Environmentally Friendly and Safe Technologies for Quality of Fruits and Vegetables

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The papers contained in this book report some of the peer reviewed Proceedings of the International Conference "Environmentally friendly and safe technologies for quality of fruit and vegetables", but also other papers related with the subject were included. The manuscripts were reviewed by the Editor and Editorial Board, and only those papers judged suitable for publication were accepted. The Editor wish to thank to all the reviewers and authors for their contribution.

Authors are responsible for content and accuracy of their papers.

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SECTION 5. NEW APPROACHES TO ENHANCE SAFETY AND QUALITY OF MINIMALLY PROCESSED FRUITS AND VEGETABLES

30. TECHNOLOGICAL INNOVATIONS TO PRESERVE QUALITY AND SAFETY OF FRESH-CUT HORTICULTURAL PRODUCTS

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Abstract

Fresh-cut or minimally processed fruit and vegetables have strongly increased their market share all over the industrialized countries. For that reason this market is currently very competitive, and forces the specialized industry to develop improved sustainable techniques in order to satisfy new consumers' requirements, and guarantee safety as well as nutritional and sensory quality. The most important goal for keeping overall quality of these commodities is the control of microbial spoilage flora improving safety. Every step in the production chain will influence the microbial load. In this way, the implementation of a proper disinfection program should be the main concern. Washing and disinfection is the only step that reduces microbial load throughout the production chain and chlorine is commonly used as an efficient sanitation agent, but the undesirable byproducts generated when it reacts with organic matter, force to find alternatives. Moreover, efficacy of chlorine is limited on some products. For that reason, several ecofriendly innovative techniques as alternative antimicrobial washing solutions (peroxyacetic acid, ClO_2, acidified sodium chlorite, O_3, electrolysed water, etc.), pre-treatment with UV-C radiation or packaging under high O_2 or non conventional gas mixtures (N_2O, noble gases, etc) alone or combined, seem to be promising to preserve overall quality. However, industrial changes for replacing conventional with innovative techniques request a fine knowledge of the benefits and restrictions as well as practical outlook. This work review some recent results obtained with these emergent techniques on quality changes of fresh-cut horticultural products.

Keywords: ecoinnovative techniques, minimal processing, noble gases, ozone, sanitizers, superatmospheric oxygen, UV-C

Introduction

The current worldwide healthier style of life has led to a rising demand of convenient fresh foods, free from additives, with high nutritional value, antioxidant and free-radical scavenging properties. In this way, the suitability of minimally processed or fresh-cut fruit and vegetables offers great advantages for consumers (Artés *et al.* 2009). Although this kind of processing maintains alive the commodities, it destroys plant structure and therefore increases the rate of senescence of tissues and reduces their resistance to microbial spoilage (Artés *et al*. 2007) which also may increase the number of microorganisms, some of which may be potentially harmful to human health (Leistner & Gould 2002). Moreover, shelf-life of fresh-cut horticultural products is affected by pre-processing factors (crop varieties, cultivation conditions, harvesting, ripening stage, handling, transportation), processing factors (precooling, trimming, conditioning, cutting, peeling, washing, disinfecting, draining, rinsing, drying, packaging) and distribution conditions (temperature, relative humidity, atmosphere composition and duration). In order to achieve fresh-cut plant produce with fresh-like quality, safety and high nutritional and sensory quality, the industry needs to implement improved ecoinnovative techniques. Currently, the major preservation techniques applied to prevent or delay overall quality loss are chemical coadjutants like antimicrobial solutions, acidulants, antioxidants, etc., combined with chilling throughout processing and shelf life and modified atmosphere packaging (MAP) until consumption (Leistner & Gould 2002).

The aim of the present work is to review the main emergent techniques which can be used at industrial level for keeping quality and safety of fresh-cut plant commodities.

Antimicrobial Solutions

Washing with chlorinated (NaClO) water is the most common way to minimize the transmission of pathogens by the food industry due to its powerful oxidizing properties and because it is generally effective and comparatively inexpensive (Nieuwenhuijsen *et al*. 2000). Its effectiveness against microorganisms depends on pH, temperature, concentration, organic matter present in the washing water and produce, time of exposure, and initial microbial load (Boyette *et al.* 1993). Their efficacy increases when increasing concentration of available chlorine, but high levels may cause product tainting and sodium residue on the product and equipment (Adams *et al.* 1989).

