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Oxidation stress and antioxidant levels in tangerine pericarp

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Titolo
Stress ossidativo e livelli di antiossidanti nel pericarpo del mandarino tangerino

Key words
Ozone, ozonated water, ultra violet radiation, oxidant

Parole chiave
Ozono, acqua ozonizzata, radiazione ultravioletta, ossidante

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Summary
Tangerine peel is a common traditional Chinese medicine and an herb for Chinese food preparation. In order to study the influence of oxidative stress, ozonated water, gaseous ozone and UV radiation were applied to potted tangerine plants and the antioxidant levels in fruit pericarp were compared. After being watered daily with 500 mL ozonated water (0.84 mg/L dissolved ozone) for two weeks; a 19.5% increase in pericarp antioxidant level was detected. After plant samples were exposed to 6 ppm ozone, 2.88 KJ/m^2 UV or a combination of both for 30, 60 and 120 min daily for the same period; decreases in antioxidant level were found and were generally in proportion to the time course of treatment. The results indicated that the oxidants depleted the antioxidant in the pericarp of mature tangerine. Ozonated water application to soil, however, as a systemic response increased the antioxidant level in the pericarp.

Riassunto
La buccia del mandarino tangerino è un ingrediente comune nella medicina tradizionale cinese e nella preparazione del cibo cinese. Al fine di studiare l'influenza dello stress ossidativo, le piante in vaso sono state sottoposte ad acqua ozonizzata, ozono gassoso e radiazioni UV e sono stati confrontati i livelli di antiossidanti nel pericarpo del frutto. Dopo essere state innaffiate giornalmente con 500 ml di acqua ozonizzata (0,84 mg/L di ozono dissolto) per 2 settimane è stato rilevato un aumento dei livello di antiossidanti nel pericarpo del 19,5%. In seguito campioni di piante sono stati esposti a 6 ppm di ozono, 2,88 KJ/m^2 di UV o una combinazione di entrambi per 30, 60 e 120 minuti al giorno per lo stesso periodo; è stata riscontrata una diminuzione del livello di antiossidanti ed era generalmente in proporzione alla durata di trattamento. I risultati hanno indicato che gli ossidanti impoveriscono gli antiossidanti nel pericarpo del mandarino tangerino maturo. L'applicazione di acqua ozonizzata nel suolo, tuttavia, come una risposta sistemica aumentava il livello di antiossidanti nel pericarpo.

Introduction

Tangerine pericarp is both a food and a traditional Chinese medicine. It is a good source of antioxidant i.e. ascorbic acid, folate and β-carotene. It is considered that fruits and vegetables rich in antioxidants will reduce the risk of chronic diseases, such as cardiovascular diseases, cancer, hypertension and type-2 diabetes (1). Antioxidant quantity and quality in plants can vary significantly according to different intrinsic and extrinsic factors such as plant genetics and cultivar, soil composition and growing conditions, and maturity state (2). Antioxidant's synthesis can also be stimulated under stress conditions, such as temperature, UV exposure and pathogenic attacks (3).

Ozone can be also used as an oxidative stress to induce the production of antioxidants in fruits or vegetables. Ozone fumigation might have other superior functions, for example, Tzortzakis et al. (4) reported that ozone-enrichment maintained tomato fruit firmness and did not affect fruit weight. In sunflower, reduced plasticity of cell wall induced by ozone exposure may oppose an unspecific mechanical resistance against abiotic stress (5). However, different doses and modes of ozonation treatments have different effects. Ozone gas fumigation influences the biological activities of plant directly as it may penetrates into the mesophyll through stomata. Ozonated water irrigation to soil may affect plant growth indirectly. This study attempted to differentiate between the influence of oxidative stress to the aerial plants part and to the rhizosphere.

Methodology

Two-year old citrus plants, 1m tall with 20 kg soil bail in pots, were employed for this two-week study.

Oxidative stress applied to roots

To each of the potted plants, 500 mL of ozonated water (0.84 mg/L dissolved O_3) was applied daily to the plant soil. For the control experiment, pots were irrigated with 500 mL tap water.

Oxidative stress applied to shoots

Three oxidative stresses were applied to the aerial plant parts, including ozone gas fumigation (OG), UV irradiation (UV) and the combination of ozone gas and UV (OUV).

O_3

Ozone fumigation study was conducted inside 1.5 m^3 fumigation chambers with laminar air flow control. Ozone was generated by a PoSeng model 990-B generator. Ozone level in the chambers was monitored by ECOSENSORS model UV-100 ozone monitor and regulated to 6 ppm (±2 ppm) within 5 min after placing the potted plants into the chambers. There were three time courses of treatment: 30, 60 and 120 min employed to study the effects of extended ozonation. Surplus ozone was vented from the chamber by slightly negative pressure and removed by an activated charcoal filter. After daily fumigation in the morning, treated plant pots were returned to the rooftop of the laboratory building with shelter to avoid excessive precipitation, and irrigated daily with 500 ml tap water.

