÉS et al. 1999). However, despite of the many advantages of conventional MAP to reduce deterioration of fresh processed vegetables (KADER 1986; GORRIS and PEPPELENBOS 1992; NGUYEN-THE and CARLIN 1994; AHVENAINEN 1996; ARTÉS and MARTÍNEZ 1996; ARTÉS 2000a), it is well known that some fresh-cut vegetables under conventional MAP can progressively generate anaerobic conditions, reducing the microbial and sensory quality of the products (DOSTAL-LANGE and BEAUDRY 1991; BEAUDRY et al. 1992; EXAMA et al. 1993; CAMERON et al. 1993, 1995; NGUYEN-THE and CARLIN 1994; VAROQUAUX and WILEY 1994; BEAUDRY 1999; SMYTH et al. 1998; JACXSSENS et al. 2001; BRECHT et al. 2003; SALTVEIT 2003).

An alternative to conventional Controlled Atmosphere (CA) and MAP is the use of argon instead of nitrogen as major component of the gas composition. It has been reported to improve the storage life of fresh vegetables by reducing microbial growth and improving product quality retention (BURG and BURG 1965; BERNE 1994; DAY 1996, 1998; JAMIE and SALTVEIT 2002).

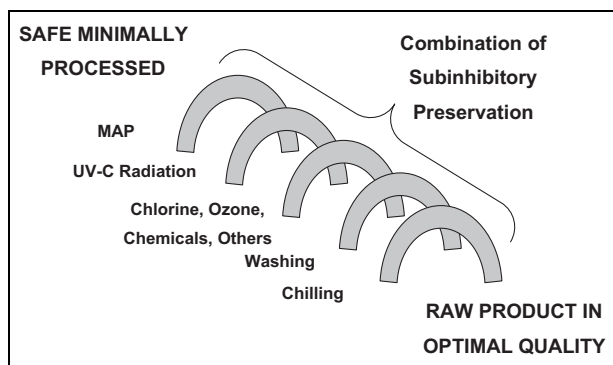


Fig. 5. The hurdles concept involving subinhibitory treatments. (Adapted from SCOTT 1989).

#### Superatmospheric oxygen MA

The improved effect of superatmospheric  $O_2$  atmosphere (higher than 70 kPa  $O_2$ ), when combined with increased  $CO_2$  concentration, in the inhibition of microbial growth and decay of minimally fresh processed vegetables has been recently demonstrated (HEIMDAL et al. 1995; AMANATIDOU et al. 1999, 2000; DAY 2001; ALLENDE et al. 2001, 2002, 2003a, b, 2004b). Therefore, it could be considered as a good alternative to conventional MAP with moderate-to-low  $O_2$  and high  $CO_2$  levels (DAY 1996).

Superatmospheric  $O_2$  levels have been shown to affect metabolism and different properties of vegetable commodities such as respiration rate, colour, and texture, microbial growth and decay (GREGORY and FRIDOVICH 1973; GONZALEZ RONCERO and DAY 1998; AMANATIDOU et al. 1999; KADER and BEN-YEHOSHUA 2000; WSZELAKI and MITCHAM 2000; JACXSSENS et al. 2001; ALLENDE et al. 2001, 2002, 2004b).

The toxic effect of  $O_2$  on microbial growth due to the formation of superoxide radicals ( $O_2^-$ ), have already been explained (GREGORY and FRIDOVICH 1973; GONZALEZ RONCERO and DAY 1998; AMANATIDOU et al. 1999). The  $O_2$  molecule is known to have a low reactivity; therefore, its toxicity stems mostly from its excited state (singlet  $O_2$ ) or its semi-reduced radical forms that can cause deleterious or lethal oxidative damage to cells (GILLE and SIGLER 1995). The Reactive Oxygen Species (ROS), notably  $O_2^-$  and hydroxyl ( $OH^-$ ) radicals,  $H_2O_2$  and singlet oxygen ( $^1O_2$ ), generated during the aerobic cellular metabolism induce DNA and nucleoproteins damage as well as lipid and protein damages in microorganisms (MORADAS-FERREIRA et al. 1996). Many microorganisms possess enzymatic and non-enzymatic antioxidative mechanisms and minimize generation of ROS to levels that are not harmful to the cells (AMANATIDOU 2001) but when ROS levels exceed the antioxidant capacity of the cells, an oxidative stress resulted (MORADAS-FERREIRA et al. 1996).