When NaClO is added to water, it increases pH and generates hypochlorous acid (HOCl), which is the active antimicrobial species. The acid dissociates readily to hypochlorite ions (OCl^-) at high pH, or chlorine gas (Cl_2) at low pH, thus the pH must be kept in the range of 6.5 to 7.5 for HOCl to be stable and efficient (Suslow, 1997). However, NaClO may oxidize food constituents that contain natural organic materials to produce unhealthy byproducts in water, such as trihalomethanes (THM) like chloroform ($CHCl_3$) or bromoform ($CHBr_3$), haloacetic acids (mono, di and trichloroacetic, and mono and dibromoacetic), haloacetonitrils or others, that have known or suspected carcinogenic or mutagenic potential effect (Nieuwenhuijsen *et al.* 2000). Consequently, these concerns have encouraged the search for alternatives to NaClO in water solutions. Among them some emergent sanitizing solutions water have been tested.

Peroxyacetic Acid

Peroxyacetic acid (CH_3COOOH) is a promising sanitizer due to it is dissociated in water in acetic acid and H_2O_2. Its breakdown products, water, O_2 and acetic acid are biodegradable. It is applied for surface cleaning in concentrations ranging from 85 to 300 ppm, and the U.S. Food and Drug Administration has set a minimum of 85 ppm (FDA 1997). Because of peroxyacetic acid tolerance to several factors like temperature, pH (from 1 to 8), and hardness and soil contamination, its current main area of application is in fruit and vegetables processing. For the treatment of plant surfaces, recommended formulations combine 11% H_2O_2 and 15% CH_3COOOH at 80 ppm (Suslow 1997). It has been reported that it was effective for controlling *Escherichia coli* and *Listeria monocytogenes* in fresh-cut products (Rodgers *et al*. 2004). Compared to 150 ppm NaClO, 68 ppm of peroxyacetic acid reduced the psychrotrophic counts by 2 log units and mesophilic counts by 1 log unit in fresh-cut 'Galia' melon after 10 d (d) at 5 ºC (Silveira *et al*. 2007). A similar effect of NaClO was found in fresh-cut Tatsoi baby leaves after 9 d at 5 ºC where a decrease in the mesophilic and enterobacteria load of 1.2 and 1.0 log cfu g^{-1} respectively without detrimental effect on the sensory attributes was found (Tomás-Callejas *et al*. 2008)

Ozone (O_3)

Ozone is a highly unstable tri-atomic oxygen molecule (O_3) formed by the addition of an oxygen atom ($O^{\cdot}$) to a molecular diatomic oxygen (O_2) and acts as a strong oxidizing agent effective in destroying microorganisms (Guzel-Seydim *et al.* 2004). O_3 destroys microorganisms by the progressive oxidation of vital cell components, preventing the microbial growth and extending the shelf-life of many fruit and vegetables (Parish *et al.* 2003). Washing with ozonated water has been suggested as an interesting alternative to traditional sanitizers due to its efficacy at low concentrations and short contact times as well as the breakdown to non-toxic products. It is remarkable that the efficacy of ozonated water is closely related to O_3 solubility, which increases as the temperature of water decreases. Kim *et al.* (1999b) reduced between 1.5 and 5.0 log cfu g^{-1} *E. coli* O157:H7, *Pseudomona fluorescens, Leuconostoc mesenteroides* and *L. monocytogenes* counts by using 1.5 ppm of ozonated water (pH = 6, 25 ºC) for 15 s. Khadre & Yousef (2001) found effectiveness of O_3 in disinfecting food-contact surfaces. Zhang *et al*. (2005) reported that in fresh-cut celery sticks dipped in 0.18 ppm O_3 water for 5 min browning was inhibited and sensory quality was improved. After 9 d at 4 ºC, bacterial population was reduced 1.69 log cfu g^{-1} compared to control water. Selma *et al*. (2007) treated shredded lettuce with 5 ppm O_3 water for 5 min reaching a reduction of 1.8 log cfu g^{-1} in *Shigella sonnei* counts. Silveira *et al.* (2007) reported a similar sanitizer effect between 150 ppm NaClO and O_3 dips (0.4 ppm, 3 min) on fresh-cut 'Galia' melon after 10 d at 5 ºC.

