Chapter 2

Fresh-Cut Fruit and Vegetables: Emerging Eco-friendly Techniques for Sanitation and Preserving Safety

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http://dx.doi.org/10.5772/intechopen.69476

Abstract

The current high demand of minimally processed or fresh-cut fruit and vegetables results from the consumer's desire for healthy, convenient, fresh, and ready-to-eat plant food-derived commodities. Fresh-cut fruits and vegetables are usually packaged under active- or passive-modified atmosphere packaging, while its shelf life must be under refrigerated conditions. The most important goal to preserve quality and safety focuses on releasing the microbial spoilage flora, since every unit operation involved will influence the final load. Sanitation in the washing step is the only unit operation able to reduce microbial load throughout the production chain. Chlorine is widely used as an efficient sanitation agent, but some disadvantages force to find eco-friendly emerging alternatives. It is necessary to deal with aspects related to sustainability because it could positively contribute to the net carbon balance besides reducing its use. Several innovative techniques seem to reach that target. However, industrial changes for replacing conventional techniques request a fine knowledge of the benefits and restrictions as well as a practical outlook. This chapter reviews the principles of emerging eco-friendly techniques for preserving quality and safety of fresh-cut products in order to meet the expected market's demand.

Keywords: minimally processed, ready-to-eat, sanitizing, pathogens, food safety

1. Introduction

The benefits of fruit and vegetables consumption on human health are well known, being linked with prevention of a grand array of diseases such as degenerative disorders, cancer, and cardiovascular, among others [1]. Consequently, their intake has been promoted among consumers by nutritionists, researchers, and even at a governmental level (i.e., campaigns like *five-a-day*, etc.). However, the actual consumer's demand of new food was elaborated by the industry with the following characteristics: freshness, healthiness, and easy- or ready-to-eat. Particularly, minimally processed or fresh-cut (FC) fruit and vegetables connect well within such consumer needs. The main advantage of FC plant foods is that they have nearly the same properties as the whole intact product, but they do not need much elaboration time and are with a uniform and consistent quality [2]. NaOCl has been widely used in the FC industry as a strong sanitizing agent due to its powerful oxidizing properties [3]. Among the main NaOCl advantages are high effectiveness, comparatively inexpensive, and that they may be implemented in any size operations [4]. Nevertheless, NaOCl may produce unhealthy by-products in processed water (chloramines, chloroform, haloacetic acids, or other trihalomethanes) that have been reported to present carcinogenic or mutagenic effects, with proven toxicity to liver and kidneys [3]. Therefore, NaOCl use in the FC industry has been forbidden in some European countries [5].

This chapter summarizes the principles and development of eco-friendly techniques for preserving safety of FC products in order to meet the expected market's demand.

2. Antimicrobial washing solutions

2.1. Peroxyacetic acid

Peroxyacetic acid (PAA), or peracetic acid, is a colorless organic peroxide that is a mixture of acetic acid and hydrogen peroxide. It is an eco-friendly sanitizer whose breakdown products are acetic acid, O_2, CO_2, and water. PAA is approved by the European Union as a disinfectant for drinking water and food areas and as an in-can preservative [6]. PAA is also permitted by the U.S. Food and Drug Administration as an additive for food [7]. The surface-cleaning concentrations range from 85 to 300 ppm, although 50 ppm has been reported to be enough [8]. PAA is mainly used in fruit and vegetable processing due to tolerance to several factors such as temperature, pH (1–8), hardness, and soil contamination. A recommended combination of 11% hydrogen peroxide (H_2O_2) and 15% PAA, at 80 ppm, was proposed for the disinfection of plant surfaces [4]. *Escherichia coli* O157:H7 and *Salmonella* spp. reductions of 2–3 log CFU g^{-1} were reported in apples and melons treated with 70 ppm of PAA [9, 10]. Similarly, *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* inoculated on FC carrot shreds were reduced after PAA washing at 40 ppm for 2 min [11]. Mesophilic and psychrotrophic loads of FC Galia melon were reduced by 1 and 2 log CFU g^{-1}, respectively, using PAA (68 ppm) [12]. The nutritional and sensory quality of FC iceberg lettuce was not affected by PAA (120 ppm), while natural microflora was reduced by approximately 1 log CFU g^{-1} [13]. A similar *Salmonella typhimurium* reduction was achieved after the PAA treatment (40 ppm) in inoculated lettuce [14]. A PAA treatment of 80 ppm was more effective than 106 ppm of NaOCl to reduce *E. coli* O157:H7 and *Salmonella enterica* Montevideo on mung bean sprouts [15]. *E. coli* and *Salmonella enteritidis* reductions of 2–3 log CFU g^{-1} were achieved in the kailan-hybrid broccoli with 100 ppm of PAA being more effective than 100 ppm of NaOCl [16].

http://dx.doi.org/10.5772/intechopen.69476

2.2. Chlorine dioxide

Chlorine dioxide (ClO_2) is a yellowish-green stable dissolved gas that has been used for the last decades for water treatment as a potential alternative to NaOCl. ClO_2 has higher effectiveness over a broad range of pH, higher water solubility (10 times higher than NaOCl), higher oxidant capacity, lower reactivity with organic matter, and higher effectiveness at low concentrations than NaOCl [17]. Nevertheless, ClO_2 is a very unstable substance and is highly explosive as a concentrated gas when concentrations ≥10% are reached in air. Hence, ClO_2 must be generated on-site by two different procedures: reacting an acid with sodium chlorite or the reaction of sodium chlorite with chlorine gas then being obtained in either aqueous or gaseous forms, respectively [18]. The ClO_2 is classified as a non-carcinogenic product since it does not ionize to produce weak acids (as occurred for chlorine and bromine) or to form carcinogenic by-products like trihalomethanes [19]. Gaseous ClO_2 treatment (100 ppm) of several fresh products (tomatoes, lettuce, cantaloupe, alfalfa sprouts, oranges, apples, and strawberries) did not leave any chemical residues on them [20]. ClO_2 is approved in the USA for usage in washing whole fresh fruits and vegetables and shelled beans and pears with intact cuticles at maximum levels of 5 ppm and 1 ppm for peeled potatoes [19].

ClO_2 is considered as a strong microbicide at low levels such as 0.1 ppm, achieving also a rapid removal of biofilms which avoid bacterial re-growth [21]. The bactericidal effect of ClO_2 is explained by the interruption of several cellular processes (proteins production and changes in the cell structure) when organic substances in bacterial cells react with ClO_2. On viruses, ClO_2 reacts with peptone to prevent the protein formation being more effective than chlorine or ozone [21]. Inoculated pathogens like *Salmonella* spp., *E. coli* O157:H7, and *L. monocytogenes* were reduced on cabbage, carrot, lettuce, strawberry, and melon with ClO_2 concentrations of 4–5 ppm [22–26]. ClO_2 treatment at 100 ppm of FC cucumber, lettuce, carrot, apple, tomato, and guava reduced total bacterial and coliform counts up to 3.5–4.0 log CFU g^{-1} being more effective than the same NaOCl concentration [27]. A ClO_2 treatment of 3 ppm substantially prevented *E. coli* O157:H7 cross-contamination but was not effective for the inoculated *Salmonella* in FC Red Chard [28]. The effectiveness of ClO_2 treatment of tomato processing water under a range of water quality and temperature was studied, which showed that an increase in temperature and ClO_2 concentration reduced the contact time achieving a 6-log reduction of *S. enterica* within 2 min of contact time [29]. Acidified sodium chlorite (100–500 ppm) at low-moderate doses showed an initial antimicrobial efficacy on natural microflora and *E. coli* of FC tatsoi baby leaves as effective as that of 100 ppm NaOCl [30].

