Effects of Ozone Treatment on Postharvest Strawberry Quality

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The effect of ozone treatment on the postharvest quality of strawberry was evaluated. Strawberry fruits (*Fragaria* × *ananassa* Duch. cv. Camarosa) were stored at 2 °C in an atmosphere containing ozone (0.35 ppm). After 3 days at 2 °C, fruits were moved to 20 °C to mimic retail conditions (shelf life). The changes in several quality parameters such as fungal decay, color, sugar and acids distribution, and aroma were evaluated during the strawberries' shelf life. Ozone treatment was ineffective in preventing fungal decay in strawberries after 4 days at 20 °C. Significant differences in sugars and ascorbic acid content were found in ozone-treated strawberries. At the end of cold storage, the vitamin C content of ozonated strawberries was 3 times that of control fruits. A detrimental effect of ozone treatment on strawberry aroma was observed, with a 40% reduced emission of volatile esters in ozonated fruits.

Keywords: *Ozone; strawberry; fruit quality; vitamin C; off-flavor; aroma*

INTRODUCTION

Ozone, the triatomic form of oxygen (O_3), is an unstable compound that decomposes either spontaneously, producing hydroxyl radicals and other free radical species, or in contact with oxidizable surfaces. Due to its high oxidation potential, ozone can oxidize contaminants in air and water. In this sense, ozone has been used for decades as a safe disinfectant agent in water treatment plants in Europe. In most countries, ozone has also been used in different applications in the food industry, and more recently an expert panel has recomended a GRAS (generally recognized as safe) classification of ozone as disinfectant or sanitizer for foods in the United States (Graham, 1997). Ozone, applied as a gas or as ozonated water, has been tested for postharvest treatment of fruits and vegetables. Besides other ozone storage uses, such as odor elimination, the main focus of these studies was ozone's capacity for controlling spoilage caused by microbial and fungal pathogens (Rice et al., 1983). Contradictory data have been reported on the efficacy of ozone as a bactericidal and fungicidal agent. Ogawa et al. (1990) reported the inactivation of *Botrytis cinerea* spores after ozone treatment of tomato fruits, whereas Liew and Prange (1994) concluded that the effect of ozone on *B. cinerea* was fungistatic but not fungicidal in treated carrots. Discrepancies on the positive or negative disinfectant effects of ozone in various commodities can be found in the literature (Norton et al., 1968; Brooks and Csallany, 1978; Spotts and Cervantes, 1992; Barth et al., 1995; Sarig et al., 1996). Assessment of quality attributes, other than fungal growth, was often not included in previous studies, and the effects of ozone exposure on fruit and vegetable quality merited further investigation.

Strawberry is a main produce export of Spain, cultivated mainly in Huelva province (southwestern Spain). This fruit is one of the most delicate and perishable of fruits, being susceptible to mechanical injury, physiological deterioration, water loss, and decay caused by fungi, mainly gray mold rot caused by *B. cinerea*. Thus, effective postharvest procedures are required to prevent deterioration during strawberry packing and transport to European markets. Installation of ozone generators in strawberry cooling rooms has increased over the past few years, with little objective information on their effect on strawberry quality. In addition, contradictory results previously reported on ozone effects suggest that the efficacy of ozone must be individually assessed for each commodity, taking into account its recommended postharvest handling and storage practices. The main objective of this study was to evaluate the effects of ozone treatment on the quality of strawberry fruits under simulated postharvest practices currently used by strawberry producers in southwestern Spain. Changes in several quality parameters such as anthocyanin content, sugar and organic acid distribution, volatile composition, off-flavor formation, and enzymatic activities were assessed during the postharvest life of strawberries stored in an atmosphere containing ozone.

EXPERIMENTAL PROCEDURES

Materials. Camarosa strawberries (*Fragaria* × *ananassa* Duch.) were grown in San Bartolomé de la Torre (Huelva). Fruit was harvested, selecting for uniformity of size and color development, packed in polypropylene berry baskets (500 g), and placed in two cold rooms controlled at 2 °C, 90% relative humidity, and ozone concentrations of 0 (control) and 0.35 ppm, respectively. Ozone was produced using an ozone generator (model ODM 100, Ozodiex). Ozone concentration was measured using a portable ozone detector (model MODP, Murco). After 3 days in the cold room, simulating transport at 2 °C, fruits were moved to a 20 °C room for 4 days to mimic retail conditions (shelf life).