However O_3 seems to be not always highly successful. Aguayo *et al.* (2003) found no clear sanitizing effect on fresh-cut 'Amarillo' melon after 10 d at 5 ºC and fresh-cut 'Thomas' tomato after 10 d at 5 ºC Aguayo *et al.* (2006). Beltrán *et al*. (2005) found no evidence of browning in fresh-cut potatoes dipped in O_3 water (20 mg L^{-1} min^{-1}) or in O_3 plus peroxyacetic acid (300 mg L^{-1}) after 14 d at 4 ºC. However, the ozonated water alone was not effective in reducing total microbial populations. Rico *et al.* (2006) found that ozonated water (1 mg L^{-1} at 18-20 ºC) reduced in fresh-cut lettuce the enzyme activity and enzymatic browning which also reduced the activity of the texture-related pectin methylesterase that was correlated with a lower crispiness.

Chlorine Dioxide (ClO_2)

ClO_2 is a stable eco-friendly dissolved gas, with a higher oxidation and penetration power than NaClO, being more effective against spores (FDA 1998). With minimal contact time, it is highly effective against pathogenic organisms such as *Legionella, amoebal cysts, Giardia cysts, E. coli*, and *Cryptosporidium* (Xie 2003). The ClO_2 does not ionize to form weak acids (as chlorine) or form carcinogenic by-products like THM. This allows ClO_2 to be effective over a wide pH range. One of the most important qualities of ClO_2 is its high water solubility, especially in cold water. However there are very few reports about the use of ClO_2 in fresh-cut products. Some of them have shown that for apple, lettuce, strawberry and cantaloupe a solution of 5 ppm was effective for inhibiting inoculated *E. coli* and *L. monocytogenes* (Rodgers *et al.* 2004). In fresh-cut faba bean (*Vicia faba* L.) dipping for 2 min in 4 ppm ClO_2 was as effective as 150 ppm NaClO in reducing *enterobacteriacea* and mesophilic counts after 8 d at 5 ºC, decreasing browning and improving sensory quality (Artés *et al.* 2007).

Acidified Sodium Chlorite ($NaClO_2$)

Acidified sodium chlorite (ASC) chemistry is principally that of chlorous acid ($HClO_2$), which forms on acidification of chlorite. Once formed, $HClO_2$ gradually decomposes to form chlorate ion, chlorine dioxide, and chloride ion. It is hypothesized that the mode of action of ASC derives from the uncharged $HClO_2$, which is able to penetrate bacterial cell walls and disrupt protein synthesis by virtue of its reaction with sulfhydryl, sulfide, and disulfide containing amino acids and nucleotides. The undissociated acid is thought to facilitate proton leakage into cells and thereby increase energy output of the cells to maintain their normal internal pH thereby also adversely affecting amino acid transport. ASC in the range 500-1200 ppm has been shown to have stronger efficacy against pathogens and spoilage bacteria than NaClO and does not form carcinogenic products. A 1200 ppm ASC washing treatment showed a good efficiency for inactivation *Salmonella* spp. in bell peppers and tomato (Hun-Gyun *et al*. 2005, 2006). However, ASC in this level can aggravate tissue damage. An ASC pre-washing controlled microbial growth in Chinese cabbage preserving quality (Inatsu *et al.* 2005). Fresh-cut bell peppers washed with 250 ppm ASC registered 2 log reductions after 7 d at 5 ºC in mesophilic, psychrotrophic and *enterobacteriaceae* counts when compared to 150 ppm NaClO at pH 6.5, but a best retention of total antioxidant activity for ASC was recorded (Conesa *et al*. 2007c).