2.3. Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a strong oxidizer able to generate other cytotoxic-oxidizing species, like hydroxyl radicals, with strong bactericide (including spores) effect [31]. H_2O_2 is an eco-friendly disinfectant since it is rapidly decomposed into water and oxygen in the presence of catalase. Likewise, it is colorless and non-corrosive. H_2O_2 is allowed for use in food processing and packaging but not as a sanitizing agent for fresh produce by the FDA [5]. However, high H_2O_2 concentrations are needed to achieve good santising effects in FC products. However, such high concentrations may lead to browning being necessary thge use of anti-browning agents like sodium erythorbate [32]. Accordingly, 2–3 × 10^4 ppm H_2O_2 were needed to reduce *E. coli* O157:H7 by 1.6 log CFU g^{-1} in baby spinach [33]. *L. monocytogenes* reductions of 2.0–3.5 log CFU cm^{-2} were reported in melon surfaces after 5 × 10^4 ppm H_2O_2 treatment [34]. Effectiveness of H_2O_2 treatment (3%) on inoculated *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in whole cantaloupe rind surfaces was enhanced when applied at 80°C for 300 s [35]. H_2O_2 has been also found to extend the shelf life and reducing native microbial and pathogen populations in whole grapes, prunes, apples, oranges, mushrooms, melons, tomatoes, red bell peppers and lettuce, and in FC cucumber, zucchini, bell peppers, and melons [5, 36]. Nevertheless, the cross-contamination may not be avoided with H_2O_2, since it may still occur in the product washing water and its breakdown is rapid with low disinfection kinetics [37].

2.4. Weak organic acids

Weak organic acids have been widely used as preservatives for the prevention of several quality degradation processes such as enzymatic and nonenzymatic browning, texture deterioration, and microbial spoilage. Contrary to NaOCl, weak organic acids do not produce toxic or carcinogenic compounds when they interact with organic molecules [38]. Therefore, several weak organic acids are considered as GRAS (Generally Recognized as Safe) by the FDA and European Commission being well accepted by consumers. The antimicrobial effect of weak organic acids is related to the cytoplasm acidification, osmotic stress, disruption of proton motive force, and synthesis inhibition of macromolecules [39]. Weak organic acids are more effective for bacteria than for yeasts and molds because of the low pH (2.1–2.7) of the applied solutions. Citric, acetic, lactic, and ascorbic acids are the most common acids applied in the food industry.

Citric acid, contrary to other acids, acts as a chelating agent of metallic ions of the medium, avoiding microbial growth [40, 41]. Citric acid treatment (0.52 mM) maintained microbial safety and visual quality of FC "Amarillo" melon during a shelf life of 10 days at 5°C [42]. A solution of 0.1 M citric and 0.5 M ascorbic acid achieved the same effectivity as 100 ppm NaOCl to control microbial growth and maintain quality of green celery crescents [43]. Citric and lactic acid dippings of 0.5–1 × 10^4 ppm achieved comparable *E. coli* reductions of 1.9–2.3 log CFU g^{-1} to 100 ppm NaOCl in inoculated FC lettuce without significant efficacy enhancement from incrementing dipping times from 2 to 5 min [44]. Likewise, acetic and citric acid dippings of 0.5–1×10^4 ppm achieved similar *L. monocytogenes* reductions of 0.8–1.0 log CFU g^{-1} to 100 ppm NaOCl in inoculated FC lettuce [44]. However, acetic acid and ascorbic acid dippings of 0.5–1×10^4 ppm achieved lower *E. coli* reductions than 100 ppm NaOCl in inoculated FC lettuce [44]. The effectiveness of citric, acetic, lactic, malic, and propionic acid dippings (1×10^4 ppm) for inoculated *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* onto fresh lettuce was studied with reductions of 1.9–2.9, 1.1–1.7, 1.9–2.5, 2.3–3.0, and 0.9–1.5 log CFU g^{-1}, respectively [45].

2.5. Calcium, sodium, and potassium-derived salts

Several salts are recognized as GRAS being a low-cost material for the food industry with high acceptance by the consumers, since it is not toxic. Calcium is used to retain the firmness of plant commodities by interaction with pectin to form calcium pectate maintaining then the cell wall structure. FC lettuce treated with 15 × 10^3 ppm calcium lactate showed higher crispness than samples treated with 120 ppm NaOCl after 1 day at 4°C [46]. Latter authors hypothesized that such finding could be owed to the activation of texture-related enzymes, like PME, by the calcium absorption in the lettuce, or an increase in diffusive processes by temperature, including the calcium. Similar results were obtained by 15×10^3 ppm calcium lactate treatment at 50°C of sliced FC carrots to maintain the cortex turgor of plant cells and reduce the lignification degree in cut surfaces [47]. However, the calcium lactate antimicrobial properties have been scarcely studied. FC lettuce and carrots treated with 3×10^4 ppm calcium lactate showed similar microbial loads than 120 ppm NaOCl after 10 days at 4°C [48]. Similarly, 3×10^4 ppm calcium lactate treatment at 60°C of FC melon induced 1–2 log CFU g^{-1} lower bacterial and yeasts and mold loads after 8 days at 5°C, while texture of such melon pieces was better maintained than samples washed with water at the same temperature [49]. Latter authors also found that other calcium salt treatments (calcium chloride and calcium propionate) maintained lower microbial loads in the FC melon samples after 8 days at 5°C. Calcium pre-treatments may also help to prevent enzymatic browning reactions during high-pressure processing (HPP) of peaches if the penetration of Ca^{2+} reaches the target area, which is the tonoplast or vacuolar membrane as observed in peaches [50].

Sodium bicarbonate, sodium carbonate, sodium silicate, potassium bicarbonate, potassium carbonate, and potassium sorbate have been studied [51]. Among them, sodium carbonate and sodium bicarbonate (at 3% w/v) reduced up to 100% disease (*Penicillium* sp.) on inoculated fresh clementines and oranges [51]. Calcium carbonate maintained lower microbial loads in the FC melon samples after 8 days at 5°C [49]. However, little is known about the mode of action of these salts, although other possible mechanisms, apart from the high salt pH, like the induction of host defence responses might be involved [52].

