The use of ozone to extend the shelf-life and maintain quality of fresh produce

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Abstract

Fresh produce has been recognised as a healthy food, thus there is increasing consumer demand for fresh fruit and vegetables. Their shelf-life, however, is relatively short and is limited by microbial contamination or visual, textural and nutritional quality loss. There are many methods to reduce/eliminate microorganisms present in food and ozone treatment is one of them. The use of ozone by the fresh produce industry is a good alternative to chemical treatments, e.g. the use of chlorine. The effectiveness of ozone as an antimicrobial agent has previously been reviewed and has been updated here, with the latest findings. The main focus of this review is on the effects of ozone on the fresh produce quality, defined by maintenance of texture, visual quality, taste and aroma, and nutritional content. Furthermore, ozone has been found to be efficient in reducing pesticide residues from the produce. The treatments that have the ability to reduce microbial contamination of the product without having an adverse effect on its visual, textural and nutritional quality can be recommended and subsequently incorporated into the supply chain. A good understanding of all the benefits and limitations related to the use of ozone is needed, and relevant information has been reviewed in this paper.

Keywords: ozone, fresh produce, quality and safety, sensory evaluation, storage

INTRODUCTION

The fresh produce industry is constantly growing, due to increasing consumer demand. Consumers care more and more about what they eat and fresh produce has been recognised as a healthy food, for example being rich in antioxidants.[1-3] The shelf-life of fresh produce, however, is limited and determined by initial quality at harvest[4,5] and subsequent storage conditions.[6] New techniques for reducing undesired microbial contamination, spoilage and decay, as well as maintaining product's visual, textural and nutritional quality are required in all steps of the production and distribution chain. One of the options could be the use of ozone, owing to its potential to reduce microbial contamination of the produce. A good understanding of all the benefits and limitations related to ozone use is needed[7] and this review aims to collate and discuss all the latest findings within this subject area.

OZONE AS AN ANTIMICROBIAL AGENT

Ozone (O_3) is a well-known strong oxidizing agent that has been used by the fresh produce industry as an antimicrobial agent for a number of years and has been generally recognised as safe (GRAS) (US FDA, 2001 (http://www.fda.gov/)). In contrast to other sanitizers, it does not leave chemical residues on the surface of the produce,[8,9] thus the use of ozone has the potential benefits to the food industry. It has been demonstrated in number of studies that microbial contamination can be reduced by applying ozone in either aqueous[10-13] or gaseous[14-17] form (Table 1). Physicochemical properties of ozone and its antimicrobial mechanisms of action (effect on cellular constituents such as proteins, lipids, nucleic acids, effect on enzymes and bacterial cell walls) have been described in more depth elsewhere,[8,9,18] and thus will not be detailed here.

Bacteria

Treatment of fresh produce with ozonated water prior to storage has been found efficient in reducing microbial counts on numerous products, including apples,[10] carrots,[19] celery,[20] lettuce,[11,13,21,22] peppers,[12,13] spinach[23,24] and strawberries.[12,25] Exposure to gaseous ozone, on the other hand, was found efficient in reducing microbial counts on blueberries,[26] carrots,[27] papaya,[28] peppers,[15,29] spinach[17,30] and tomatoes.[14,31,32] It is worth mentioning, that treatment with ozone reduced human health risk associated with foodborne pathogens, i.e. *Escherichia coli*,[10,23,24,33] *Listeria* sp.[12,23,24] and *Shigella* sp.[34] by reducing their numbers on fresh produce. Some authors,[31,35,36] however, did not observe reductions in microbial counts in response to ozone treatment. This could be due to cut surfaces that promoted the leaching of organic matter from the product, e.g. from fresh-cut green peppers[36] or tissue damage if the dose of ozone was too high.[37,38] Ozone would in that case rather react with organic matter than act as an antimicrobial agent,[39] thus being less efficient against the microorganisms. Ketteringham *et al*.[36] suggested that whole peppers rather than fresh-cut fruits would be more suitable for ozone treatment.

The level of reduction in microbial counts on carrots and lettuce[31] was less when compared with tomatoes and was explained by better attachment of bacteria cells to porous and rough surfaces in which bacteria were protected from ozone action. The lack of effect of gaseous ozone treatment at 5 ppm for 3-15 min on *E. coli* in carrots,[31] can be explained by the dose of ozone being too low. It has been previously reported by others[19,27] that to reduce microbial contamination of carrots, ozone needs to be applied at 1,000 ppm for 5 min in its gaseous form,[27] or about 10 ppm for at least 10 min when used in the aqueous form.[19,27] Singh *et al*.[27] however did not assess organoleptic properties of the produce, whereas ozone at the dose used in their study is likely to adversely affect other quality characteristics of carrots.[40] In lettuce, on the other hand, the lack of effect of gaseous ozone treatment at 5 ppm for 3-15 min[31] was probably due to the dose of ozone being too high, so that it caused tissue damage and counteracted the beneficial antimicrobial action. Microbial reductions on lettuce have been observed by others when ozonated water was used at 0.5 ppm for 5-30 min,[13] at 0.5-4.5 ppm for 0.5-3.5 min,[38] at 2.5-4 ppm for 5 min,[21] at 4 pm for 2 min[11] and at 2.5-7.5 ppm for 10 min.[22] In some of these studies the doses of ozone were also too high,[13,22] however sensory evaluation of the produce was either not conducted,[13] or carried out by a random, untrained panel,[22] who nonetheless were not pleased with the quality of lettuce treated with ozone, as indicated by their lack of willingness to purchase the product. Olmez and Akbas[38], who focused on optimization of ozonated water treatment for lettuce, reported that overall visual quality declined with the concentration of ozone being increased above 2.5 ppm and exposure time extended above 2.5 min.