Estimation of Fungal Decay. Fungal infection was visually estimated during the course of the experiment. Strawberry fruits showing surface mycelial development were considered decayed. Fungal decay was expressed as percentage of decayed fruits.

Total Anthocyanin Content. Strawberry tissue was ground with methanol, 0.1% HCl (10:1 v/w), using an Omni-

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mixer (Sorvall) and centrifuged at 2000*g* for 15 min at 4 °C. Anthocyanin concentration was determined as pelargonidin 3-glucoside in a Beckman DU 640 spectrophotometer at 510 nm, using a molar absorptivity coefficient of 36000.

Sugar and Acid Analysis. *Preparation and Fractionation of Fruit Extracts for HPLC Analysis.* Sixteen pieces from 16 different fruits were blended in the dark with 95% ethanol for 3 min with an Omni-mixer (Sorvall). The homogenate was vacuum-filtered through Whatman No. 1 filter paper and the residue washed twice with 80% ethanol. The filtrates were combined and adjusted to 5 mL/g of fresh weight (FW); 10 mL of this extract was evaporated in the dark to dryness at 50 °C. The dry residue was redissolved in 1 mL of 0.2 N H_2SO_4 and 0.05% EDTA, loaded onto a Sep-Pak C18 cartridge (Lida, Kenosha, WI), and eluted with up to 4 mL of the same solution. These extracts containing sugars and organic acids were filtered through 0.45 μm nylon filters before HPLC analysis.

HPLC Analysis. Sugars and organic acids were analyzed in a Hewlett-Packard 1090 liquid chromatograph equipped with a photodiode array detector and a Waters 410 differential refractometer (Millipore) connected in series. Isocratic separations of the compounds were made on a stainless steel Ion-300 (300 mm × 7.8 mm, 10 μm) column, according to the method developed by Pérez et al. (1997).

Off-Flavor Determination. Acetaldehyde, ethanol, and ethyl acetate contents, indicators of off-flavor formation in fruits, were determined in strawberry purees. Sixteen portions of 16 different strawberries were blended, and 3 mL aliquots of the obtained puree were placed in 11 mL headspace vials. The vial was transferred to an automatic headspace sampler (Hewlet-Packard 19395A), where a 15 min equilibrium time was set at 60 °C to allow the volatiles to enter the gas phase. Volatiles were analyzed by GLC in a gas chromatograph (Hewlett-Packard 5890A) equipped with an FID and a glass column (2 mm × 1.0 m) containing 5% Carbowax on 60/80 Carbopack as stationary phase. Oven temperature was held isothermally at 70 °C. Four vials per sample were analyzed.

Analysis of Volatile Compounds. *Headspace Sampling.* Strawberries (150 g) were placed in a desiccator housed within a thermostated water bath (25 °C). Methyl octanoate was added to the sample as internal standard. The vessel was continuously flushed with purified air (99.9% pure) at a flow rate of 337.5 mL/min. For sampling, a Tenax TA trap (150 mg) was attached to the outlet of the desiccator. Sampling time was 30 min.

Desorption and GLC/MS Analysis. The desorption of volatiles trapped in the Tenax was carried out by using a Chrompack thermal desorption cold trap injector (TCT). Identification of compounds in the headspace was made by means of GLC/MS. Volatiles were analyzed using a GLC (HP-5890) equipped with a fused silica capillary column DB-Wax (60 m × 0.25 mm i.d.); carrier gas was helium at flow rate of 30 cm/s. The column was held for 15 min at 40 °C and then programmed at 2 °C/min to 160 °C. An MS-30/70-VG mass spectrometer was directly coupled to the gas chromatograph described above. Identification of volatile compounds was made by matching against the Wiley/NBS library and by GLC retention time against standards. Relative quantitation was performed by normalization of the values obtained from the integrator to that of methyl octanoate. Peak areas were converted to nanograms per gram of strawberry fruit per 10 L of headspace.