2.6. Electrolyzed oxidizing water

Electrolyzed oxidizing water (EOW) is formed in an electrolysis chamber by the electrodialysis of a NaCl solution between an anode and a cathode [53]. Acidic EOW (pH 2.5–3.5; oxidation-reduction potential (ORP) 1000–1200 mV) is produced in the anode and alkaline EOW (pH 10–11.5; ORP −800 to −900 mV) is produced in the cathode. HCl, HOCl, Cl_2, OCl^-, and O_2 are formed in the anode, while the cathode produces hydroxyl ions, which can react with Na ions generating NaOH. EOW contains free chlorine as the main microbial inactivation agent showing higher microbicidal effect against pathogens and spoilage microorganisms than NaOCl [16, 54, 55]. EOW is considered as an eco-friendly technology that presents the following advantages over other sanitizing methods: easy-to-find and cheap materials (NaCl and water), simple and on-site production, and low operational expenses and trihalomethanes formation. Additionally, some cell electrodes, like boron-doped diamond electrodes, are able to oxidize organic matter, reducing then the environmental footprint of wastewater from fresh produce industry [56]. Nevertheless, EOW has a short shelf life, in some cases, being necessary to be produced on-site and recommended in a ventilated area due to the Cl_2 and H_2 production [5]. EOW has been approved at maximum concentration of 200 ppm by the FDA [57]. Neutral EOW (pH 7; ORP 700 mV) can be produced by the mixture of acidic and alkaline EOW [58]. The additional advantages from using neutral EOW are that it does not affect surface color, general appearance, or pH of FC vegetables [54].

EOW has been used in several works as an excellent disinfestation method of food equipment surfaces and tools reaching up to 9 log CFU cm^{-2} reductions for several pathogen biofilms like *L. monocytogenes, E. coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* [59–61].

The EOW (15–50 ppm free chlorine) was early studied on FC carrots, spinach, bell pepper, potato, and cucumber being considered as an effective disinfectant able to reduce microbial loads by 0.6–2.6 log units without product discoloration [54]. Neutral EOW (100 ppm free Cl; pH 7) achieved 0.5, 1.3, and >2.1 log mesophilic, psychrophilic, and yeast and mold reductions, respectively, in FC kailan-hybrid broccoli, showing similar microbial loads and good sensory quality to NaOCl-treated (100 ppm) samples after 19 days at 5°C [62]. Neutral EOW also showed similar microbial effectiveness to NaOCl to reduce natural microflora of FC lettuce with no impact on its physical and sensory quality [63–65]. Acidic and neutral EOW treatments at 70 and 100 ppm free Cl were studied on two broccoli varieties showing neutral EOW (100 ppm) the best microbial reductions after shelf life comparing to NaOCl (100 ppm) [58]. Furthermore, EOW-treated samples showed higher (up to 30%) total phenolic content and more stabilized myrosinase activity (the enzyme responsible for the formation of the bioactive isothiocyanates (ITC) in broccoli) than NaOCl-treated samples after shelf life [58]. Microbial reductions of 1–2 log units were observed in FC mizuna baby leaves treated with acidic EOW (40–100 ppm free Cl) and neutral EOW (40–100 ppm free Cl), with similar microbial effectiveness to NaOCl 100 ppm, showing neutral EOW better bacteriostatic effects than acidic EOW in some cases [66]. The sensory quality, physical structure, and health-promoting compounds of EOW-treated FC mizuna baby leaves were not significantly affected [66]. Neutral EOW (100 ppm free Cl) treatment reduced counts of inoculated *E. coli* and *S. enteritidis* in FC kailan-hybrid broccoli by approximately 2.6 log CFU g^{-1}. Nevertheless, the effectiveness of the last treatment was not increased when it was combined with ultraviolet (UV)–C (7.5 kJ m^{-2}) treatment. Similarly, neutral EOW treatment (306 ppm free Cl) of romaine lettuce reduced inoculated *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* loads by 2.0 log CFU g^{-1} [67]. *E. coli* O157:H7 grew slower in FC lettuce treated with NEW (50 ppm free Cl) during storage at 13–16°C, while no microbial growth was observed if the product was stored at ≤8°C [68].

The use of different organic and inorganic salts has been studied to increase electrolysis efficacy and avoid the corrosive effects of NaCl on equipment. Particularly, electrolyzed sodium bicarbonate allowed to control postharvest citrus rots as a result of direct inhibition and the induction of fruit resistance-response mechanisms showing normal electrolyzed water a less marked effect [69].

2.7. Ozone

Ozone (O_3) is a colorless gas with a pungent odor having an oxidizing potential (+2.07 V) 1.5 times higher than that of chlorine, which oxidizes the cell components of the microbial cell wall [70]. Ozone half-life is very short, from seconds to hours depending on temperature and water quality [71]. Thus, O_3 is commercially generated on-site by submitting oxygen, or air, to ultraviolet radiation (285 nm) or through an electrical charge leading to the cleavage of oxygen molecules to form ozone [5, 72].

Ozone solubility is 12 times lower than that of NaOCl. Nevertheless, ozone concentrations as low as 1–5 ppm are enough to reach good antimicrobial reductions. Nevertheless, higher O_3 concentrations are needed when it is applied as a gas treatment since its penetration into the cells, to achieve the disinfection effect, is affected by the humidity of the air [40, 73]. The O_3 effectiveness may be increased at lower pH (more stable) and temperature (higher residual ozone concentrations), higher relative humidity of the storage room (increasing its solubility on the moisture present on produce surfaces), and purity of the water (ozone is consumed by the matrix components reducing its efficacy) [74]. Ozone is spontaneously decomposed to the non-toxic O_2 when applied. Accordingly, O_3 has been approved as a GRAS product by the FDA to be used in the food industry [75]. However, O_3 can cause irritation to eyes and throat (at concentration higher than 0.2 ppm) of plant operators, is highly corrosive to the equipment, and the physicochemical properties of treated produce may be altered [5].

An ozonated water treatment at 0.4 ppm for 3 min has been recommended to maintain microbiological quality and firmness of tomato slices while it did not affect the physicochemical quality and organic acid contents [76]. The levels of *Salmonella* spp. inoculated in melons were reduced between 4.2 and 4.8 log CFU/rind-disk (12 cm^2) after a gaseous ozone treatment (10,000 ppm for 30 min under vacuum system) [77]. Counts of inoculated *E. coli* O157:H7 in spinach leaves were also decreased under a novel gaseous system capable of generating O_3 inside the sealed package at various geometries [78]. Respiration rate and browning of FC celery were reduced, while sensory quality was well maintained using ozonated water at 0.18 ppm for 5 min [79]. Ozonated water at 1 ppm reduced both enzyme activity and enzymatic browning of shredded lettuce [80]. Nevertheless, the latter enzyme inactivation showed a negative effect, as the reduction in activity of the texture-related pectin methyl esterase was correlated with a lower crispiness. FC rocket treated with ozonated water at 5 ppm for 10 min showed better sensory scores and microbial quality (psychrophilic and yeast and molds) than untreated samples after 12 days at 5°C, while total chlorophylls and carotenoids contents were unchanged [81]. Mesophilic, psychrotrophic, and yeast counts of FC tomato slices treated with ozonated water (3.8 ppm for 3 min) showed 1.9, 1.6, and 0.7 lower log units, respectively, than untreated samples after 10 days at 5°C [82]. The enzymatic antioxidant system of FC green peppers treated with gaseous ozone (6.42 mg cm^{-3} for 15 min) was induced during storage at 5°C, while polyphenol oxidase activity was reduced. A synergistic effect was even observed when the latter ozone treatment was combined with modified atmospheric packaging (3% O_2, 4% CO_2, and 93% N_2) of green peppers [83].