Allende *et al*.[35] reported that treatment with gaseous ozone at 5,000 mg L^{-1} for 6 days had no significant effect on microbial counts in strawberries. However, in their study Allende *et al*.[35] observed only low microbial counts (10^2–10^4 colony forming units (cfu) g^{-1}) on both control and ozone-treated strawberries, and microbial growth was not a critical parameter of produce quality, explaining why difference between treatments could not be detected. In contrast, when initial microbial counts on strawberries were higher (10^7 cfu g^{-1}), reduction by 1.21 log unit was observed in strawberries washed with ozonated water at 0.3 ppm for 2 min[25] and by 2.30 log unit at 2 ppm for 3 min.[12] The difference between these studies may also be due to the method of ozone application (gaseous[35] vs. aqueous[12,25]).

The efficiency of ozone treatment in reducing microbial counts on fresh produce depends on the dose of ozone being used (dose - ozone concentration x time of exposure) and on initial microbial counts/inoculum.[29,32] In the case of aqueous ozone solutions (pre-ozonated water or continuously ozonated water), time of exposure is often limited,[10,11,13] for practical reasons. Continuously ozonated water was found to be more efficient in reducing microbial counts[13] compared to pre-ozonated water, which is not surprising, since in pre-ozonated water the concentration of ozone is depleted during the processing stage, e.g. due to contact of ozone with organic matter, while in continuously ozonated water its concentration was maintained throughout the treatment period. With gaseous treatment, on the other hand, fruit and vegetables can be treated with high ozone concentration prior to storage[28,41] or they might be continuously/intermittently exposed[14,42] to lower ozone concentrations during storage.

The highest microbial reductions are often observed at the highest doses of ozone,[12,26,43] however, as the sensitivity to ozone varies among different commodities, it is necessary to establish an optimal treatment (dose) for each product[44] to avoid tissue damage, that among other things leads to an increased susceptibility to microbial infection. A number of studies[9,36,45] focused on the antimicrobial efficacy of ozone treatment and not on the effects of ozone on nutritional and sensory quality of the product. Only those treatments that reduce microbial contamination of the product without having an adverse effect on product's visual, textural and nutritional quality[46,47] can be recommended and subsequently incorporated into the supply chain.

Fungi

Treatment of fresh produce with ozone has reduced fungal development, measured as lesion size, on a number of products, e.g. in apples[48] exposed to gaseous ozone at 450 ppb for 2 days, in broccoli[37] continuously exposed to ozone at 200 and 700 ppb for 12 days, in carrots exposed to gaseous ozone at 450 ppb for 2 days,[48] at 1 ppm for 4 days,[49] intermittently (8 h per day) exposed to ozone at 15 ppm for 4 weeks[50] or continuously exposed to low level of ozone at 50 ppb over a 6 month storage period.[40] Fungal development was also reduced in kiwi[51,52] continuously exposed to ozone at 300 ppb over 4 month storage period, in papaya[53] exposed to ozone at 0.04, 1.6 and 4 ppm for 48-144 h, in peaches[54] continuously exposed to ozone at 300 ppb over a 4 week period, in tangerine[55] exposed to gaseous ozone at 200 ppm for 4-6 h, and in tomatoes[56-58] continuously exposed to ozone at 0.2, 1 and 5 ppm for up to 13 days, but not in plums[59] where dose of ozone at 0.1 ppm for 8 days was too low to prevent fungal growth.

It has been demonstrated[59] that efficiency of ozone exposure in reducing fungal development depends on the product type and inoculum concentration. Tzortzakis *et al*.[59] inoculated clementines, tomatoes and plums with *Botrytis cinerea* spores at low (2×10^3), intermediate (2×10^5) or high (2×10^7) spore concentration. Fruit were subsequently exposed for 8 days to clean air (control) or ozone at 100 ppb. Fungi development was significantly reduced in ozone-exposed clementines at all spore concentrations, whereas in tomatoes a positive effect of ozone was only observed at the highest (2×10^7) inoculum. In contrast, in plums ozone had no effect at either low or high spore concentration. Clearly, not all commodities would benefit from ozone exposure as high doses that might be needed may cause damage to fruit epidermis, facilitating fungal penetration.