Nevertheless, exposure to superatmospheric  $O_2$  may inhibit, have no effect, or even stimulate growth of different microorganisms from the same genus. Because of the different behaviour of microorganisms under this atmosphere, it is necessary to study the effect

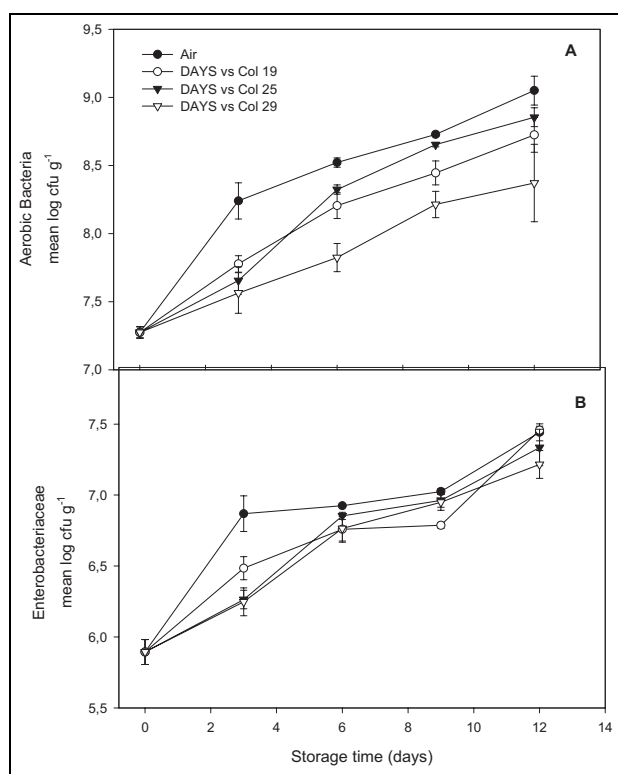


Fig. 6. Growth of aerobic bacteria (A) and Enterobacteriaceae (B) ( $\log_{10}$  CFU/g) on fresh processed baby spinach packaged under air, conventional MAP, and superatmospheric  $O_2$  (80 and 100 kPa  $O_2$ ) at 5 ° for 12 days. Bars indicate a 95 % confidence interval of the measurements (Adapted from ALLENDE et al. 2004b).

of superatmospheric  $O_2$  on the microflora of each microorganism and vegetable product. We recently found that the effect of superatmospheric  $O_2$  on the growth of aerobic microflora was variable (Fig. 6). The overall visual appearance (mainly color) of the mixed vegetable salads and baby spinach was better maintained and the shelf-life prolonged when packaged under  $O_2$  levels higher than 50 kPa (ALLENDE et al. 2002, 2004b).

On the other hand, it was reported that increased  $O_2$  concentrations around and within commodity may cause damage to the tissue due to the higher levels of free radicals (FRIDOVICH 1986) increasing the respiration rate, ethylene production, peel breakdown, synthesis and accumulation of some volatile compounds and physiological disorders (KADER and BEN-YEHOSHUA 2000). Concentrations higher than 50 kPa  $O_2$  are considered to be explosive and special precautions have to be taken on the work floor (BCGA 1998).

### Alternative surface decontamination methods

The food industry is currently searching for new alternative technologies to assure the safety of the fresh processed products (KHADRE et al. 2001; LADO and YOUSEF 2002; MENG and DOYLE 2002). Most of these emerging technologies are less damaging to product quality, more cost-efficient and environmentally friendly than current techniques, in reaction to the changing

requirements of consumers (GOULD 1996; PIYASENA et al. 2003). Therefore, there is a move away from using high concentrations of individual food preservatives towards increasing reliance on an intelligent mix of hurdles like combinations of sub-lethal levels of antimicrobial compounds or processes (ROLLER 1999). This phenomenon is known as the hurdle effect (Fig. 5) (LEISTNER 1978, 1995, 2000; BRUL and COOTE 1999) and it implies a good understanding of the physiological responses of microorganisms to stresses imposed during food preservation (KNOCHEL and GOULD 1995). Therefore, more research is needed in view of microorganisms behaviour considering the homeostasis, metabolic exhaustion, and stress reactions of microorganisms in relation to hurdle technology, due to the current danger of the emergence of microorganisms capable of exploiting these 'new' environments (LEISTNER 2000; THOMAS and O'BEIRNE 2000), known as 'emerging pathogens'.