Preparation of Enzymatic Extracts. Three enzymatic activities were determined: lipoxygenase (LOX), hydroperoxide lyase (HPL), and alcohol acyltransferase (AAT). Due to their different subcellular distributions, two different extraction buffers were used: 0.1 M Tris-HCl, pH 8/1 M KCl buffer was used for LOX extraction, and the same buffer with Triton X-100 (0.1%) was used to extract HPL and AAT activities. Strawberry tissue (25 g) was blended in a Waring blender with 4 g of PVPP and 33 mL of the appropriate extraction buffer. The resulting homogenate was vacuum-filtered through Whatman No. 1 filter paper and the residue washed two times with 8 mL of the appropriate buffer. The extract was centrifuged at 35000*g* for 20 min, and the supernatant was used as crude extract.

LOX Assay. LOX activities were determined by continuously monitoring the formation of conjugated diene at 234 nm (Axelrod et al., 1981). The standard assay mixture consisted of 1.25 mL of 0.1 M sodium phosphate buffer, pH 6, 25 μL of substrate solution (10 mM linolenic acid), and 25 μL of enzyme solution. One unit of LOX activity is defined as the amount of enzyme catalyzing the formation of 1 μmol of product/min.

HPL Assay. The reaction mixture contained 1 mL of 200 mM sodium phosphate buffer, pH 6.0, 50 μM 13-hydroperoxylinolenic acid (13-LNAOOH), and the appropriate amount of enzyme (10−50 μL). Changes in absorbance at 234 nm were recorded for 60 s, and one unit of HPL activity was expressed as the amount of enzyme consuming 1 μmol of 13-LNAOOH/min; 25000 M^{-1} cm^{-1} was used as the extinction coefficient for 13-LNAOOH.

AAT Assay. The standard assay mixture consisted of 0.85 mL of 0.5 M Tris-HCl, pH 8.0, buffer containing 11.6 mM $MgCl_2$, 0.3 mM acetyl-CoA, 10 mM butanol, and 0.15 mL of the enzyme solution. The mixture was incubated at 35 °C for 15 min, and then 50 μL of 20 mM 5,5′-dithiobis(nitrobenzoic acid) (DTNB) was added and allowed to stand at room temperature for 10 min. The increase in absorbance at 412 nm over time, due to a yellow thiophenol product formed by reaction of DTNB with the free CoA-SH liberated during the catalytic reaction, was meassured by means of a spectrophotometer (Pérez et al., 1996).

Analysis of Results. The data were statistically evaluated using CoStat statistical software (CoHort Software, 1995; CoStat, Minneapolis, MN). Analysis of variance (ANOVA) was used, and comparison of means was done by the Student−Newman−Keuls/Duncan test, at a significance level of 0.05.

RESULTS AND DISCUSSION

Estimation of Fungal Decay. Ozone treatment (0.35 ppm) for 3 days at 2 °C was partially effective in preventing fungal growth after 2 days at 20 °C (day 5), with 15% less fungal decay in treated fruits. Nevertheless, after 4 days at 20 °C (day 7), a higher incidence of *B. cinerea* rot was observed in ozone-treated strawberries, with similar rates of gray mold proliferation in ozonated and nonozonated fruits. Probably, a higher ozone concentration could have caused a greater reduction of fungal growth. Nevertheless, the ozone concentration used in this study, 0.35 ppm, was selected as a compromise between treatment efficacy and safety of the process, taking into account OSHA limits of exposure to ozone (0.3 ppm for 15 min) and results obtained with similar ozone levels in other berries (Barth et al., 1995). A prestorage exposure to ozone had proved to be ineffective in preventing decay of other fruits (Spott and Cervantes, 1992), and the need of prolonged ozone exposure to achieve effective protection against *B. cinerea* could be deduced from data reported by Barth et al. (1995).