Interestingly, both Liew and Prange[50] and Sharpe *et al*.[48] observed reduced growth of gray mould (*B. cinerea*) and white mould (*Sclerotinia sclerotiorum*) on ozone treated carrots, whereas others[40,49] observed better resistance to *B. cinerea* but not to *S. sclerotorium*. The level of isocoumarin 6-methoxymellein (3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin; 6-MM), which is associated with resistance to *B. cinerea*, was found to be significantly increased in carrots continuously exposed to ozone, even at the dose as low as 50 ppb.[40] Other mechanisms, however, such as other compounds could have contributed to the improved resistance.[49] The results reported by different authors[40,48-50] may vary for several reasons. First of all, the ozone dose of 7.5-60 ppm (8 h per day) for 4 weeks used by Liew and Prange[50] was higher than 1 ppm for 4 days used by Forney *et al*.[49] and 50 ppb over 6 months storage period used by Hildebrand *et al*.[40] Even though, the dose of ozone used by Liew and Prange[50] was found to be fungistatic, it was injurious to the produce as indicated by increased respiration rate, electrolyte leakage, colour changes (orange-red colour appeared to be bleached by the treatment) and surface pitting. Secondly, the duration of storage differed between the studies. In the study of Sharpe *et al*.[48] and Liew and Prange[50] growth of fungal pathogens (*B. cinerea* and *S. sclerotorium*) was assessed over 12 and 28 days, respectively, whereas Forney *et al*.[49] and Hildebrand *et al*.[40] conducted long-term storage trials of up to 6 months, and observed that the growth of *S. sclerotiorum* was slightly reduced immediately after treatment with ozone at 1 ppm for 4 days[49] or by continuous exposure to ozone at 50 ppb,[40] but this effect was lost after 4 weeks of storage.

Ozone exposure decreased the disease incidence (% of fruit that show any degree of spoilage due to fungal infection) in apples treated with ozone at 25 ppm for 30-90 min,[60] in grapes[41,54,61-64] exposed to gaseous ozone at 200 ppm for 15 min[62] or at 2,500 ppm for 2 h, 5,000 ppm for 1 h and 10,000 for 30 min.[41] Similarly, decay of grapes was significantly reduced in produce continuously exposed to ozone at 100 ppb for up to 60 days[64], at 300 ppb over 7 weeks[54] and in grapes continuously or intermittently (12 h per day) exposed to ozone at 2 ppm for 72 days,[61] in tangerine exposed to gaseous ozone at 200 ppm for 4-6 h[55], in strawberries exposed to gaseous ozone at 1.5 ppm for 3 days, [65] but not when the dose of ozone at 0.35 ppm for 3 days[66] was too low to prevent decay of strawberries or in blueberries exposed to gaseous ozone at 450 ppb for 2 days.[48] Sharpe *et al*.[48] found that no effect in blueberries was due to their high susceptibility to fungal infection, when compared with apples, grapes and carrots. Disease incidence in grapes, however, was not reduced when produce continuously exposed to low dose of ozone at 100 ppb[64] or 300 ppb[54] was transferred to ambient temperature, simulating retail conditions.

Minas *et al*.[52] observed reduced disease incidence only in kiwi fruits exposed to ozone at 300 ppb for 8-144 h prior to inoculation with *B. cinerea*, whereas post-inoculation treatment with ozone had no effect. This finding indicates that exposure of kiwi fruit to ozone may affect fruit-pathogen interaction and enhance their disease resistance. Increased resistance to diseases has also been observed in tomatoes[58] exposed to gaseous ozone at 50 ppb for 6 days prior to inoculation. This implies that ozone may induce defence responses, such as the synthesis of phytoalexins,[63] i.e. resveratrol and pterostilbene.

Lower disease incidence in fruit and vegetables exposed to ozone might be partially explained by reduced spore production and viability observed in the majority of studies,[48,52,56-58] which is of high importance for fresh produce industry, reducing disease spread from injured and infected produce. Care must be taken, however, as ozone exposure - even at high doses - does not provide adequate control of fungal development in wound inoculated fruit, thus often having no effect on disease severity,[52,54,67] which is usually only delayed by the ozone treatment. Fungal structures already developed within wounds remain protected from the oxidizing effect of ozone due to its limited penetration.

It is apparent that ozone efficiency against fungal pathogens is not only affected by the dose of ozone used but also by several other factors including skin characteristics of the produce (e.g. roughness), sensitivity of the specific fungi to ozone[57,62] and storage conditions, e.g. temperature[48,50] and relative humidity.[62] Ozone applied at 60 ppm (8 h per day) for 4 weeks was found to be more efficient in reducing the growth of *B. cinerea* and *S. sclerotorium* on carrots at 2 °C when compared with 8 °C.[50] The reduction rate for both pathogens was 57 and 56% at 2 °C, and 42 and 37% at 8 °C, respectively,[50] which may be associated with slower growth rate of fungi at lower storage temperature. Ozkan *et al*.[62] on the other hand, observed that ozone efficiency against conidia of *Penicillium digitatum*, *Penicillium italicum*, and *B. cinerea* depended on relative humidity (RH). Treatment with gaseous ozone at 200 ppm for 15 min was sufficient to inhibit conidia germination of all three pathogens at 95% RH, whereas at 75% RH and 35% RH the dose of ozone required to achieve similar inhibition had to be increased two and more than ten times, respectively. Ozone efficiency was clearly reduced with decreasing relative humidity.