### Hot water treatments

In recent years there has been an increasing interest in the use of heat postharvest treatments due to its effectiveness against fungal pathogen (ARTÉS 1995; LURIE 1998) and to reduce browning of minimally processed vegetables (LOAIZA-VELARDE et al. 2003; DELAQUIS et al. 2004).

Wounding during fresh processing fruit and vegetables induces the synthesis of certain proteins and subsequent tissue browning (KE and SALTVEIT 1989; BRECHT 1995; LÓPEZ-GÁLVEZ et al. 1997; SALTVEIT 1997). Therefore, inhibitors of protein synthesis could be effective in reducing browning by interfering with the synthesis of enzymes involved in phenolic metabolism (LOAIZA-VELARDE and SALTVEIT 2001).

The cellular defence against hostile environments by the activation of heat shock proteins (hsp) is an important resistance mechanism, which indicates the activation of the cell integrity pathway (BRUL et al. 2002) that protects the induced tissue from subsequent high temperature stress (VIERLING 1991). It means that exposure of plant tissue to temperatures about 10 ° above the normal growing temperature induces the synthesis of hsp. It was reported that a heat-shock treatment (e.g. 90 s at 45 ° or 55 °C) that reduces browning in fresh-cut lettuce, may work by redirecting protein synthesis from the production of wound-induced enzymes of phenolic metabolism to the production of innocuous hsp (OUGHAM and STODDART 1986; FOURRE and LHOEST 1989; SOMERS et al. 1989; DELAQUIS et al. 1999; SALTVEIT 2000).

Besides controlling browning, heat shock treatments have additional benefits. Lower centrifugal forces to de-water vegetable products are needed because water at 45 °C drains away faster than water at 0 °C. Furthermore, since phenolic compounds are not synthesised, the use of expensive barrier bags to maintain MA could not be required to prevent browning and an inexpensive polyethylene or polypropylene bag could be used to reduce moisture loss and maintain the cleanliness of the tissue.

On the other hand, it should be taken into account that the slight heat stress that prevents the rise in PAL activity and browning in lettuce does not eliminate the

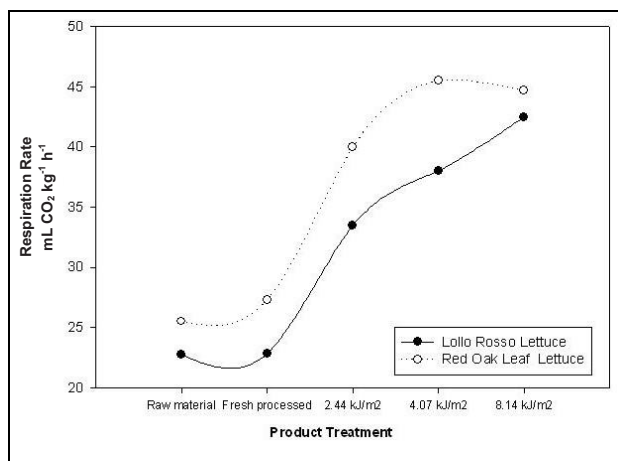


Fig. 7. Mean values of the respiration rate for 'Lollo Rosso' and 'Red Oak Leaf' lettuce. Values belong to the raw material, fresh processed product, and fresh processed and radiated with different UV-C radiation product and stored for 6 days at 5 °C. (ALLENDE and ARTÉS 2003a, b).

activity of enzymes involved in tissue browning (e.g. PAL, PPO or POD) that are already present in the tissue (LOIZA-VELARDE et al. 1997). Therefore, alternative (e.g. higher temperature, longer treatment time) and combined (e.g. antioxidants, low O<sub>2</sub> MAP) treatments may be necessary to control browning in lettuce that have high levels of phenolic compounds and activities of enzymes of phenolic metabolism (SALTVEIT 2000).