Nevertheless, O_3 seems to be not always highly effective. In that sense, low (<0.5 log units) inhibitory effect on mesophilic, psychrophilic, enterobacteria, molds, and LAB loads of FC "Galia" melon washed with ozonated water (0.4 ppm, up to 5 min) was observed after 10 days at 5°C, registering even higher yeast and molds compared to untreated samples [84]. However, FC 'Galia' melon washed for just 1 min with 80 ppm PAA showed the lowest microbial loads after 10 days at 5°C. However, the latter PAA antimocrobial effect was reduced when it was combined with ozonated water treatment. On the other side, FC "Galia" melon samples treated with ozonated water reduced respiration rates, while sugar contents and vitamin C were better maintained [84]. Shredded "Iceberg" lettuce treated with ozonated water (1 ppm, 120 s) showed lower sensory and microbiological quality than samples treated with NaOCl (200 ppm, 120 s) [85].

2.8. Essential oils

High antimicrobial properties have been studied with several plant essential oils (EOs) [86]. Generally, EOs possessing the strongest antibacterial properties are those that contain phenolic compounds such as carvacrol, eugenol, and thymol [87, 88]. In general, the mechanism of action of EOs against microorganisms involves the interaction of phenolic compounds with the proteins (porins) in the cytoplasmic membrane that can precipitate and lead to ions leakage of and other cell contents causing cell lysis [89]. Carvacrol solutions have shown good antimicrobial effects in different FC fruit and vegetables like lettuce, kiwifruit, apples, and melons treated with carvacrol-containing washing solutions [90–92]. Thymol (from thyme and oregano) and eugenol (from clove, *Syzygium* spp.) have also shown high antimicrobial and antioxidant effects on MAP-stored table grapes [93].

The EOs concentrations should be increased when tested *in vivo* in order to reach the same effectiveness than that observed *in vitro*. However, such high EOs concentrations may lead to EOs-related off-flavors transmission to the product. Furthermore, the lipophilic nature of EOs difficult their solution in the water-based washing solutions [86]. Therefore, the reduction of the EOs particle size (<100 nm) has been proposed as an alternative to improve EO antimicrobial efficiency through two important targets: (i) the possibility of enhancing physicochemical properties and stability; and (ii) the ability of improving biological activity of lipophilic compounds by increasing the surface area per unit of mass [94]. The antimicrobial and physical properties of different EOs (lemongrass, clove, tea tree, thyme, geranium, marjoram, palmarosa, rosewood, sage, or mint) have been reported to be enhanced when they were processed to nanoemulsions [94]. Such nanotechnology has been recently applied in FC carrots using nanoencapsulated carvacrol particles' incorporation in a washing solution, which reduced the characteristic off-flavors and EOs oxidation while keeping good microbial quality of the product during shelf life [95]. Furthermore, EOs may be included as antimicrobial agents in packaging films as reported of FC vegetables as proposed in a study using a carvacrol–polylactic acid film to inhibit *E. coli* ATCC 8739, *Fusarium oxysporum, Geotrichum candidum*, and *Phytophthora* spp. [96].

2.9. Isothiocyanates

Isothiocyanates (ITC) are sulfur compounds that can be formed in the *Brassica* vegetables after hydrolysis of glucosinolates by plant myrosinase. Antimicrobial activity of ITC has been reported for a wide range of foodborne microorganisms [97, 98]. The antimicrobial mechanism of ITC is not still clear, although it is hypothesized to be owed to the electrophilic nature of the central carbon atom located in the N=C=S group [99]. Among them, allyl-isothiocyanate has also shown high antimicrobial activity being listed as a GRAS [100]. FC lettuce treated with a washing solution of 75 ppm of benzyl-isothiocyanate (5 min) achieved a complete removal of total bacteria and inoculated *Salmonella* in the wash water, which proved to persist such antimicrobial effects in the processed water up to 48 h [101]. However, the low solubility of ITC highly limits its application as a sanitizing water treatment in the FR industry. Accordingly, integration of ITC in edible coatings of FC produce has been proposed with a longer antimicrobial effect due to slower release from the coating [96].

3. Biological-based methods

3.1. Bacteriocins

Bacteriocins are toxins of protein nature that are synthetized by bacteria to inhibit the microbial growth of similar or closely related bacterial strains. The solubility of bacteriocins may increase at lower pH, facilitating diffusion of bacteriocin molecules [102]. Nisin is a food-grade bacteriocin produced by *Lactococcus lactis* that is widely used in the food industry. The antimicrobial activity of this bacteriocin is owed to its action on the cell membrane forming pores leading to the microbial cell death [103]. Nisin is principally effective against Gram-positive bacteria, while it is not active against Gram-negative bacteria due to their outer membrane [104]. However, the nisin efficacy may be increased also due to Gram-negative bacteria using chelating agents (e.g., ethylene diaminotetracetic acid [EDTA]), acids, or osmotic shock, with the outer membrane destabilized before the nisin application [103]. Attending to natural microflora, a mesophilic reduction of approximately 2 log CFU g^{-1} was reached in FC "Galia" melon after a nisin (0.250 g L^{-1}) treatment combined with EDTA (0.100 g L^{-1}) and citric acid (2.0 g L^{-1}) [105]. Nisin and other bacteriocins (pediocin, coagulin, plantaricin C, and lacticin 481) were also tested in FC lettuce inoculated with *L. monocytogenes*, inducing nisin and coagulin a reduction of pathogen viability by 1.2–1.6 log units [106]. The inclusion of nisin (IU mL^{-1}) in a pectin coating applied on FC "Rojo Brillante" persimmon completely inhibited the growth of mesophilic bacteria and inoculated *E. coli, S. enteritidis*, and *L. monocytogenes* [107]. Nisin treatment (0.03% for 10 min) controlled microbial growth and maintained quality of Chinese yam during storage at 4°C [108]. Bacteriocin RUC9 produced by *L. lactis* reduced by 2.7 log units, the *L. monocytogenes* loads inoculated in FC lettuce, while nisin only achieved a pathogen reduction of 1.9 log units [109].

3.2. Biological control

The use of biological preservation (strains of *Enterobacteriaceae*, lactic acid bacteria, yeasts, and molds) has also been studied in several products like lettuce, apples, peaches, and strawberries [110–113]. Recently, the application of the strain CPA-7 of *Pseudomonas graminis*, isolated from apples, could reduce the foodborne pathogens on FC apples, peaches, and melon [114, 115]. Such antimicrobial effects of these antagonists may be explained by the triggered activity of defence-related enzymes [116].

3.3. Bacteriophages

Bacteriophages are viruses that infect and replicate in a bacteria causing their lysis and death [117]. Bacteriophages were earlier studied on FC fruit like apples and melons [118]. *S. typhimurium* and *S. enteritidis* inoculated in lettuce were highly reduced by 3.9 and 2.2 log units, respectively, using different lytic bacteriophages treatments for 60 min at room temperature [119]. Nevertheless, bacteriophages treatments should be optimized due to the impractical application in the FC industry. Accordingly, integration of bacteriophages in edible coatings of FC produce has been proposed as an effective antimicrobial coating in tomatoes, with such phages being stable up to 1 week at 4°C [120].

4. Physical-based treatments

4.1. Mild heat treatments

The use of mild heat treatments is a promising sanitizing technique for the FC industry, which may extend the FC product shelf life through microbial destruction and partial enzymatic inactivation. However, the treatment temperature and exposure time should be carefully selected for each product due to possible undesirable changes of sensory and nutritional quality. Fruit and vegetables treated by heat treatments within the range 40–60°C for 1–5 min, depending of the commodity, are still considered as a fresh product as it is defined [17, 121].