In summary, ozone reduces microbial contamination of the produce, being more efficient against bacteria than fungi. The disease incidence may not always be reduced in ozone-treated produce; nonetheless ozone exposure reduces the spread of the disease by reducing spore production and viability. Furthermore, it has been reported to induce changes in the produce, i.e. in grapes, kiwi and tomatoes, increasing their disease resistance.

PESTICIDE RESIDUES

Pesticides are frequently used to improve crop productivity and health by controlling pests. There is, however, an increasing public concern about health risks associated with the presence of these chemicals on fruit and vegetables.[68] Furthermore, pesticide residues may affect international trade, due to differences in food policies regarding pesticide use among various countries.[69]

Ozone cannot penetrate deeply into the fruit because fruit surface generally contains many readily oxidizable materials that ozone will react with. The majority of pesticide residues, however, are located in the skin.[44] The efficiency in pesticide residues removal by ozone varies among different commodities, due to their surface characteristics.[70]

A number of studies have found reductions in pesticide residues in apples washed with ozonated water at 1 and 3 ppm for 5-30 min[71] or at 250 ppb for 5-30 min,[72] in grapes treated with gaseous ozone at 10,000 ppm for 1 h[41] or continuously exposed to ozone at 0.3 ppm for 36 days,[42] in lettuce[70,73] washed with ozonated water at 0.5-2.0 ppm for 5-20 min, in pak choi[74] washed with ozonated water at 1.4 or 2.0 ppm for 15-30 min, in strawberries[70] washed with ozonated water at 2.0 ppm for 10 min, in tomatoes[70,73] washed with ozonated water at 0.5-2.0 ppm for 5-20 min, and citrus fruit,[75] i.e. grapefruit, lemon and orange washed with ozonated water at 4-10 ppm for 5 min. Treatment with ozonated water was very efficient in reducing several pesticide residues, including captan (above 92% reduction in apples),[72] mancozeb (up to 97% reduction in apples),[71] fenitrothion (up to 58, 48 and 25% reduction in lettuce, tomatoes and strawberries, respectively),[70,73] cypermethrin (up to 60% reduction in pak choi)[74], parathion (up to 55% reduction in pak choi)[74] and diazinon (up to 50% reduction in pak choi).[74] The efficiency of the washing was temperature dependent,[72,73,75] with higher efficiency being observed with increasing temperature in case of apples,[72] lettuce[73] and tomatoes.[73] Increase in temperature, on the other hand reduced the efficiency in pesticide residues removal from citrus fruit.[75] In case of apples, the rate of change in captan degradation with temperature increase from 21 to 44 °C was low (around 7%), due to 100% removal of the pesticide at both temperatures; the only difference was in time needed - at 21 °C treatment time had to be 10 min longer than at 44 °C. In lettuce and tomatoes, with temperature increase from 15 to 30 °C, the rate of reduction of fenitrothion was increased by ~30%. In case of citrus fruit, with temperature increase from 10 to 40 °C, the efficiency of ozone treatment at 4-10 ppm for 5 min in removal of Tetradifon was reduced by ~50% in lemon and grapefruit and by ~20% in orange, whereas removal of Chlorothalonil was not affected by changes in wash water temperature. Nonetheless, difference among the commodities in pesticides removal was observed, e.g. ozonated water at 4-10 ppm for 5 min removed 100, ~90 and ~40% of Chlorothalonil from orange, lemon and grapefruit, respectively. This was explained by differences in the diffusion of adsorbed pesticides into the matrix.

The reduction rates were much higher when ozone was used at higher concentration (2,500 - 10,000 ppm) for a short time (up to 2 hours),[41] but this treatment led to significant damage of the fruit, thus continuous exposure to ozone at low concentration of 300 ppb was suggested[42] as a more feasible solution for pesticide residues removal. Although gaseous ozone was found to be efficient in reducing pesticide residues, including pyrimethanil, cyprodinil and fenhexamid from grapes,[41,42] it had no effect on boscalid and iprodione residues, which means that not all pesticides can be removed with ozone treatment. Treatment with gaseous ozone at 10,000 ppm for 1 h reduced pyrimethanil, cyprodinil and fenhexamid by ~84, 75 and 69% respectively, whereas the residues of boscalid and iprodione were only reduced by 17 and 5%. Similarly, in the presence of gaseous ozone at 300 ppb, residues of pyrimethanil, cyprodinil and fenhexamid were reduced by ~35, 22 and 23% respectively, while the residues of boscalid and iprodione were only reduced by 7 and 1%.