Anthocyanin Content. The initial value of anthocyanin content determined in Camarosa strawberries was 959.34 ± 11.12 nmol/g of FW. After 3 days at 2 °C, a decrease in the anthocyanin content of treated and nontreated samples was observed, with a significantly ($p < 0.05$) lower value in ozonated fruits (639. 08 ± 11.01 nmol/g of FW) than in nontreated fruits (811.34 ± 6.81 nmol/g of FW). When strawberries were placed at 20 °C, a slight increase in anthocyanin accumulation was determined in both treated and nontreated fruits. No significant differences were found after 4 days at 20 °C. Similar results were found in blackberry fruits stored over 12 days at 2 °C in a 0.3 ppm ozone atmosphere

Table 1. Effect of Ozone Treatment on Sugar and Organic Acid Contents (Milligrams per Gram of FW) during Postharvest Life of Strawberries

days of storage	treatment	sucrose	glucose	fructose	malic acid	citric acid	ascorbic acid
0		19.29 ± 0.3[a]	15.55 ± 0.33	15.83 ± 0.27	1.16 ± 0.02	6.77 ± 0.11	0.07 ± 0.010
3	control	16.78 ± 0.12	17.34 ± 0.12	17.98 ± 0.20	1.43 ± 0.05	7.73 ± 0.11	0.07 ± 0.010
	ozone	14.06 ± 0.32	13.76 ± 0.32	15.08 ± 0.18	1.28 ± 0.14	6.83 ± 0.21	0.22 ± 0.004
5	control	8.33 ± 0.37	18.38 ± 1.31	18.12 ± 0.26	1.25 ± 0.08	6.84 ± 0.13	0.09 ± 0.002
	ozone	9.01 ± 0.24	17.38 ± 0.57	18.49 ± 0.37	0.90 ± 0.08	7.12 ± 0.49	0.12 ± 0.002
7	control	4.00 ± 0.12	14.61 ± 0.42	16.63 ± 0.49	0.75 ± 0.05	7.11 ± 0.17	0.18 ± 0.006
	ozone	7.99 ± 1.03	14.29 ± 0.15	14.04 ± 0.17	1.11 ± 0.03	8.43 ± 0.15	0.13 ± 0.010

[a] Values represent the mean and standard deviation of three analyses.

(Barth et al., 1995); a sharp decrease in anthocyanin levels of ozonated blackberries was determined after 4 days of storage.

Sugar and Organic Acid Content. An equilibrated balance among main sugars and organic acids determines strawberry flavor. Ascorbic acid, better known as vitamin C, is also present in strawberry fruits, giving an added value to this fruit due to its important nutritional implications. Changes in sugars and organic acids, including vitamin C, from strawberries are shown in Table 1. Sucrose content decreased with storage time from an initial value of 19.9 mg/g of FW at day 0 to <45% at the end of shelf life (day 7) in both treated and nontreated fruits. Concomitant with the decrease in sucrose content, an increase in glucose and fructose levels was observed from day 0 to day 5. Nevertheless, the pattern of this conversion of sucrose into glucose and fructose is significantly different in treated and nontreated strawberries. Thus, the lower content of sucrose in ozonated fruits on day 3 was not correlated with a higher accumulation of glucose and fructose, the contents of which were also significantly lower in ozone-treated fruits. As sugar contents at day 3 were measured immediately following ozone treatment, the low sucrose, glucose, and fructose contents could be due to an activation of other sucrose degradation pathways in response to oxidative stress caused by ozone. In this sense, data on vitamin C content could provide important information. On day 3 vitamin C content of ozonated strawberries was 3-fold that of control fruits (Table 1). Vitamin C content was also significantly ($p < 0.05$) higher in ozonated fruits on day 5. Due to its high oxidative capacity and its ability to generate toxic molecular species, ozone acts as a potent phytotoxic agent that elicits plant defense reactions (Sanderman et al., 1998). In this sense, ozone and ozone-derived oxyradicals may be scavenged by low molecular weight antioxidants of the plant cell such as ascorbic acid or polyamines (Schrauchner et al., 1992). The increase of ascorbic acid levels in leaves in response to ozone exposure has been previously reported by several authors (Luwe et al., 1993; Ranieri et al., 1996). It can be assumed that elevated levels of vitamin C in regions of high metabolic activity other than the chloroloplast may perform a similar function. Thus, changes in sugars and vitamin C contents in ozone-treated strawberries could be the result of an antioxidative system that promotes the biosynthesis of vitamin C from carbohydrate reserves of the fruit. No significant differences ($p < 0.05$) were found in malic and citric acid contents in both treatments.

