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Effect of ozone treatment on postharvest disease and quality of different citrus varieties at laboratory and at industrial facility

Juan Francisco García-Martín[a], Manuel Olmo[b], José María García[b,*]

[a] Departamento de Ingeniería Química, Facultad de Química, Universidad de Sevilla, C/Profesor García González, s/n. 41012 Sevilla, Spain
[b] Instituto de la Grasa (CSIC), Departamento de Fisiología y Tecnología de Productos Vegetales, Carretera Sevilla – Utrera Km 1, Campus de la Universidad Pablo de Olavide, Edificio 46, 41013 Seville, Spain

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ABSTRACT

The effects of continuous and intermittent (simulating a day-night cycle) exposure to ozone enriched atmosphere (from 1.6 to 60 mg kg^{-1}) at 5 °C for 28 d and subsequent shelf life at 20 °C for 15 d on six citrus varieties (two mandarins: Fortune and Ortanique; and four oranges: Navelate, Lanelate, Salustiana and Valencia) were investigated. *In vitro* and *in vivo* growth of *Penicillium digitatum* and *Penicillium italicum* was first assessed. Based on the results obtained, continuous 60 mg kg^{-1} ozone and intermittent 1.6 mg kg^{-1} ozone were selected for industrial trials, and decay and oleocellosis incidence, colour, firmness, weight losses and juice (content, soluble solids, pH, titratable acidity and vitamin C) were analysed. Results showed that the application of ozone was not detrimental to fruit quality. Furthermore, the application of both continuous and intermittent ozone delayed decay and oleocellosis incidence and slowed down the development of the colouring process, while reducing firmness and weight losses. For industrial applications, the advantage of using ozone 12 h d^{-1}, simulating a day-night cycle, is that workers would not be exposed to ozone inside the cold storage room during the day shift.

1. Introduction

Citrus industry, including harvesting, handling, transport, marketing and delivery, provides nowadays millions of jobs in more than 137 countries around the world. Citrus ranks first among fruits in the world, due to their huge annual production (90–110 million tons) (Karaca, 2010). The importance of citrus arises from their nutritional and antioxidant properties. However, this nutrient composition along with their higher water content makes them susceptible to infection by microbial pathogens, mainly fungi because citrus fruits are quite acidic, from harvesting to consumption (Talibi et al., 2014). Economic losses caused by fungi are especially damaging for citrus fruit and may even reach 5 to 10% of the total production, thus limiting the overall profitability of the citrus industry. A number of postharvest diseases are responsible for citrus losses during storage and shelf life. Some of them are the result of preharvest infections while others are produced during postharvest, mainly due to injuries. *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold) are the major pathogens of citrus fruits. The source of both fungal infections is practically continuous during the season, and can infect citrus in the grove, the packinghouse and during distribution and marketing (Palou et al., 2008). Traditionally, these losses have been efficiently controlled by the application of fungicides, including benzimidazole (thiabendazole, benomyl and carbendazim) and sterol inhibitors (imazalil, prochloraz and propiconazole) sodium orthophenyl phenate and different mixtures of these compounds (Eckert and Brown, 1986; Eckert, 1990; Palou et al., 2008; Talibi et al., 2014). The postharvest use of these fungicides is subject to registration and permission in various countries (Talibi et al., 2014). However, consumers are becoming increasingly aware of the fact that many of these chemical treatments represent a potential risk for human health and environment. The application of these substances during the postharvest manipulation of the fruit can lead to the presence of certain residual quantities of these chemical products or their metabolites, potentially harmful for human health, in the treated fruits. The residuals of the fungicides used before or after harvest may also contaminate the environment. Furthermore, the chemicals used become inefficient, due to the selection of resistant pathogenic stocks. A vicious circle begins: Once resistance has been developed in the pathogens, new, more effective chemicals have to be produced to maintain the same level on the control of produce decay, which leads to the increase of the risk of possible toxicity for environment and human health, until once again resistant pathogens occur, and so on. For these reasons there has been an increasing interest in the development of effective, non-harmful physical procedures for decay control of horticultural products,

* Corresponding author.
E-mail address: jmgarcia@cica.es (J.M. García).

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including physical treatments (curing, hot water and irradiation treatments), chemical treatments (sodium bicarbonate, calcium polysulfide and ammonium molybdate treatments, borax baths, and addition of natural compounds such as volatiles and essential oils, plant extracts, peptides, proteins, chitosan and chitosan derivatives), biological treatments (utilisation of microbial antagonists, application of naturally derived bioactive compounds, and induction of natural resistance) and combinations of the above-mentioned treatments for integrated disease management (García et al., 2016).

Ozone, the three atomic form of oxygen, is a gas with a strong oxidant capacity that was granted GRAS (Generally Recognized As Safe) status in 1997 by the U.S. Food and Drug Administration and approved for use as disinfectant or sanitizer in food processing (Karaca, 2010). Its main advantage is that ozone does not present safety concerns about consumption of chemical residues in the treated produce so it is accepted by many organic grower organizations (Horvitz and Cantalejo, 2014). Ozone may therefore constitute a non-contaminant alternative for reducing the use of fungicides during fruit storage. Although high concentrations show toxic effects and predispose the vegetables to *Botrytis* infection (Wukasch and Hofstra, 1977), ozone used at a suitable level in the storage atmosphere may provide disease protection with a minimum of physiological damage (Liew and Prange, 1994). Such atmospheres delay mycelial growths of *Sclerotinia sclerotiorum*, *Botrytis cinerea* or *Rhizopus stolonifer* (Liew and Prange, 1994; Sarig et al., 1996; Tzortzakis et al., 2007). Furthermore, ozone may induce resistance of plants to pathogens (Kangasjarvi et al., 1994). With the aim of reducing decay incidence in citrus and other fruits, ozone in aqueous and gaseous environments has been assayed. For the former, the low solubility of ozone in water may be a hindrance for aqueous ozone applications. Thus, Smilanick et al. (2002) reported that green mold and sour rot on citrus fruit, caused by *P. digitatum* and *Geotrichum citri-aurantii*, respectively, were not reduced by 20 min immersion in 10 mg kg^{-1} ozone. On the contrary, gaseous ozone used at proper concentrations in the storage atmosphere can protect several fruits and vegetables against diseases with minimum physiological damage (Nadas et al., 2003). From the information available in literature, it can be concluded that citrus exposure to gaseous ozone provides better results, as it will be discussed later, and thereby the use of an ozone-enriched atmosphere was selected for this research.