WEIGHT LOSS

Weight loss was reduced in kiwi continuously exposed to ozone at 300 ppb,[76] in papaya exposed to ozone at 1.5-5.0 ppm for 4 days[77] and strawberries exposed to ozone at 1.5 ppm for 3 days,[65] while in the majority of studies ozone exposure had no effect. Ali *et al*.[77] suggested that this response might be due to thick cuticle of papaya fruit, which prevented the damage of epidermal tissues by ozone action.[18] It has been shown in several other studies that weight loss was unaffected, for example in broccoli[37] continuously exposed to ozone at 200 ppb, in carrots treated with ozone at 0.3-1 ppm for up to 4 days[49] or continuously exposed to ozone at 50 ppb for 6 months,[40] in grapes[54] continuously exposed to ozone at 300 ppb, in peppers[15] treated with ozone at 1 ppm for 1-5 min, in rocket leaves[78] washed with ozone at 10 ppm for 1 min, and in tomatoes[79] treated with ozone at 10 ppm for 10 min, i.e. when fresh produce was exposed to relatively low concentration of ozone. On the other hand, high dose of ozone caused damage to fruit epidermis, thus leading to higher weight loss in broccoli[37] continuously exposed to ozone at 700 ppb, grapes[61] exposed to ozone at 2 ppm for 72 days and tomatoes[56] exposed to ozone at 1 ppm for 6 days. These findings suggest that for each commodity there is a threshold in ozone concentration, above which, the exposure may cause the damage to the produce. Based on the limited information available in the literature, it can be concluded that these thresholds lie somewhere between 200 and 700 ppb for broccoli,[37] 400 ppb and 2 ppm for grapes,[54,61] and 50 ppb and 1 ppm for tomatoes.[56]

RESPIRATION

Quality loss during the storage of fresh produce may be accelerated by changes in the metabolic activity of the product. Respiration rate (consumption of O_2 and production of CO_2), which is a measure of physiological activity[80] increases in response to tissue damage (e.g. during processing stage). Thus, it is not surprising that several authors have reported higher respiration rate as a result of tissue damage due to cutting, e.g. in lettuce[81] or in tomato slices when compared with whole fruit.[14]

Most studies that used low doses of ozone have shown that ozone treatment did not result in a higher respiration rate in asparagus,[82] broccoli,[37] carrots,[19] celery,[20] lettuce,[11,21,83] peach,[54] peppers,[15] rocket leaves,[78] strawberries[35] and tomatoes[14,56,79] unless the dose of ozone used was too high, e.g. 700 ppb in broccoli[37] which caused damage to the produce.

TEXTURE

Texture loss during storage is a serious problem because it reduces marketability of the product. A number of authors have studied the effect of ozone on texture maintenance. Most of them found no effect on textural changes in apples,[84] blueberries,[48] cantaloupe,[85] grapes,[61,63,64] lettuce,[11,21,83] pears,[84] peppers[15] and rocket leaves.[78] Several studies, on the other hand found better firmness retention, e.g. in cucumbers continuously exposed to ozone at 40 ppb,[84] in kiwi continuously exposed to ozone at 300 ppb,[76] in papaya exposed to ozone at 1.5-3.5 ppm for 4 days,[77] strawberries washed with ozonated water at 300 ppb for 2 min[25] or exposed to gaseous ozone at 1.5 ppm for 3 days[65] and in tomatoes cyclically exposed to gaseous ozone at 4 ppm for 30 min every 3 h,[14] exposed to gaseous ozone at 50 ppb[56,58] or 1ppm[56] for 6 days, or treated with gaseous ozone at 10 ppm for 10 min,[79] where softening of the fruit, associated with ripening, was delayed in ozone-exposed samples. Rodoni *et al*.[79] conducted analyses of the cell wall and found a decreased activity of pectin methylesterase (PME) in ozone-exposed tomato fruit. These authors suggested that delayed fruit softening might be due to reduced solubilisation and depolymerisation of pectin polysaccharides. There’s clear evidence in literature that ozone may affect both ripening[76,77] and enzymes, e.g. through signalling molecules.[86]

In a few studies, i.e. in asparagus[87] and carrots[19,49] ozone treatment delayed tissue toughening. These changes were associated with changes in cellulose, hemicellulose and lignin content, namely due to reduced lignification of cell walls. The mechanism may involve decreased activity of phenylalanine ammonia lyase (PAL; EC 4.3.1.5) as reported in asparagus[87] washed with ozonated water at 1 ppm for 30 min or reduced activity of polyphenol oxidase (PPO; EC 1.14.18.1) and/or peroxidase (POD; EC 1.11.1.7) as observed in carrots[19] washed with ozonated water at 10 ppm for 10 min. It could also be associated with changes in the proportion of uronic acids and neutral sugars being present in the water-soluble fraction of pectin.[79]

VISUAL QUALITY

Visual quality of the product is important because fresh produce with a good appearance is preferred by customers. Any colour alteration might be recognised as a symptom of senescence,[6] reducing its marketability. Monitoring colour changes during storage is used for determining visual quality loss. Commonly used parameters of colour in 3D colour space are: either (i) hue angle which describes the basic colour, luminance and chroma, i.e. colour saturation[88] or (ii) lightness (from black to white), greenness/redness and yellowness (from blue to yellow) values.[89] These parameters have been used to assess colour changes during the storage of various products, e.g. in apples,[48] broccoli,[37] carrots,[31,48] grapes,[64] lettuce[11,31] and tomatoes.[90]