Hot water and vapor treatments have been studied in several fresh-cut commodities like pomegranate arils [122, 123], kiwifruit [124], lemons [125], peaches [126], apples [127], sunchoke [128], lettuce [46, 129, 130], rocket [131], spinach [132, 133], celery [134], eggplants [135], and onions [136]. PPO activity of fresh-cut pomegranate arils was 1.3-fold reduced by a hot water treatment at 55°C for 30 s, while total anthocyanins contents were similar to those of untreated samples after 7 days at 5°C [137].

Short vapor treatments, usually up to 15 s, usually steam jet-injection systems, have been also used as an alternative to hot water treatments due to less impact on sensory quality of the FC product. Accordingly, vapor treatment (95°C; 7–10 s) kept better quality of FC pomegranate arils [138] compared to the hot water (55°C; 30 s) treatment [139] during storage at 5°C, increasing the shelf life from 7 to 18 days. Furthermore, vapor heat treatments reduced up to 2-fold the total antioxidant capacity losses observed in fresh-cut pomegranate arils sanitized by conventional NaOCl treatment during shelf life [138]. Steam jet-injection treatment of fresh-cut lettuce for 10 s reduced respiration rate, partially inactivated browning-related enzymes and kept the mesophilic load as low as with a conventional NaOCl treatment [46]. Nevertheless, further research is needed to optimize the exposure conditions for FC commodities.

Heat treatments by microwave (750 W for 45–60 s) have been recently proposed to reduce natural microflora of FC carrots, which also prevented whitening and surface drying of samples during storage up to 7 days at 5°C [140]. The increased microbial growth due to plant cell disruption after the heat treatment may be controlled with the use of combined storage under modified atmosphere packaging.

4.2. UV radiation

Ultraviolet (UV) light is an electromagnetic radiation divided in four groups: UV-A, UV-B, UV-C, and vacuum UV [141]. UV-C radiation (λ = 190–280 nm) is a promising sanitizing technology for FC products, which offers several advantages: it does not leave any residue, no legal restrictions, easy to use, and it does not require extensive safety equipment to be implemented [142, 143]. UV-C is a non-ionizing radiation, which means it is an electromagnetic radiation that does not carry enough energy/quanta to ionize atoms or molecules and is represented mainly by visible light, UV rays, microwaves, and infrared. UV-C radiation in the range 250–260 nm is lethal to most microorganisms, including bacteria, viruses, protozoa, mycelial fungi, yeasts, and algae, showing the maxima germicidal effectiveness at 254 nm [144]. UV-C germicidal effect is based on the ability of this radiation to alter microbial DNA through dimer formation [142]. If the damage goes unrepaired, the accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription, which ultimately result in reproductive cell death. Nevertheless, it has been also stated that UV-C may lead to the conversion of bacteria in the viable but non-cultivable state as a strategy of protection against the UV-C germicidal effect (to economize on energy, induction of repair mechanisms, inhibit the generation of mutant bacteria, etc.) [145]. UV-C is a superficial sanitizing treatment with low penetration in the plant tissue as observed in carrot tissue where a transmittance below 20% was observed in a 0.1-mm layer of the carrot epidermis [146]. Accordingly, a UV-C treatment of 0.4 kJ m^{-2} applied to iceberg lettuce internally inoculated (using vacuum system) with *Salmonella* Montevideo P2 did not achieve significant pathogen reduction, while the same UV-C dose achieved a 2-log CFU g^{-1} reduction on the surface-inoculated lettuce [147]. UV-C effectiveness appears to be dependent on the treatment temperature, distance between sample and lamp, direction of lamp, UV intensity, and exposure time [148, 149]. Cell permeability may be changed with UV-C, depending on the tissue and UV dose, leading to increase of electrolytes, amino acids, and carbohydrates leakage, which can enhance the microbial growth [150]. Accordingly, the crucial point is to apply an appropriate UV-C dose that achieves the maximum microbial reduction without damaging the product.

The UV dose (D; usually expressed in kJ m^{-2}) is directly proportional to the product of UV intensity (I; usually expressed in W m^{-2}) and exposure time (t) according to the equation: $D = I \times t$. The three pathogens regulated for FC products (according to the European Regulation [151]), *E. coli, Salmonella* spp., and *L. monocytogenes*, were inoculated in the kailan-hybrid broccoli, and the inactivation rates with UV-C doses up to 15 kJ m^{-2} were modeled [149]. The inactivation curves showed a pronounced tailing effect achieving a UV-C dose of 2.5 kJ m^{-2} *E. coli, S. enteritidis*, and *L. monocytogenes* reductions of 1.22, 2.61, and 0.72 log cycles, respectively. Hence, *S. enteritidis* was the most sensitive microorganism, while *L. monocytogenes* was the most resistant. Doses higher than 2.5 kJ m^{-2} led to further additional inactivation in the inoculated kailan-hybrid broccoli, although the most important inactivating effect was achieved in the range 0–2.5 kJ m^{-2} as similarly found in other FC products [149, 152].

The UV-C effectiveness on natural microflora has also been studied in several FC fruit and vegetables. UV-C treatments (0.49, 4.9, and 9.8 kJ m^{-2}) of FC zucchini slices reduced microbial activity and deterioration during subsequent storage at 5 or 10°C [153]. Mesophilic loads of FC pomegranate arils were reduced by approximately 1 log units after 4.5 kJ m^{-2}, while yeasts were reduced >1.8 log units [139]. Similarly, UV-C radiation doses (4.5–9 kJ m^{-2}) of the kalian-hybrid broccoli reduced mesophilic loads by approximately 1.2 log units while enterobacteria and psychrophilic were unaffected [62, 154]. However, combination of UV-C with NEW in kalian-hybrid broccoli or with hot water (55°C for 30 s) in FC pomegranate arils did not achieve further microbial inactivations [62, 139]. FC tomatoes treated with a UV-C dose of 4 kJ m^{-2} and stored for 21 days at 12°C under MAP (5 kPa O_2 +1 kPa CO_2) retarded ripening and maintained better firmness and sensory attributes than UV-C treated samples stored under air conditions [155]. The range of 0–2.5 kJ m^{-2} UV-C dose achieved the most important mesophilic reductions in treated date palm [156]. The UV-C sanitizing effect has been studied in a wide FC products such as tomato [157], strawberry [158], watermelon [159], potatoes [160], and lettuce [161], among others.

Increases in bioactive compounds of several fruit and vegetables after UV treatments have been reported. Such enhancements of health-promoting compounds have been reported to be a consequence of the free radicals generated during irradiation that might act as stress signals and trigger stress responses leading to the observed bioactives increments [162]. Broccoli exposed to several UV-C doses (1.5–15 kJ m^{-2}) registered increases in its polyphenols content (up to 25%) after 19 days at 5°C [154]. FC tomatoes UV-C treated (0.97 kJ m^{-2}) showed higher total phenolic content than other samples treated with hot water (40°C, 30 min), ultrasounds (45 kHz; power of 80%; 30 min), or its combination, after 30 days at 10°C [157]. The lycopene content of watermelon was preserved with a UV-C dose of 2.8 kJ m^{-2}, although a lower dose of 1.6 kJ m^{-2} did not show the same benefit [159]. However, immediate bioactive increments after UV-C have been observed in several FC fruit and vegetables, being probably attributed to an enhanced compound extraction due to plant cell disruption as a consequence of the UV radiation. Therefore, phenolic compounds and flavonoid contents of FC mangoes were increased after UV-C doses of 2.46 and 4.93 kJ m^{-2} [163]. Hydroxycinnamoyl acid derivatives of FC broccoli were also increased by 4.5–4.8-fold after UV-C treatments (4.5–9.0 kJ m^{-2}) [154]. Total phenol and flavonoid contents of banana and guava were enhanced after UV-C treatment [164]. FC carrot treated with UV-C (9 kJ m^{-2}) showed higher chlorogenic content on total antioxidant capacity than untreated samples on processing day [146]. Then, besides the interest of UV-C radiation as a microbial safety method, this non-ionizing radiation may also be used as a tool to enhance or better preserve the health-promoting compounds of plant products during shelf life [165].