Aroma Quality. To evaluate the effect of ozone treatment on strawberry aroma, three different aspects have been studied: off-flavor formation, headspace volatile composition, and some enzymatic activities related to aroma biosynthesis (LOX, HPL, and AAT). As an index of off-flavor formation, concentrations of acetaldehyde, ethanol, and ethyl acetate were analyzed during simulated transport and shelf life of ozone-treated and nontreated fruits (Table 2). No significant ($p < 0.05$) differences in ethyl acetate content could be attributed to ozone treatment of strawberries, although slightly lower levels of this compound were found in ozone-treated strawberries. No significant differences ($p < 0.05$) were found in the acetaldehyde content of treated compared to control fruits. The levels of these two compounds, ethyl acetate and acetaldehyde, determined in all analyzed samples might be regarded as acceptable (weak off-flavor) according to the concentration limits predicted by Ke et al. (1991). Ethanol was the most abundant off-flavor (alcoholic flavor) related compound found in this study. The amounts of ethanol determined for ozone-treated strawberries were significantly lower ($p < 0.05$) than the levels found in control fruits (50% on days 5 and 7). The final ethanol concentration values determined for control and treated fruits (203.04 ± 6.99 and 105.51 ± 6.62 μL/kg) were clearly above the tolerance limits determined by Ke et al. (1991) and could be correlated with strong and medium off-flavors, respectively. Data presented in Table 2 indicate a possible effect of ozone treatment on preventing off-flavor formation in strawberry.

Table 2. Effect of Ozone Treatment on Off-Flavor Formation during Postharvest Life of Strawberries

days of storage	treatment	acetaldehyde (μL/kg of FW)	ethanol (μL/ kg of FW)	ethyl acetate (μL/kg of FW)
0		23.95 ± 0.26[a]	108.82 ± 5.25	9.24 ± 0.57
3	control	6.25 ± 0.04	92.72 ± 7.65	2.15 ± 0.11
	ozone	5.40 ± 0.04	82.96 ± 3.82	3.02 ± 0.61
5	control	9.99 ± 0.23	37.74 ± 1.52	1.93 ± 0.18
	ozone	9.19 ± 0.17	14.49 ± 2.86	2.14 ± 0.49
7	control	17.61 ± 0.73	203.04 ± 6.99	12.20 ± 1.43
	ozone	17.63 ± 0.67	105.51 ± 6.62	7.08 ± 1.75

[a] Values represent mean and standard deviation of four analyses.

Reduced emission of volatile compounds has been reported as the factor most likely responsible for diminished flavor of fruits (Fallik et al., 1997). One of the main objectives of this study was to determine whether ozone treatment affects volatile composition of strawberry fruits. Table 3 shows the main volatile compounds identified in treated and control fruits. Methyl and ethyl esters could be considered the most significant components of strawberry aroma, and the ratio methyl/ethyl esters is characteristic of each strawberry cultivar (Pérez et al., 1992). In Camarosa strawberries this ratio is 2:1, with methyl butanoate as the most abundant volatile compound determined in the headspace of this strawberry cultivar (Table 3). A considerable decrease after 3 days of simulated transport at 2 °C was observed

Table 3. Effect of Ozone Treatment on Volatile Production (Nanograms per Gram of FW per 10 L) during Postharvest Life of Strawberry

	storage duration and treatment				
volatile compound[a]	0 days	3 days, control	3 days, ozone	5 days, control	5 days, ozone
methyl acetate	3.35[b]	1.11	1.16	4.83	0.21
ethyl acetate	11.02	9.80	16.54	44.36	13.74
2-methylethyl acetate	5.11	2.42	1.39	6.06	0.87
methyl propanoate	2.09	0.60	0.50	6.42	0.55
ethyl propanoate	0.002	0.006	0.08		
methyl butanoate	122.41	71.81	46.56	139.36	105.18
methyl 2-methyl-butanoate	5.50	0.58	0.28	6.07	1.35
methyl 3-methyl-butanoate	5.10	0.67	0.42	6.17	1.59
ethyl butanoate	59.14	44.86	18.80	143.44	106.28
2-methylethyl butanote	12.01	1.87	2.21	28.64	11.43
dimethyl disulfide	0.62	0.41	0.04	0.31	
methyl esters	138.45	74.77	48.93	162.85	108.88
ethyl esters	70.14	54.60	35.42	188.76	120.02
total	225.70	133.99	87.94	386.25	240.30