Ozone treatment had no effect on colour changes during storage in apples,[48] cucumber,[84] cilantro,[91] persimmon,[92] rocket leaves,[78] tangerine[55] and tomatoes.[31,73,79] Zambre *et al*.[90] however, observed delayed development of red colour during the storage of tomatoes treated with gaseous ozone at 20 to 50 ppm for 10 min. Similar findings have also been reported by others,[77] who observed that peel colour changes of papaya fruit were affected by exposure to gaseous ozone at 2.5 ppm for 96 h. Exposure to ozone delayed ripening of the fruit, thus extending the shelf-life of papaya and tomatoes, however full colour was not always developed during the storage period.

Continuous exposure to ozone in the range from 40 ppb[84] to 200 ppb[37] slowed the process of yellowing of broccoli florets, whereas the concentration of 700 ppb was found to be injurious,[37] leading to desiccation and tissue browning. Visual quality maintenance was also improved in grapes kept in the presence of 100 ppb of ozone,[64] when product was subsequently transferred from 0 to 15 °C (simulating typical retail conditions). In control clusters extreme browning of rachis was observed, grapes became darker and appearance was scored as “poor”, whereas in grapes continuously exposed to ozone at 100 ppb, only slight browning of rachis was reported, the change in colour was less pronounced and appearance was scored between “moderate” and “good”.

In carrots treated with ozone at the concentration from 5 to 60 ppm[31,50] lightness value increased significantly, suggesting that typical orange-red colour was bleached by the treatment. This was further confirmed by the increase in whiteness index.[31] On the other hand, ozone treatment at 450 ppb had no effect on colour of carrots.[48] Sharpe *et al*.[48] however, did not measure other quality characteristics, while it was previously reported[40] that ozone at the concentration as low as 50 ppb may cause an injury to carrot tissue appearing as blotches of brown discoloured periderm. Unfortunately, Hildebrand *et al*.[40] did not assess colour changes in their study.

In lettuce, the colour parameters were not affected when samples were treated with ozonated water at concentrations of up to 2 ppm.[38,73,83] Olmez and Akbas[38] reported that visual quality of lettuce declined when concentration of ozone was above 2.5 ppm and lettuce leaves became translucent when ozonated water at 4.5 ppm was used. Thus, it is not surprising that leaf lightness and whiteness index significantly increased in lettuce leaves, which became translucent after being treated with gaseous ozone at 5 ppm for 15 min.[31]

In several fruit and vegetables, e.g. blueberries,[26] peppers[15] and strawberries[25,35,65,66] the response to ozone depended on the exposure method. Interestingly, colour alterations were observed when ozone was applied at 30,000 ppm for 64 min in the gaseous form in case of blueberries, which became darker, more red and less blue when compared with untreated samples, and in aqueous form at 1 ppm for 1-5 min in case of peppers which became lighter, whereas ozone treatment had no effect on colour changes in blueberries washed with ozonated water at 21 ppm for 64 min and peppers treated with gaseous ozone at 700 ppb for 1-5 min, respectively. Colour evaluation of blueberries was conducted after the treatment, and it is likely that high dose of ozone used caused significant changes in pigments content, e.g. anthocyanin. In peppers, change in colour due to washing with ozonated water could either be a result of bleaching the pigments from the produce and/or reduced browning due to oxidative processes during storage. In strawberries better colour retention, considered as total colour difference, was found in samples treated with ozonated water at 300 ppb for 2 min,[25] however, individual colour characteristics, e.g. lightness value, were not provided. Exposure to gaseous ozone at 1.5 ppm for 3 days, on the other hand, had no effect on colour characteristics,[65] whereas visual quality of strawberries declined, as a result of calyx browning, below the limit of acceptance from the consumers point of view, as assessed by sensory evaluation panel, at high concentration of ozone - 5,000 mg L^{-1} for 6 days.[35]

TASTE AND AROMA

The taste of the product can be affected by a number of factors, including sugar content and composition, organic acids, acidity and texture-related mouth feel,[93,94] whereas changes in aroma are related to changes in the composition of volatile compounds.[66,95,96] Both taste and aroma together, define the product's flavour, i.e. the way it is perceived by the customers.

The level of soluble solids is associated with sugar content and fruit maturity. It increases during fruit ripening, and starts to decline when fruit overripe. Most studies have found that ozone treatment had no effect on total soluble solids content in apples,[84] cantaloupe,[85] carrots,[40,49] celery,[20] grapes,[61,64] pears,[84] persimmon,[92] tangerine[55] and tomatoes.[14] On the other hand, a decrease in soluble solids was reported in kiwi fruit continuously exposed to 300 ppb ozone,[76] which suggests that ripening was delayed in ozone-treated fruit. Similarly, delayed increase in soluble solids due to delayed ripening was observed in papaya fruit exposed to a low dose of ozone, between 1.5 and 3.5 ppm for 96 h,[77] but not when the concentration of ozone was higher,[97] inhibiting the development of full ripe colour and resulting in tissue damage due to its strong oxidizing activity and thus supporting the growth of fungal pathogens. This underlines the fact that only at certain levels of ozone shelf-life may be extended.