Furthermore, some problems have been associated with the use of hot water treatments. LI et al. (2001) suggest that heat (50 °C) treatment combined with 20 mgL<sup>-1</sup> free chlorine for 90 s may have delayed browning and reduced initial populations of some groups of microorganisms naturally occurring on iceberg lettuce, but enhanced microbial growth during subsequent storage due to tissue damage. Too high temperatures stimulate respiration and damage the tissue, increasing browning and reducing shelf-life of vegetable products (DE BELIE et al 2000; SALTVEIT 2000).

#### UV-C radiations

The knowledge of the ultraviolet radiation (UV-C) as a technique to preserve food was discovered in the 1930 s and since then, several studies have addressed the effect of UV-C on microorganisms (ABSHIRE and DUNTON 1981; EL-GHAOUTH and WILSON 1995; SOMMER et al. 1996, 2000; BINTSIS et al. 2000; GARDNER and SHAMA 2000; MARQUENIE et al. 2002a). The damage inflicted by UV-C probably involves specific target molecules and a dose in the range from 0.5 to 20 J m<sup>-2</sup> leads to lethality by directly altering microbial DNA (BINTSIS et al. 2000).

Currently, UV-C radiation is widely used in the industry of fruit juices for disinfection of water supplies and food contact surfaces. This recent uses have demonstrated a beneficial effect when low doses were applied to different food products (STERMER et al. 1987; LIU et al. 1993; MAHARAJ et al. 1999; STEVENS et al. 1999; DUFFY et al. 2000; ERKAN et al. 2001; MERCIER

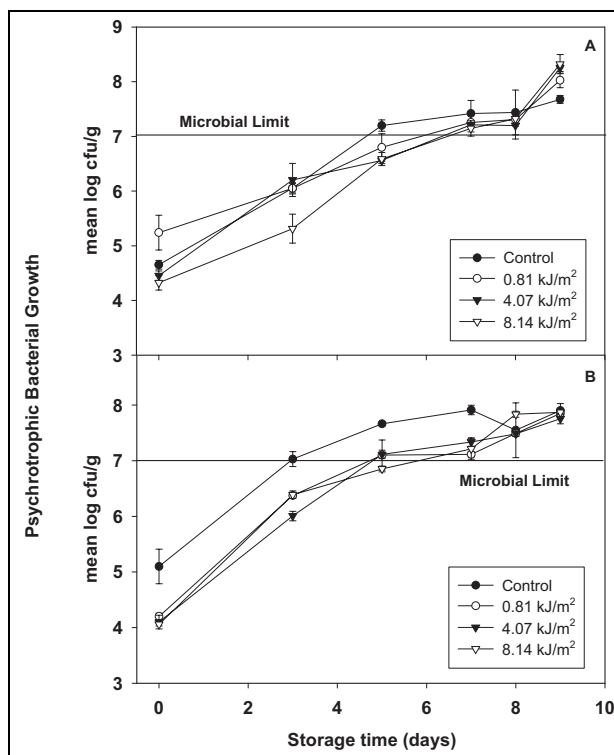


Fig. 8. Psychrotrophic bacterial growth (log<sub>10</sub> CFU/g) from fresh processed 'Lollo Rosso' (A) and 'Red Oak Leaf' (B) lettuces depending of UV-C doses, stored under passive MAP at 5 °C for 9 days. Error bars indicate a 95 % confidence interval. The straight horizontal line indicates the microbial criteria of the Spanish legislation for total aerobic growth, 7 log cfu/g (ALLENDE and ARTÉS 2003a, b).

et al. 2001). However, JAGGER (1965) and LUCKEY (1980) reported that UV-C radiation is mainly a surface treatment because it penetrates only 50–300 mm into the plant tissue. Therefore, UV-C light cannot penetrate through water, glass, and other substances and they can only be successfully used for surface microbial populations.

The UV energy is a non-ionising radiation with germicidal properties at wavelengths in the range of 200–280 nm, with a maximum at 254 nm. It has been found that UV radiation damages microbial DNA and to a lesser extent denatures proteins (KUO et al. 1997; LUCHT et al. 1998). Furthermore, it induces the formation of pyrimidine dimers, which distort the DNA helix and block cell replication. In addition, UV radiation cross-links aromatic amino acids at their carbon-carbon double bonds. The resulting denaturation of proteins contributes to membrane depolarisation and abnormal ionic flow (MOSELEY 1990). Cells unable to repair their radiation-damaged DNA die. Particularly, exposure of microbial cells to UV induces enzymatic photorepair and expression of excision-repair genes that may restore DNA integrity (LADO and YOUSEF 2002).