UV

The fumigation chambers were also provisioned with UV light source. Plant samples for UV treatment were placed inside the fumigation chamber exactly as for ozone treatment; however, ozone stress was replaced by UV stress and the intensity was adjusted to 2.88 KJ/m^2 on the leaf surface level, determined by a UNP Digital Radiometer UV monitor model UVX /16890.

O_3 and UV

To study the synergetic effects, if any, three pots of plant samples were subjected to the combination of the ozone and UV treatments.

Control experiment

All tangerine plant samples were divided into two parts in advance, one side was treated with oxidants; the symmetric opposite side was temporarily covered by a heavy duty black plastic bag with the opening carefully tightened. It was proven and recorded that the plastic bag was effective to protect the plant parts inside; neither O_3 nor UV were detected inside the bag.

Total antioxidant content (FRAP) assay

The FRAP test Modify Fe-TPTZ method (6) was used. After each treatment, 1g fresh tangerine pericarp was homogenized using a pestle and mortar in 1 mL distilled water. The extract was then centrifuged at 13,000 rpm for 10 min and the supernatant was diluted 10 times. Each diluted supernatant (50 μL) was mixed with 1.5 mL FRAP reagent. The absorbance was then read after 5 min at 593 nm.

Results

An increase of 19.5% of the total antioxidant activity was found in ozonated water (0.84 mg/L)-treated samples; compared to the control (Fig. 1). The results indicate that oxidant application to the rooted soil was beneficial to the plant and caused an increase in antioxidant level in the fruit pericarp. However, the effect of oxidants applied directly on pre-harvest fruits was different. After ozone fumigation the content of antioxidant in pericarp decreased with the increasing treatment time. The relative total antioxidant concentration compared to control was 87.8%, 76.7% and 51.0% in OG group, respectively (Fig. 2). In OUV group, the relative antioxidant concentration compared to control was 78.1%, 63.6% and 35.2%, respectively. Although UV reduced the content of total antioxidant (Fig. 3) as well, the relative reductions were not exactly inversely proportional to the length of time for the treatment, as the relative total antioxidant concentration compared to control was 76.6%, 68.0% and 86.1% respectively; making it different from the OG and OUV treatments (Fig. 4).

Discussion

Negative effects

These results indicate that excessive exposure to ozone gas and/or UV light decreases the antioxidant content of the samples, as the sam-

Figure 1 - Effect of ozonated water on total antioxidant content of pericarp of tangerine tree

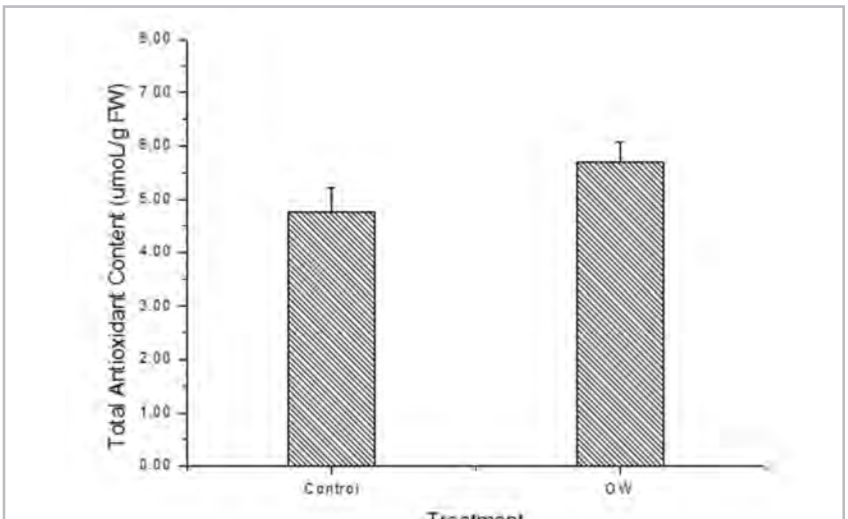


Figure 2 - Effect of ozone gas on total antioxidant content of pericarp of tangerine tree