Ozone had no effect on sugars in carrots,[40,49] celery,[20] grapes[64] and kiwi,[51] however, in some cases sugar content (glucose, fructose) and/or composition was found to be altered in ozone-exposed tomatoes.[14,56] In the case of whole tomatoes cyclically exposed to gaseous ozone at 4 ppm for 30 min every 3 h, the content of fructose and glucose was not affected after 15 days of storage. In another study,[56] where tomatoes were stored in the presence of ozone at 0.05 or 1.0 ppm for 6 days, no difference in sugar content was found until the samples were transferred to "clean air" for additional 6 days, when content of fructose, glucose and total soluble sugars was better maintained in ozone-exposed tomatoes, which were perceived sweeter by the sensory evaluation panel when compared with control.[56] Interestingly, in the case of tomato slices no significant difference in taste was found between treated and untreated samples, even though the content of glucose was significantly higher in ozone-exposed tomatoes.[14]

Regarding organic acids composition, more detailed analyses have only been conducted for kiwi[51] and tomatoes.[14,56] In kiwi stored for 29 weeks at 0 °C in the presence of ozone supplied at 4 mg h^{-1}, no difference was found in ascorbic and tartaric acid when compared with samples stored without ozone. A significant decline, however, was found in citric, malic and quinic acid in ozone-exposed fruit close to the end of the storage period, suggesting that ozone-exposed fruit became overripe and organic acids were used as respiratory substrates. In tomatoes exposed to ozone, no difference was observed in ascorbic, citric, malic and succinic acid,[14,56] while fumaric acid content increased.[14]

Even though some changes in organic acids have been observed,[14,51] in general, ozone had no effect on acidity of the product in apples,[84] kiwi,[51,76] persimmon,[92] tangerine,[55] tomatoes[79] and strawberries.[65] Artes-Hernandez *et al*.[64] did not observe differences in acidity between ozone-exposed (100 ppb for 60 days) and control “Autumn seedless” grapes; however, Cayuela *et al*.[61] have demonstrated that these changes depend on the grape variety and method of exposure (continuous, intermittent). In “Regina Victoria” grapes acidity significantly increased in samples continuously or intermittently (12 h per day) exposed to ozone at 2 ppm during storage for 72 days at 5 °C. In “Cardinal” grapes intermittent exposure to ozone had no effect on produce acidity, whereas in continuously exposed grapes acidity increased. Interestingly, in “Superior Seedless” grapes, produce acidity was reduced in response to ozone; reduction being more pronounced in continuously exposed grapes.

The typical aroma of the product was reversibly reduced in tomatoes[14] cyclically exposed to gaseous ozone at 4 ppm for 30 min every 3 h and strawberries[65,66] continuously exposed to gaseous ozone at 0.35 and 1.5 ppm for 3 days, but not affected in cantaloupe[85] treated with gaseous ozone at 10,000 ppm for 30 min, in grapes[64] continuously exposed to ozone at 100 ppb for 60 days, and in papaya[77] treated with ozone at 1.5-5.0 ppm for 4 days. These differences could be due to oxidation of volatiles released by the fruit by ozone molecules[65] or changes in volatile composition by suppressing their emission,[66] which is partly associated with delayed ripening. In case of carrots, that were found to be very sensitive to ozone exposure, production of stress volatile compounds (i.e. ethanol, hexanal) has been reported when carrots were exposed to 300-1000 ppb ozone.[49]

NUTRITIONAL QUALITY

Plants produce reactive oxygen species (ROS) during cellular metabolism; however, in response to environmental stresses, e.g. ozone,[86] ROS production, as well as the activity of antioxidant enzymes - ascorbate peroxidase (APX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6) and superoxide dismutase (SOD; EC 1.15.1.1) may increase.[98,99] ROS include such compounds as superoxide radicals (O_2^-), singlet oxygen ($^1O_2$) and highly reactive hydroxyl radicals (OH^-). SOD catalyses the dismutation of superoxide (O_2^-) to H_2O_2 which is then transformed to H_2O and O_2 by simultaneous action of APX and CAT. To mitigate ROS, plants may also induce the biosynthesis of antioxidants, including ascorbic acid (AsA) which is involved in the reduction of ROS through the ascorbate-glutathione cycle.[100,101] Other antioxidants, such as carotenoids and flavonoids have also been suggested to play an important role as ROS scavengers.[102,103] ROS have also been shown to play a role of signalling molecules. Thus, as a result of cross-talk, they may induce different defence responses within plants, e.g. in response to pathogens or to multiple stresses.[101,104]

The content of ascorbic acid (AsA) was not affected in carrots[47] and lettuce[11] washed with ozonated water at 4 ppm for 2 min and in lettuce and spinach washed with ozonated water at 12 ppm for 15 min.[23] AsA content was also not affected in whole tomatoes cyclically exposed to gaseous ozone at 4 ppm for 30 min every 3 h or continuously exposed to ozone at 1 ppm for 6 days[14,56] but increased in tomato slices.[14] On the other hand, ozone treatment decreased vitamin C content in banana[1] and pineapple.[1]