Application of UV-B radiation (280–320 nm) has also been proposed as a friendly and cheap nonmolecular tool to enhance the phenolic compounds in carrots and other horticultural crops during postharvest life [166–168]. FC carrot shreds treated with a UV-B dose of 1.5 kJ m^{-2} showed 23% higher total phenolic content (mainly chlorogenic acid) than untreated samples after 72 h at 15°C [169]. UV-A has also been reported to induce biosynthesis of anthocyanins in cherries [170], although its effects has not been widely reported, and further research must be conducted.

Low-light conditions during storage have been recently proposed as an innovative and eco-friendly postharvest technique to highly prolong the shelf life of FC products. Accordingly, the shelf life of FC lettuce (butterhead and iceberg) was highly extended when it was stored under low-light conditions (≈5 μmol m^{-2} s^{-1} PAR; using either fluorescent or LED light) compared to samples stored under dark conditions [171]. Thus, lighting delayed cut-edge browning, reduced ascorbic acid degradation while carbohydrates levels were highly increased, although light samples did not show net photosynthesis according to photosynthetic activity measurements. Latter authors hypothesized that the observed prolonged shelf life in lit samples could be due to the higher levels of sugar and ascorbate that may act as antioxidants, may maintain membrane integrity, and may supply enough respiratory substrate to prevent ATP depletion.

4.3. Pulsed light

Pulsed light (PL) is a preservation technology that involves the use of intense short duration (1 μs–0.1 s) pulses of polychromatic light from UV to near infrared (100–1100 nm) emitted by an inert gas (e.g., xenon) lamp [172]. The microbicidal action of PL has been attributed to different mechanisms: photochemical, photothermal, and photophysical [173]. Several studies have shown the effects of PL treatments on inoculated microorganisms, native microflora, and quality aspects of FC spinach, lettuce, cabbage, carrot, mushrooms, avocado, and watermelon [174–179]. Microbial reductions up to 2.2 log units have been reported in different products such as lettuce, celery, spinach, bean sprouts, white cabbage, and green bell pepper, with the different antimicrobial effectiveness in different produce dependent on the location of microorganisms as well as the presence of protective substances present in the product [179, 180]. Further investigation may cover the reported microbial photoreactivation after PL treatments [181–184] and the PL efficiency due to shadow effects that is one of the main industrial limitations of PL technology.

4.4. Pulsed electric fields

Pulsed electric fields (PEFs) are based on the application of DC voltages for very short periods of time, usually μs, to the food material which is placed between two electrodes. PEF equipment consists of a treatment chamber, pulse generator, control system, data acquisition, and material-handling equipment [185]. PEF has been successfully applied for microbial inactivation in liquid food systems, although its application in plant tissues is limited due to the PEF-related plant cell disruption processes known as membrane breakdown, membrane permeabilization, or electroporation of the membrane [186]. Accordingly, PEF treatment (wave bipolar pulses at 2 kV cm^{-1} electric field strength, 1 μs pulse width, and 100 pulses s^{-1}) applied to blueberries (immersed in a saline fluid for PEF transmission) for just 2 min achieved 1 and 2 log unit reductions of inoculated *E. coli* and *L. innocua*, respectively [187]. Latter authors reported no PEF effects on color and appearance of blueberries, and the nutritional quality even enhanced, although PEF caused fruit softening. However, high electric field strength (333 V cm^{-1}) applied on a single pulse has been reported to not alter structure-related properties of FC onions, while such undesirable effects were observed using several pulses ($n \geq 10$) [185]. Accordingly, PEF is a promising technology to be used in FC produce, although the tissue changes as a function of the electrical field strength, and the number of pulses for each plant produce must be further investigated.

4.5. Cold plasma

Plasma is generated when an inert gas is in contact with electricity and is being considered as the fourth state of matter. Plasma is composed by charged particles, excited molecules, reactive species, and UV photons which induce microbial inactivation [188]. Plasma is generally classified as cold (non-thermal) and thermal plasma. Thermal plasma generation requires temperature and high pressure with heavy electrons. Cold plasma is generated at temperatures of 30–60°C under atmospheric or vacuum requiring low energy [189]. Among the main advantages of cold plasma are lower cost operating temperature and water consumption, together with timely production of the acting agents and lack of residues during production when compared to thermal and chemical treatments [190–192]. Cold plasma can be generated using either of the following devices: resistive barrier discharge, dielectric barrier discharge, corona discharge, radio frequency discharge, glow discharge, and atmospheric pressure plasma jet [193]. Plasma jet may be considered as the fastest plasma generation method to achieve microbial inactivation (4.3 ± 6.5 min) [194]. The most widely used gas in the published research has been air, followed by pure Ar, mixtures of He/O_2 and Ar/O_2, and pure N_2 [194]. The plasma inactivation capacity depends on several factors like the type of technology used to generate the plasma, the voltage, the feed gas, the treatment time, the species, the direct or indirect exposure and the concentration of the tested microorganisms, and the structural characteristics of the produce [192]. Cold plasma has a high potential to be used in the industry for fruit and vegetables according to studies of the last few years as recently reviewed [194]. Generally, plasma treatments are able to achieve microbial reductions of 2.7 ± 1.4 log units, ranging from 1.5 ± 1.0 log units for bacilli and spores to 3.3 ± 1.6 Log for *Listeria* spp., with treatment times of 22.2 ± 7.5 min for bacilli and spores and 3.5 ± 3.8 min for *E. coli* spp. [194]. Cold plasma has been also reported to highly (42–89%) reduce enzymatic browning of FC apples and potatoes [195, 196]. Nevertheless, the bactericidal mechanisms of cold plasma are still unclear being dependent on lots of factors related to processing parameters, environmental elements, and microbial properties [197].