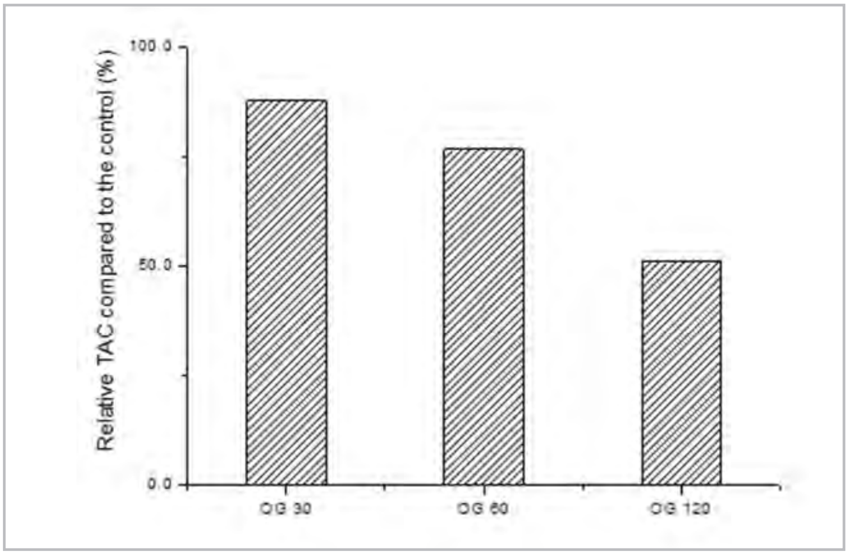
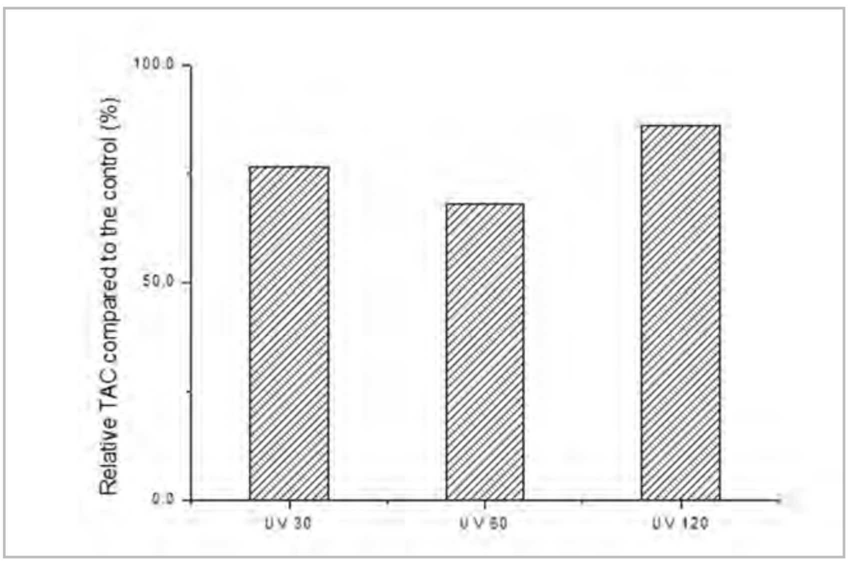


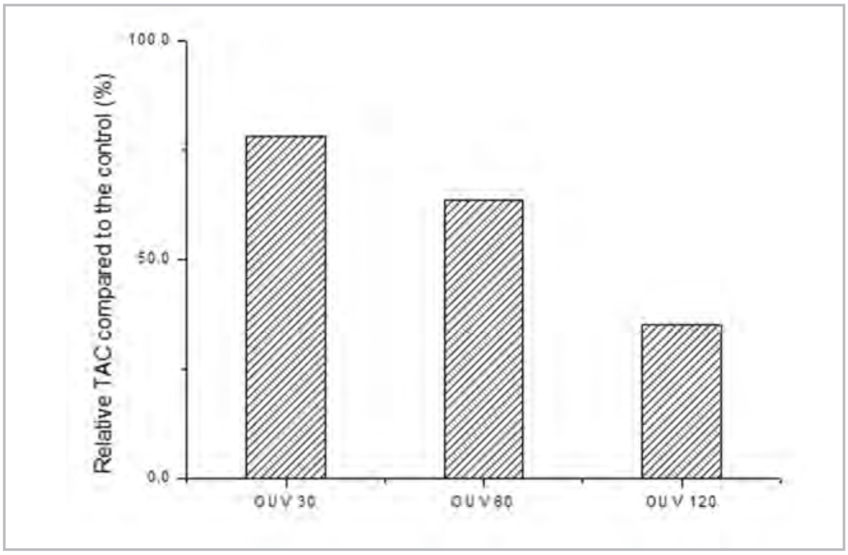
Figure 3 - Effect of UV irradiation on total antioxidant content of pericarp of tangerine tree



ples subjected to oxidative stress in the form of gaseous ozone and/or UV light all exhibited significant decreases in antioxidant level. The percentage decreases compared to the control samples ranged from around 10% (sample exposed to ozone gas for 30 min per day) to over 60% (sample exposed to ozone gas and UV light for 120 min daily). Ozone, as a strong oxidant, has been proven to be effective in removing plant antioxidants and phenolics (7-9). It is expected that an excessive dose of oxidants will consume the antioxidant in mature fruits where the pericarp is already well developed. Pasqualini et al. (10) also reported that atmospheric ozone decreased the gallic acid and vanillin levels in Aleppo pine.