Increase in AsA content was observed in kiwi fruit stored at 0 °C for up to 3 months in the presence of ozone at 300 ppb.[76] Higher AsA content was also found in strawberries treated with low dose of ozone (300-350 ppb) either in gaseous[66] or aqueous[25] form and in papaya fruit exposed to gaseous ozone at 1.5-5 ppm for 4 days.[77] The increase in vitamin C was observed in celery washed with ozonated water at 30-180 ppb for 5 min.[20] On the other hand, AsA content was reduced when strawberries were exposed to gaseous ozone at 5,000 mg L^{-1} for 6 days[35] and carrots exposed to ozone at 10 ppm for 10 min[19] but not affected in carrots washed with ozonated water at 4 ppm for 2 min.[47] These findings clearly suggest that changes in AsA content in response to ozone are dose dependent. Changes in AsA content are not surprising as AsA is a key antioxidant in plant tissue[101,105] and its role is to scavenge ROS that are produced in excess under stress conditions, e.g. high dose of ozone.

Ozone exposure at 2 or 4 ppm for 2 min had no effect on β-carotene content in lettuce[11,38] and β-carotene and lycopene content in tomatoes continuously exposed to ozone at 1 ppm for 6 days[56] but reduced carotenoid content in carrots washed with ozonated water at 10 ppm for 10 min.[19]

Total phenolic content was found to increase in ozone treated banana,[1] grapes,[61,63] kiwi[76] and pineapple.[1] Increase in total phenolics was also observed in response to ozone at 10 ppm for 10 min in tomatoes,[79] while ozone at 1 ppm for 6 days had no effect.[56] The content of total phenolics was found to be higher in papaya exposed to gaseous ozone (1.5-5 ppm for 96 h prior to ambient storage) when compared with control fruit stored in clean air.[77] Increase in phenolics was also reported[28] when papaya was treated with gaseous ozone at 9.2 ppm for 10 or 20 min prior to storage, but not when the exposure time (30 min) was too long. This may be explained by antioxidant capacity of phenolic compounds, i.e. if the dose of ozone is too high it could result in excess oxidative stress and production of ROS which then need to be scavenged by antioxidants, e.g. phenolic compounds. Total phenolic content also declined in strawberries exposed to gaseous ozone at 5,000 mg L^{-1} for 6 days,[35] these findings suggest that similarly to changes in AsA, the content of phenolic compounds can be affected in response to ozone in a dose dependent manner. Increase in phenolic compounds in response to ozone exposure may be associated with increased activity of phenylalanine ammonia lyase (PAL) or reduced activity of polyphenol oxidase (PPO) and/or peroxidase (POD), which are all involved in polyphenol biochemistry,[106] e.g. reduced activity of PPO and POD has been reported in carrots washed with ozonated water at 10 ppm for 10 min.[19]

Antioxidant activity measured as 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity and ferric reducing/antioxidant power (FRAP) was found to increase in ozone-exposed banana,[1] kiwi,[76] papaya[28,77] and pineapple.[1] The increase in antioxidant activity was associated with changes in phenolic compounds. On the other hand, Tzortzakis *et al*.[56] did not observe changes in antioxidant activity in tomatoes exposed to 1 ppm ozone for 6 days, which is not surprising as content of AsA, β-carotene, lycopene and phenolic compounds was not affected. No difference in antioxidant activity was also observed in fresh-cut lettuce and spinach washed with ozonated water at 12 ppm for 15 min,[23] where the contents of AsA and total phenolics were not affected by the treatment.

INTERACTION WITH ETHYLENE

In addition to its effect on the produce, it has been observed that ozone can be used in storage rooms to reduce the level of ethylene[67,84] in the air, delaying the ripening and senescence process and in this way extending the shelf-life of fruits and vegetables. The effect of ethylene on fresh produce quality has been reviewed elsewhere.[107]

CONCLUSIONS

The use of ozone seems to be a simple and feasible solution for the fresh produce industry. Ozone reduces microbial contamination of the produce and has also been shown to be efficient in removing pesticide residues. Care must be taken because some concentrations of ozone used to reduce microbial contamination and pesticide residues on the fresh produce were higher than those used for produce quality preservation. When used at the proper dose, not too high to cause the damage, ozone treatment may be beneficial to the produce by reducing weight loss, improving texture maintenance and visual quality or enhancing its nutritional content. The purpose of this review was to give a clear overview of the findings reported so far on the use of ozone as a postharvest technology to extend the shelf-life and maintain the quality of fresh produce. It is apparent from this work that in number of studies ozone had no adverse effect on the produce quality, while some commodities can clearly benefit from ozone exposure. Thus, further research is necessary to determine the optimal dose of ozone for each commodity of commercial importance. This knowledge would clearly benefit the industry and could be incorporated within the supply chain to extend the shelf-life and/or improve the quality of the produce.

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