4.6. Ultrasounds

Ultrasounds (US) are sound waves with amplitude higher than the upper audible limit of human threshold (above 20 kHz) that generate cavitation bubbles [198]. The antimicrobial properties derived from US is based on the combination of mechanical (responsible for the disinfection action leading to detachment) and chemical energy (responsible for the free radicals formation leading to destruction), produced from the collapse of latter generated bubbles, which increase the cell membranes permeability [40]. Consequently, DNA modifications of microbial cells are formed due to formed hot spots (due to collapse), with high temperatures and pressure, and released free radicals [199]. The US treatment should always ensure that US pressure levels (70 dB at 20 kHz or 100 dB at ≥25 kHz) are not surpassed according to UK Health Protection Agency recommendations [200]. The effectiveness of microbial inactivation achieved with US is influenced by microbial cell shape (high resistance of coccus), size (bigger cells are less resistant), Gram type (negative are less resistant), and cellular metabolism (anaerobes are less resistant) [201]. Among treatment parameters, US effectiveness is influenced by fluid temperature (optimum at 60°C which may be reduced to 20°C to avoid losses of produce sensory quality without highly affecting microbial US inactivation), water hardness, and dissolved gases content [202]. US treatment (40 kHz, 50 W) for 5 min of strawberries initially reduced natural mesophilic microflora by approximately 1 log unit [203]. However, latter authors showed that such initial antimicrobial effect was lost after 5 days at 8°C since similar reduction logs (regarding unwashed samples) to sterile water-washed (5 min) samples were achieved. On the other hand, such antimicrobial effects were maintained up to 9 days at 8°C when US treatment was combined with PAA (40 ppm) even showing a synergistic effect on mesophiles and the highest initial reductions on inoculated *S. enterica* subsp. Enterica, with sensory, physicochemical, and nutritional quality highly maintained [203]. No statistical differences among the effects of different frequencies (25, 32, and 70 kHz; 10 min) on achieved log reductions (1.5 log) were observed in FC lettuce inoculated with *S. typhimurium* [202]. Inoculated *E. coli*, *S. enteritidis*, and *L. innocua* loads of FC lettuce were reduced by 2.3, 5.7, and 1.9 log units with a US treatment for 30 min at 37 kHz without high color changes, although sensory quality was not studied in a parallel experiment with non-inoculated samples [204]. However, the produce matrix is highly important for US effectiveness since lower pathogen inactivations were observed in different products [204, 205].

4.7. High-pressure processing

High-pressure processing (HPP) uses elevated pressures, with or without the addition of heat, also called high-hydrostatic pressure processing since water is the most used pressure-transmitting fluid [206]. HPP is a promising eco-friendly sanitation treatment that may have a potential application in the FC industry. HPP may reach very high microbial inactivations as reviewed [207] while maintaining, or even enhancing, sensory properties of food products like aroma and taste. However, although the texture of tissues of firm FC products with low amounts of entrapped air remains unaffected, HPP may induce some alterations like water-soaked appearance of the product [208]. Furthermore, HPP may either enhance (leading to enzymatic browning reactions due to loss of membrane permeability and sub-cellular compartmentalization) or inhibit the activity of enzymes related to cell wall degradation in FC products [209]. HPP treatment (200–400 MPa, 3 min, 25°C) of FC persimmon induced changes in physicochemical quality (electrolyte leakage, texture, total soluble solids, pH, and color), which were a function of the amount of applied pressure compromising the consumer acceptance of the product [210]. However, latter authors reported that HPP may improve carotenoid extractability and tannin polymerization of FC persimmon, which could enhance its functionality and eliminate astringency, respectively. Plant cell membranes of FC peaches have been tried to be stabilized prior to HPP treatment (200 MPa for 10 min; 23–28°C) through penetration of Ca^{2+} into the plasma membrane using calcium chloride or calcium lactate soaking treatments (1–2% w/v for 5 min). Nevertheless, latter authors reported that loss of cell integrity due to HPP was not avoided with the calcium soakings probably due to a low Ca^{2+} penetration into the tonoplast membrane being recommended for future research a higher calcium concentration and/or improved Ca^{2+} impregnation (e.g., using vacuum infusion). Higher pressure levels (585 MPa) were more effective to inactivate enzymes and to preserve color of FC peaches than longer times being optimized a HPP treatment of 585 MPa for 1 min [211]. Nevertheless, due to the known baroresistance of some enzymes, like polyphenol oxidase (PPO), browning reactions may not be completely avoided during FC product shelf life, although MAP could limit such enzymatic activities due to low oxygen levels.

High-pressure carbon dioxide (HPCD) treatment has been proposed as another antimicrobial method being applied in FC carrots at 12 MPa (40°C) for 15 min and leading to complete inactivation of natural microflora being maintained after 28 days at 4°C together with the enzymatic stability [212]. HPCD treatment (12 MPa; 40°C; 20 min) of inoculated FC coconut also achieved *S. typhimurium* reductions of 4 log units, which was even enhanced to 8 log units when HPCD was combined with a high-power ultrasound treatment (10 W delivered every 2 min of treatment) [213].

5. Packaging under non-conventional gas mixtures

Modified atmosphere packaging (MAP) is a postharvest preservation technique based on the packaging of a perishable product within an atmosphere that has been modified compared with air conditions. There are two types of MAP: active or passive. Active MAP consists in the replacement on the initial present gases by a desired mixture. Passive MAP is progressively generated as a result of respiration of the product and gas transfer through the film, which has a selected permeability to gases, until the desired gas equilibrium atmosphere is reached.

The use of superatmospheric O_2 concentrations (>75 kPa O_2) during modified atmosphere storage (HO—high oxygen conditions) reduces aerobic and anaerobic microbial growth, prevents anaerobic fermentation, avoids non-desirable flavor changes, and inhibits enzymatic browning. The microbial toxicity to HO may be explained due to the unfavorable effects on the oxidation-reduction potential of the system, the oxidation of enzymes having sulfhydryl groups or disulfide bridges, and the accumulation of toxic reactive O_2 species [214]. HO-controlled atmosphere (75 kPa O_2 balanced with N_2) inhibited the mesophilic count of FC lettuce during storage for 10 days at 7°C [215, 216]. Chinese bayberries, strawberries, and blueberries stored under HO-controlled atmospheres (60–100 O_2 kPa balanced with N_2) inhibited decay during storage at 5°C and subsequent 2 days at 20°C, while chemical parameters and surface color were only slightly affected compared to samples stored under air conditions [217].

High CO_2 levels (maximum limits depending on the produce due to generation of related off-flavors) have also shown antimicrobial effects which are even stronger at low temperature because of enhanced CO_2 solubility. Accordingly, controlled atmosphere with 15 kPa CO_2 + 5 kPa O_2 (balanced with N_2) showed similar inhibitory effect to high O_2 on mesophilic growth of FC lettuce than samples stored at 0 kPa CO_2 + 75 kPa O_2 (balanced with N_2) [216]. Interestingly, a combined high O_2/CO_2 effect may be obtained using active HO MAP. The latter beneficial gas conditions are a result of the produce respiratory activity that generates antimicrobial CO_2 levels, while O_2 is inevitably reduced due to a combined effect of respiration and film diffusivity processes. Hence, the addition of CO_2 is unnecessary when high O_2 atmospheres are injected during active HO MAP. Accordingly, kailan-hybrid broccoli stored under high O_2/CO_2 (initial O_2/CO_2 of 70/0.02 kPa changing to 50/30 after 19 days at 5°C) showed 2.8 log units lower natural microflora load safter 19 days at 5°C compared to samples stored under passive MAP conditions (1.5–3.0 kPa O_2 + 16–21 kPa CO_2) [62]. Similarly, active HO MAP of inoculated kailan-hybrid broccoli showed 1.4 and 2.3 lower *E. coli* and *S. enteritidis* log units, respectively, after 19 days at 5°C regarding samples stored at passive MAP conditions [16]. However, such beneficial effect of HO was not observed when FC kailan-hybrid broccoli was stored at 10°C. Yeast and mold growth in FC pomegranate arils packaged under active MAP (initial O_2/CO_2 of 70/0.02 kPa changing to 20/5.5 kPa after 14 days at 5°C) was highly inhibited (up to 1.2 log units inhibition) during storage for 14 days at 5°C [139]. Such beneficial high O_2/CO_2 effects on microbial growth of FC produce packaged under active HO MAP have also been observed in other studies [218, 219].