[a] Volatile compounds appear in order of their retention time. [b] Values represent the average of two determinations.

in total volatile compounds, declining from 225.70 ng/g of fW at day 0 to 133.99 and 87.94 ng/g of FW on day 3 for control and treated strawberries, respectively. A temporary suppression of volatile emission has been associated with postharvest conditions that delay ripening (Fallik et al., 1997). The percentage of total volatile production by ozone-treated fruits was 65% compared to nontreated fruits. Volatile emission increased after 2 days at 20 °C in both treated and control fruits, reaching a total volatile amount similar to that of day 0 in the case of ozonated strawberries (240.30 ng/g of FW) and an even higher value in control fruits (386.25 ng/g of FW). Despite this recovery in total amount of volatile compounds, the aroma of day 5 fruits showed an altered distribution of volatile esters caused by an increase in ethyl esters, with a methyl/ethyl esters ratio of 0.9 in both treated and nontreated samples. On day 5, the volatile content of ozonated strawberries was 62% compared to control fruits. Data presented in Table 3 suggest that ozone treatment could have a nonreversible effect on the fruit's ability to produce volatile compounds. To further investigate this detrimental effect of ozone on strawberry aroma, we studied the effect of ozone treatment on different biochemical activities. In previous studies, we have reported the contribution of several enzymes such as LOX, HPL, and AAT to strawberry aroma biogenesis (Pérez et al., 1993, 1996; Sanz et al., 1997). These three enzymatic activities were determined in crude extracts of control and ozone-treated fruits (Table 4). Many of the alcohols, aldehydes, and acids found in fruit aroma are generated from the oxidative degradation of linoleic and linolenic acids catalyzed by LOX and HPL (Sanz et al., 1997). The LOX and HPL activities of treated and nontreated strawberries were examined as an index of aroma formation in the fruit. No significant differences in LOX activity levels could be attributed to ozone treatment. Both control and treated fruits exhibited an initial decrease of LOX activity during simulated transport at 2 °C and a further increase when fruits were kept at 20 °C. Contradictory data on the effect of ozone on LOX pathway have been reported in the literature, with an increase of LOX activity caused by ozone treatment of

Table 4. Effect of Ozone Treatment on Flavor-Related Enzymatic Activities, LOX, HPL, and AAT (Milliunits per Gram of FW), during Postharvest Life of Strawberries

days of storage	treatment	LOX	HPL	AAT
0		400.12 ± 82.31[a]	303.21 ± 10.34	23.2 ± 2.81
3	control	270.10 ± 42.18	181.70 ± 3.37	29.11 ± 2.51
	ozone	321.51 ± 32.86	280.40 ± 6.57	12.32 ± 1.21
5	control	1010.98 ± 54.18	101.88 ± 9.76	16.60 ± 1.30
	ozone	1086.01 ± 63.22	120.82 ± 8.43	37.30 ± 2.80

[a] Values represent the mean and standard deviation of four analyses.

lentils but no effect in other commodities (Sandermann et al., 1998). The observed inhibitory effect of ozone on strawberry aroma could not be explained as a result of HPL inhibition. HPL levels determined in both samples showed an increase in ozone-treated strawberries at day 3, but no significant differences were found on day 5. Volatile esters, formed by esterification of alcohols and carboxylic acids, constitute the main group of volatiles in strawberry aroma (Pérez et al., 1992). AAT is the main enzyme implicated in ester formation in fruits, and a good correlation has been found between AAT activity and aroma quality in strawberry (Pérez et al., 1996). The effect of ozone treatment on this enzyme was evaluated as a parameter of strawberry aroma quality. Althouh a significantly ($p < 0.05$) lower AAT activity value was determined on day 3 for ozone-treated strawberries, contradictory results were obtained with day 5 fruits (Table 4). Because no clear differences were found in any of the aroma biosynthesis related enzymes under study, a physical alteration of the fruit surface could be considered as an alternative explanation of reduced volatile emission of ozonated fruits. In this regard, changes in the lipid composition of cranberry cuticle were first described by Norton et al. (1968). More recently, differences in wax deposition and cuticle thickness caused by ozone treatment have been used to explain differences in the ripening pattern of plum fruits (Crisosto et al., 1993).

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