Recently, UV-C radiation has been considered an alternative treatment for preserving vegetable products (MAHARAJ et al. 1999; ALLENDE et al. 2004; YAUN et al. 2004). In fact, UV-C radiation doses has been reported



to reduce postharvest decay of onions (LU et al. 1987), sweet potatoes (STEVENS et al. 1999), carrots (MERCIER and ARUL 1993), tomatoes (LIU et al. 1993; MAHARAJ 1995), strawberry (MARQUENIE et al. 2002a, b), apples (WILSON et al. 1997), peaches (STEVENS et al. 1998), lemon fruits (BEN-YEHOSHUA et al. 1992), table grape (NIGRO et al. 1998), and zucchini squash (ERKAN et al. 2001). The reduction in postharvest diseases and delaying decay in low UV-C treated commodities have been related to the increase in decay-resistance of tissues due to the accumulation of antifungal compounds (ERKAN et al. 2001).

UV-C radiation has been recommended as best used in combination with other preservation techniques to keep the sensorial quality and nutritional value of the product, since the accumulative damage to microbial DNA appears to be effective decreasing the overall number of bacteria, but does not result in complete sterilisation (RAME et al. 1997; BRUL and COOTE 1999; ALLENDE and ARTÉS 2003a, b). Recently, MARQUENIE et al. (2003) confirmed that the intensity of these treatments should be minimized to prevent quality loss as an undesired side effect. They proposed the combined use of UV-C and pulsed white light or mild heat treatment.

Our results showed that the use of low UV-C doses (between 0.81 and 8.14 kJ m<sup>-2</sup>) causes a respiratory stress in the 'Lollo Rosso' and 'Red Oak Leaf' lettuce tissue (Fig. 7) and reduced growth of psychrotrophic bacteria (Fig. 8), coliforms, yeast and moulds in minimally fresh processed lettuce without affect the sensory quality of the product (ALLENDE and ARTÉS 2003a, b).

The UV-C radiation in practice, has been already reported by WILSON et al. (1997), they successfully treated apples on line during processing with UV-C light for controlling postharvest decay. The equipment is relatively inexpensive, but the technique is subject to certain safety precautions easy to use, and the radiation is lethal to most types of microorganisms (BINTSIS et al. 2000). However, an on-line application for leafy vegetables could be difficult due to the rough surface of vegetables.

## Conclusions

The advances in alternative, milder, more cost-efficient and environmentally friendly processing techniques will improve the quality and safety of minimally fresh processed vegetables. Therefore, an actual knowledge of the current status of the production chain of minimally processed vegetables is necessary to clearly establish the critical points of this industry.

Washing and disinfection are the most important stages of the production chain where a reduction in the microbial load can be obtained. Conventional washing and sanitizing techniques usually get 1–2 log units or even smaller reductions in microbial population in some commercial washing systems. However, alternative sanitizer formulations and disinfection techniques can get improvements in the decontaminating procedure such as hydrogen peroxide, chlorine dioxide, and ozone. As all these disinfection agents have benefits and problems to be applied in the fresh processing industry, new decontamination methods are needed to assure safety of minimally fresh processed vegetables.

Among them, surface-sanitizing agents could represent an alternative to increase efficacy of the production chain, since they are compatible with existing industrial practices. Interesting results have been obtained with hot water treatments and UV-C radiation alone or combined with other sanitizing methods. However, it is clear that the successful application of a disinfectant agent have to be validated in each condition.

Additionally, superatmospheric O<sub>2</sub> conditions were successfully applied to improve quality and prolong the shelf-life of minimally processed leafy vegetables. However, these alternative MAP may not be recommended for all types of vegetables and all the fresh processing conditions.

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Received January 26, 2004 / Accepted May 03

Addresses of authors: F. Artés (corresponding author) and A. Allende, Postharvest and Refrigeration Group, Department of Food Engineering, Technical University of Cartagena, Paseo Alfonso XI-II, 48. 30023 Cartagena, Murcia, Spain, e-mail: fr.artes@upct.es.