Enzymatic browning of FC produce has been shown to be reduced under HO atmospheres. Accordingly, active HO MAP (80 kPa O_2 + 20 kPa CO_2) delayed browning of FC lettuce during storage for 10 days at 5°C [220]. Active HO MAP (initial O_2/CO_2 of 80/0 kPa, balanced with N_2) of FC pomegranate arils reduced enzymatic browning related to PPO [123, 137], while formed off-odors were highly controlled with active high CO_2 MAP (20 kPa CO_2 balanced with N_2) [139]. Active HO MAP of FC celeriac, mushrooms, and chicory endives (initial O_2/CO_2 of 95/0 kPa, balanced with N_2, changing to 10–20/10–50 after 7 days at 4°C) were more effective to control enzymatic browning than low O_2 atmospheres [221]. It is hypothesized that high O_2 may cause substrate inhibition of PPO or high contents of colorless quinones formed cause feedback PPO inhibition. High O_2 levels kept the initial color and firmness of fresh-cut melon retarding anaerobic fermentation better than low O_2 atmospheres [222]. Pre-treatment of whole "Spartan" apple with 100 kPa O_2 (up to 19 days at 1°C) before cutting decreased surface browning, flesh softening, and off-flavor in FC apple slices [223]. Such inhibition of enzymatic browning was related to retention of cellular integrity while in low O_2 pre-treatment (1 kPa O_2, balanced with N_2) would have another inhibitory browning mechanism on apple slices. Furthermore, the 100 kPa O_2 pre-treatment before slicing apples can reduce the dependence on antioxidant additives to inhibit slices browning. Sensory quality of produce stored under high O_2 atmospheres has been shown to be better than low O_2 atmospheres due to fermentation processes [218].

High O_2 levels are also considered as postharvest abiotic stresses able to increase PAL activity and consequently phenolic biosynthesis as observed in carrot shreds stored under high O_2-controlled atmosphere (80 kPa O_2 balanced with N_2) [146]. Furthermore, high O_2 and CO_2 levels may be tolerated by FC carrots maintaining their fresh characteristics and reducing microbial growth [224]. Such abiotic stress may be used as a tool to increase the health-promoting compounds of plant material to subsequently obtain functional beverages after correspondent thermal or non-thermal treatments to ensure microbial quality and safety [225].

The use of MAP under mixtures of non-conventional gases such as Ar, He, Xe, or N_2O has been proposed to maintain the quality of FC produce extending its shelf life. The latter gases may be chemically inert, but they have some physiological and/or antimicrobial properties, even though it does not seem to be through modification of enzyme activity [226]. Ar, He, or N_2 atmospheres mixed with low O_2 showed different diffusive properties, since Ar and He are monoatomic and smaller in size than N_2 [227]. Treatment of asparagus spears for 24 h at 4°C under an atmosphere of Ar and Xe at 2:9 (v:v) reduced RR and bract opening leading to a subsequent shelf life of 12 days at 4°C showing better quality than those samples treated with an atmosphere of 5 kPa O_2 + 5 kPa CO_2 [228]. Microbial quality and some bioactive compounds were highly preserved in FC red chard baby leaves stored under active He MAP (100%) during 8 days at 5°C [229]. N_2O has a direct effect on cell metabolism achieving shelf life extension of FC produce. It may be explained since N_2O has 77% solubility in fruit cell, while its absorption in tissues is completely reversible [230]. FC spinach leaves stored under active N_2O MAP (100%)

showed low microbial growth after 8 days at 5°C, with chlorophylls and phenolics being well preserved [231]. Different N_2O and N_2 combinations (including always 3% O_2) were used as active MAP for FC lettuce and wild rocket during storage up to 12 days at 5°C, suggesting such results that N_2O does not improve the produce quality compared to N_2 [232]. Lower microbial loads have also been observed in FC watercress and arugula leaves at the end of cold storage when active MAP containing N_2, Ar, He, Xe, or N_2O were used [233, 234].

6. Future research needs and conclusions

FC plant produce is greatly vulnerable to microbial spoilage, and cultivar selection is probably the most important factor in FC overall quality and shelf life. With the intention of better inhibition of microbial spoilage, and subsequently decrease in decay and safety problems, genetic cultivar selection should turn to retard ripening and senescence, low ethylene production and/or sensitivity, and enhanced firmness, well adapted to minimal processing and increased antioxidant systems. While chlorine is widely used by the FC industry to ensure safety, new eco-friendly techniques/technologies are needed to replace the latter chemical treatment due to the production of carcinogenic compounds. Several eco-friendly strategies such as ozone, UV-C light, natural antimicrobial substances, GRAS chemical, and biological compounds can decrease microbial loads of FC products and extend their shelf life. Other advanced techniques such as pulsed electric fields, ultrasounds, and high-pressure processing, among others, are promising sanitizing strategies for the FC industry due to high microbicidal rates. However, the treatment parameters of latter technologies to be applied in FC products need further research to be optimized for each specific commodity. Furthermore, the potential and limits of these innovative eco-friendly techniques must be well defined and included in the regulations. In that way, modeling tools to predict microbial inactivation and product shelf life are very useful, principally to optimize production and distribution. Application of nanotechnology to FC products is also a promising future in order to produce products with extended shelf life and excellent quality meeting always the food safety. Nevertheless, regulations related to nanoparticles' inclusion in food products need to be better defined by institutions. The combination of well-designed integrated production, handling, processing, and distribution chains for FC produces is crucial for achieving the high quality and safety demanded by consumers.

Acknowledgements

The authors are grateful to European Union, DG, for Science Research and Development (CRAFT Program Contracts SMT4-CT98-5530, QLK1-1999-707917, and OLK1-CT-2002-70791), 7th Framework Programme (FP7-BBBE.2013.1.2-02), Spanish Ministry for Education and Science and Spanish Ministry of Economy and Competitiveness (Projects ALI-95-0001, ALI-98-1006, AGL2005-08189-C02-01/ALI; AGL2007-63861/ALI; AGL2010-19201-C04-02-AGR; AGL2013-48830-C2-1-R), the Consejería de Educación y Cultura de la Comunidad

Autónoma de la Región de Murcia (SUE-OA 06/02-0008), and Fundación Séneca de la Región de Murcia in Spain (Project AGR/92, PS96/CA/d3, AGR/3/FS/02 and 00553/PI/04) for financial support. We also acknowledge the following companies for financing research and development contract with us: Repsol Petróleo S.A. (Madrid), Plásticos de Alzira, S.A., Sakata Seed Iberica S.L.U. (Valencia), Primaflor SAT (Almería), Canarihorta (Las Palmas de Gran Canaria), Frutas Hermanos Mira S.L. (Alicante), Kernel Export S.L., Frutas Esparza S.L., Perichán SAT, Pozosur S.L. (Murcia), and Sakata Seed (Valencia). The good skills and work of all researches whenever involved in the Postharvest and Refrigeration Group are very much appreciated.

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