The objective of this work was to evaluate first the effect of continuous exposure to 0.6, 1.6 and 60 mg kg^{-1} gaseous ozone and intermittent exposure to 1.6 mg kg^{-1} gaseous ozone (day-night cycle) on the *in vitro* microbial growth of *P. digitatum* and *P. italicum* on potato dextrose agar Petri dishes and on the *in vivo* microbial growth of *P. italicum* on artificially inoculated citrus stored at low temperature. A 10^6 conidia mL^{-1} suspension was used for both *in vitro* and *in vitro* experiments. This concentration of pathogen was chosen because it has been verified that this amount is enough to provoke complete decay of citrus fruit (Eckert and Brown, 1986) and it has been already successfully tested in previous works (Nunes et al., 2007; García et al., 2016). Once the most suitable conditions for ozone treatment are found, the next step is to assess the effects of exposing citrus fruits to ozone during storage at 5 °C (up to 28 d) and subsequent shelf life at 20 °C (up to 15 d) at industrial scale on different fruit quality parameters. The main target of this research is to check the feasibility of the application of ozone at industrial facilities, searching thereby the conditions in which decay incidence is reduced without affecting fruit quality during both cold storage and shelf life of citrus fruit.

2. Materials and methods

2.1. Citrus fruit

Six citrus varieties were used, two mandarins (*Citrus reticulata* cvs, Fortune and Ortanique) and four oranges (*Citrus sinensis* cvs, Navelate, Lanelate, Salustiana and Valencia), which were grown in the commercial orchard "El Zumajo" located in Río Tinto (Huelva, Spain) by the company Río Tinto Fruit S.A. Each variety was harvested in the moment in that the fruit showed ≥90% of skin surface degreening and the Total Soluble Solids:Titratable Acidity ratio in the juice was ≥10:1.

2.2. Pathogen microorganisms

The fungi *Penicillium digitatum* and *Penicillium italicum* were obtained from the Spanish Type Culture Collection and maintained on potato dextrose agar plates. Conidia of a 7–12 d culture grown at 25 °C were suspended in 100 mL sterile distilled water with two drops of Tween 80. The suspension was adjusted to 10^6 conidia mL^{-1}, using haemocytometer for each fungus. For *in vitro* experiments, PDA Petri dishes were inoculated in its geometrical centre using a 1.4 mm diameter steel rod, previously immersed in each conidia suspension. Similarly, for *in vivo* assays, fruits were wounded and inoculated on their flavedo using the same system and conidia concentration, but only with *P. italicum*.

2.3. Ozone treatments at laboratory scale

Ozone was produced by an OMD 100 ozone generator (Ozodiex S.A., Barcelona, Spain) in all the trials.

2.3.1. In vitro assays

In vitro inoculation at laboratory scale was performed to assess the direct effect of ozone treatment on pathogens and so determine the most suitable conditions for *in vivo* inoculation. For this purpose, 4 replicates of 10 PDA Petri dishes each one were carried out for each pathogen and treatment. After inoculating, Petri dishes were let stand for 2 h at room temperature to allow the development of the infection and immediately afterward were exposed to ozone (except for control sample, which was exposed to air) at 5 °C for 28 d. Four ozone atmospheres were assayed: continuous 0.6, 1.6 and 60 mg kg^{-1} ozone-enriched atmospheres, and intermittent exposure (12 h on, 12 h off) to 1.6 mg kg^{-1} ozone-enriched atmosphere, simulating a nocturnal ozone application. In order to monitor the fungal growth, the diameter of the resulting fungal colony placed in the centre of each plate was measured and the results presented as the percentage of the plate covered by the pathogen at each sampling date.

2.3.2. In vivo assays

After harvesting, Valencia oranges were let stand for one night at room conditions (20 ± 2 °C and 80% relative humidity). Afterwards, 4 groups of 20 oranges each (i.e. 4 replicates) were inoculated with *P. italicum*. Two hours after the inoculation citrus were intermittently exposed to 1.6 mg kg^{-1} ozone atmosphere (12 h on, 12 h off) at 5 °C for 28 d. Meanwhile, another 4 groups with the same number of inoculated fruits were stored at 5 °C for 28 d in air without ozone (control samples).

2.4. Ozone treatments at industrial scale

Industrial-scale ozone treatments were carried out using citrus not previously treated with fungicides and without pathogen inoculation. Immediately after harvesting fruit of each variety were distributed in 20 perforated plastic boxes able for 20 kg of fruit and simultaneously treated in the ozone chamber. Control samples of each variety were subjected to the same procedures but using air instead of ozone atmosphere. Three ozone treatments were performed. In the first trial, Salustiana, Lanelate and Navel oranges and Fortune and Ortanique mandarins were exposed to continuous 60 mg kg^{-1} ozone-enriched atmosphere at 5 °C for 28 d. After this period, both ozone-treated and control fruit were located at 20 °C under air, simulating shelf life during 14 d. In the second ozone trial, other two groups of 20 perforated boxes of Lanelate and Valencia oranges were exposed to continuous