Hydrogen Peroxide (H_2O_2)

H_2O_2 is a powerful bactericide and oxidant. Its efficacy has been demonstrated in extending shelf-life and reducing natural microflora and pathogens in fresh-cut cucumber, zucchini, bell peppers, and melons (Sapers 2003). Although H_2O_2 is permitted for other uses in food processing and packaging because it leaves no potentially harmful residues, it is not yet approved by the FDA as a sanitizing agent for fresh produce. A H_2O_2 solution was promising for sanitation of fresh-cut commodities, although results were inconsistent.

Washing with 5% H_2O_2 was more effective than with 1000 ppm NaClO and Na_3PO_4 in terms of reducing the microbial load on cantaloupe rinds. A H_2O_2 vapour treatment reduced microbial counts, extended shelf life and maintained quality of fresh-cut green bell pepper, cucumber, and zucchini (Sapers 2003). However, browning of shredded lettuce increased after dipping in a H_2O_2 solution (Parish *et al*. 2003).

Organic Acids & Calcium Salts

Organic acids have been largely applied for the prevention of enzymatic and non-enzymatic browning and microbial growth at levels that did not adversely affect taste and flavour of plant commodities (Yildiz 1994). They are more effective for bacteria than for moulds and yeast due to the low pH (between 2.1 and 2.7) at which they are applied. Kim & Klieber (1997) reported that citric acid (10 g L^{-1}) repressed the petiole sprouting (black speck) development of fresh-cut Chinese cabbage and prolonged their shelf life to 14 d at 5 °C. Fresh-cut 'Amarillo' melon dipped in 0.52 mM citric acid for 30 s before a MAP reached a shelf life of 10 d at 5 °C with no translucency and discoloration (Aguayo *et al.* 2003). Dipping green celery crescents in a 0.5 M ascorbic and 0.1 M citric acid solution was as effective as 100 mg L^{-1} NaClO for reducing microbial counts and improving consumers acceptability (Gómez & Artés 2004).

Calcium is related to maintain cell wall structure and firmness of plant commodities by combining with pectin to form calcium pectate (Rosen & Kader 1989). Washing with calcium lactate (15 g L^{-1}) at 50 °C was effective in keeping turgor of cortex tissue cells and reduced the extent of lignification at cutting-edge areas (Rico *et al*. 2007). Aguayo *et al*. (2008) found that fresh-cut melon dipped into $CaCl_2$ (0.5%) solution at 60 °C for 1 min, or organic acid salts like calcium propionate (0.9%) or lactate (1.4%) were very effective in reducing microbial growth and maintaining firmness during 8 d at 5 °C.

Electrolyzed Water (EW)

Electrolyzed water has a strong bactericidal effect against pathogens and spoilage microorganisms, more effective than NaClO due to its high redox potential (Izumi 1999; Koseki & Itoh 2001). Hypochlorous acid is present in EW at 6.8 pH and it is generated by electrolysis of NaCl solution, since HCl formed at the anode site neutralizes the NaOH at the cathode site. Few studies have been reported the use of EW in fresh-cut vegetables (Izumi 1999; Koseki & Itoh 2001; Wang *et al*. 2004; Rico *et al*. 2008). EW containing 15 to 50 ppm available NaClO was effective as a disinfectant for fresh-cut carrots, spinach, bell pepper, potato and cucumber, without discoloration and lowering microbial counts from 0.6 to 2.6 log units (Izumi 1999). It was also shown that EW has no impact on the sensory quality of fresh-cut lettuce (Koseki *et al.* 2001). Rico *et al*. (2008) showed that washing with EW containing 60 mg L^{-1} free chlorine (pH 6.5) resulted as effective as chlorine, with good quality retention.