There was a positive relationship between the percentage decrease in total antioxidant content and the length of time of exposure to ozone gas, both on its own and when it was coupled with UV light. This suggests that a shorter length of time of exposure to ozone gas may cause the antioxidant content to be reduced to a lesser extent. For the samples treated with UV light, the set of samples exposed to 120 min per day showed less influence, percentage-wise, as it had an approximately 10% decrease, while the samples treated for 30 minutes and 60 minutes had an approximately 20% and 30% decrease respectively. However, it is unlikely that there may be a further enhancement in total antioxidant if the length of time were to be increased.

Figure 4 - Effect of ozone gas and UV irradiation on total antioxidant content of pericarp of tangerine tree



Positive effects

Conversely, the sample watered with ozonated water showed an increase in total antioxidant content of nearly 20% compared to the control. This might be due to the oxidant stress being applied directly to the roots and thus being treated as nourishment, as opposed to threats to the well being of the plant, like ozone gas and UV light. The effects of ozone to soil are complicated, as soil matrix is heterogeneous. Johnson and Pregitzer (11), after site investigation, suggested that concentration of soluble phenolic acids and free amino acids in soil were strongly influenced by soil properties, plant and microbial activity, plant community and to a less degree, changes in atmospheric O_3.

A number of studies indicate that antioxidant and related chemicals in fruits were augmented after ozonation. The effect of ozone treatment on total phenol, flavonoid, and vitamin C content of fresh-cut honey pineapple, banana and guava was investigated by Alothman et al. (12). The antioxidant capacity of the fruits was evaluated by measuring the ferric reducing/antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH). Total phenol and flavonoid contents of pineapple and banana increased significantly after exposure to ozone for up to 20 min, with a concomitant increase in FRAP and DPPH values; however, the opposite was observed for guava. Ascorbic acid increase is used as a systemic response to ozone stress; in the study conducted by Severino et al. (13), ozone-resistant clones of *Trifolium repens* and *Centaurea jacea* showed the highest concentration of antioxidants, with 50-70% more ascorbic acid than ozone-sensitive clone. Liu et al. (14) also reported that antioxidant and total phenolic content were affected by environmental conditions. Elevated ozone increased the net photosynthetic rate of red clover leaves and antioxidant phenolic compounds before visible injuries in a large areas study (15).

Integrated effects and commercial consideration

Generation of reactive oxygen species (ROS) is characteristic for hypoxia and especially for reoxygenation. Consequences of hypoxia-induced oxidative stress depend on tissue and species, on membrane properties, on endogenous antioxidant content and on the ability to induce the response in the antioxidant system (16). In plant tissues, many phenolic compounds, flavonoids and tannins may work as ROS-scavenging compounds and antioxidants act as a cooperative network.

The age of the tangerine plants may be a hindrance to the augmentation of the antioxidant level, as the fruits have already matured and hence may find difficult to produce any more antioxidants. Torres et al. (17) reported that color degradation, rheological properties and retention of polyphenols of apple juice were significantly influenced by ozone concentration and processing time. It was concluded that while ozonation can be employed as a preservation technique for the processing of fruit juice, its impact on the nutritional and quality parameters should be considered. The present study also indicates that excessive oxidation, no matter if in the form of ozone, UV or combination of both, may reduce fruit pericarp antioxidant level. Alternatively, it can be considered that the role of fruit pericarp is to protect the fruit from oxidation damage and it performs in the presence of excessive oxidation stress. However, the role that oxidative stress can play when the fruit is still actively growing, green and unripe remains uncertain.

Although promising results of stimulatory effects on antioxidant levels have been reported (12), the present study shows rapid antioxidant consumption on fruit pericarp by atmospheric oxidants. However, fruit juice content was not tested in the samples. The preservation of antioxidant properties, storage stability and safety is of utmost importance in the fresh food industry as supplementation with antioxidant-rich foods was more effective than that with single antioxidants (18). Ozone has been looked into as an alternative sanitizing technology in the fresh produce industry. However, excessive levels should be avoided, as it is wasting energy and may reduce the nutritional value.

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