Prepackaging: UV-C Radiation and Intense Light Pulses

The use of non-ionizing and germicidal ultraviolet light (UV) at 190–280 nm (UV-C) could be effective for surface decontamination of fresh-cut products. It has been reported that UV-C affects several physiological processes in plant tissues and damages microbial DNA (Lucht *et al*. 1998). Lado & Yousef (2002) reported that 0.5 to 20.0 kJ UV-C m^{-2} inhibited microbial growth by inducing the formation of pyrimidine dimers which alter the DNA helix and blocks microbial cell replication. The effectiveness of UV-C seems to be independent of the temperature (5 to 37 °C) but depends on the incident irradiation determined by the structure and surface of the product (Bintsis *et al.* 2000). However, UV-C can change the cell permeability increasing electrolytes, amino acids and carbohydrates leakage, which can stimulate bacterial growth (Artés-Hernández *et al.* 2009). The crucial point is whether a safe dose could be found which would greatly impair pathogen growth without damaging the product (Ben-Yehoshua & Mercier 2005). It has been reported that abiotic stresses, like UV light, may enhance the nutraceutical content of fresh fruit and vegetables (Cisneros-Zevallos 2003). However, more research is needed to optimize its use.

In vitro studies have demonstrated the efficiency of UV-C illumination on microbial inhibition (Abshire & Dunton 1981). Some *in vivo* studies have reported that UV-C inhibited microbial growth, delaying decay and senescence. In zucchini squash slices, UV-C exposition reduced microbial activity and deterioration during storage at 5 or 10 °C (Erkan *et al.* 2001). Civello *et al.* (2006) reported that 4 to 14 kJ UV-C m^{-2} applied to broccoli heads delayed yellowing and chlorophyll degradation at 20ºC with an increased in total phenols, antioxidant capacity and flavonoids.

The use of two sided UV-C radiation at 1.18, 2.37 or 7.11 kJ m^{-2}, was effective for reducing the natural microflora of fresh-cut 'Red Oak Leaf' lettuce up to 10 d at 5 ºC although 7.11 kJ m^{-2} induced tissue softening and browning after 7 d at 5 ºC (Allende *et al.* 2006). Similar results were previously found for one sided UV-C radiation of fresh-cut 'Red Oak Leaf' and 'Lollo rosso' lettuces throughout 10 d at 5 ºC (Allende *et al*. 2003ab). Bell peppers sticks from integrated cultivation, showed a high reduction in mesophilic and Enterobacteria counts after 12 d at 5 ºC when 2.27 kJ UV-C m^{-2} was applied (Artés *et al.* 2006). Low to moderate UV-C radiation can be effective for sanitizing minimally processed spinach leaves preserving their quality (Artés-Hernández *et al*. 2009).

However, López-Rubira *et al.* (2005) found inconsistent results regarding the effect of UV-C (0.56 to 13.62 kJ m^{-2}) on microbial growth in fresh-cut pomegranate arils stored up to 15 d at 5 ºC. Microbial counts were not systematically reduced throughout shelf life and yeast and moulds were unaffected. In the same way, Artés-Hernández *et al.* (2009) reported that despite 4.54, 7.94 and 11.35 kJ UV-C m^{-2} showed an initial reduction in mesophilic and psychrophilic counts on processing day, no residual inhibitory effect was found after 6 to 13 d at 5 and 8 ºC compared to a control sanitizing treatment with 150 mg L^{-1} NaClO.

In whole bell peppers harvested from integrated pest management production and 2.27 kJ m^{-2} UV-C illuminated, decay was reduced after 21 d at 5 ºC + 5 d at 15 ºC (Artés *et al*. 2006). In tomatoes destined for the fresh-cut industry, a pre-treatment of 4 kJ UV-C m^{-2} and CA storage under 5 kPa O_2 + 1 kPa CO_2 at 12 ºC for 21 d retarded ripening and kept better firmness and sensory attributes (Robles *et al.* 2007). Doses of 2.15 and 4.3 kJ UV-C m^{-2} retarded the development of decay in strawberry and increased the phenolic content (Erkan *et al.* 2008).

Intense light pulses (ILP) are an innovative decontamination method for food surfaces approved by the US-FDA that could be suitable for sanitizing fresh-cut plant commodities. ILP kills microorganisms using short time (85 ns to 0.3 ms) high frequency pulses (0.45 to 15 Hz) and energy per pulse (3 to 551 J) of an intense broad spectrum, rich in UV-C light (Gómez-López *et al*. 2005). Hoornstra *et al.* (2002) showed a 2 log cfu g^{-1} reduction in aerobic counts on vegetables increasing their shelf-life in 4 d.

Non Conventional Packaging

High Oxygen

An alternative active MAP by the use of superatmopheric O_2 was described as effective to inhibit enzymatic browning, prevent anaerobic fermentation, moisture and odour losses, and reduce aerobic and anaerobic microbial growth (Day 2001). The exposure to superatmospheric O_2 levels may stimulate, have no effect, or reduce respiration rates and C_2H_4 production, depending on the commodity, ripening stage, O_2 level, storage time and temperature, and CO_2 and C_2H_4 levels in the atmosphere (Kader & Ben-Yehoshua 2000).

The combined high O_2 level and 10 to 20 kPa CO_2 may provide adequate suppression of microbial growth and prolong shelf-life of several fresh-cut plant commodities (Allende *et al.* 2004; Conesa *et al*. 2007a,b; Escalona *et al*. 2007). Fresh-cut bell peppers showed that 80 kPa O_2 combined with 15 kPa CO_2 maintained the main sensory quality attributes and inhibited growth of the spoilage microorganisms and *enterobacteriaceae* (Conesa *et al.* 2007a,b). Levels higher than 75 kPa O_2 were needed to reduce *L. innocua* growth on fresh-cut lettuce (Escalona *et al.* 2007). Allende *et al.* (2002), reported yeast counts higher than 5 log cfu g^{-1} after 3 d at 4 °C on mixed salad stored under superatmospheric O_2 MAP.

There are few reports on the effects of elevated O_2 on enzymatic browning. The *in vitro* kinetics of PPO, main enzyme responsible of browning, with respect to O_2 levels from 5 to 100 kPa, and using chlorogenic acid as substrate, has been examined. The substrate concentration as well as the O_2 level had a clear inhibitory effect on the reaction rate. Moreover, the inhibitory effect of O_2 was more evident at low final product concentration (Gómez *et al.* 2006).

Regarding sensory quality, acceptable scores were found in spinach leaves under 80 to 100 kPa O_2 combined with moderate kPa CO_2 compared to low O_2 and high CO_2, where the spinach was affected by fermentation (Allende *et al.* 2004).

Other Innovative Gas Mixtures

The use of non-conventional gases like Ar, He, Xe or N_2O has been proposed for improving quality of selected fresh-cut plant commodities. It has been shown that MAP enriched in Ar reduced microbial growth and delayed quality loss of fresh broccoli and lettuce (Day 1996). However, it has been found that 90 kPa Ar + 2 kPa O_2 did not delay the accumulation of phenolics in fresh-cut lettuce, or the loss of chlorophyll from broccoli florets beyond that of low O_2 atmospheres made with He or N_2 (Lougheed & Lee 1991). Zhang *et al.* (2008) have found promising results of combined Ar and Xe for extending shelf life of green asparagus spears.

Partial pressures of 50, 80 and 100 kPa N_2O reduced the respiration rates of onion bulbs by 50% after 5 d at 18 °C compared to air storage, increased the organics acids content and reduced decay (Benkeblia & Varoquaux 2003). Innovative MAP of 90 kPa Ar + 5 kPa O_2 and 90 kPa N_2O + 5 kPa CO_2 were applied to kiwifruit slices at 4 °C for 12 d and active 90 kPa N_2O kept the best quality of slices delaying firmness losses and browning. Slight modifications in the most important discriminated quality factors for the slices in N_2O and an acceptable quality in Ar after 8 d was found (Rocculi *et al.* 2005). An active 90 kPa He MAP showed a beneficial effect in retaining total chlorophylls and vitamin C content after 8 d at 5 °C in minimally processed Red chard baby leaves compared to 100 ppm NaClO, at pH 6.5 under conventional MAP, with no differences in development of natural microflora (Tomás-Callejas *et al.* 2009).

Conclusions

Although NaClO is being widely used for assuring safety of fresh-cut plant commodities, improved ecofriendly strategies are promissory alternatives to preserve the high quality and safety demanded by consumers. Some GRAS compounds, ozonated or electrolyzed water, UV-C illumination or non conventional gas mixtures for active MAP can reduce initial microbial load and its development prolonging shelf life. However, in order to facilitate its industrial implementation, the potential and limits of these emergent techniques must be well defined and included in the regulations.

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