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Residual pesticides reduction on table grapes in post-harvest using ozonated water washing

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Abstract. Nowadays, different systems for reducing pesticides in table grapes are being tested at different production stages either in the field or in post-harvest. The present study tested ozonated water treatments at the beginning of the cold storage on Melissa seedless table grape variety to reduce residue contents of some pesticides. An ozone generator capable of producing ozone concentrations ranging from 18 to 65 Nm^3 was utilized for obtaining three ozone concentration levels in water: 3, 5 and 10 mg/L. Ozonated water was placed into a 70 L plastic box where 500 g grape samples closed in perforated plastic clamshell containers were immersed utilizing two washing times (5 and 10 min). Overall, six ozonated water treatments were tested. After ozonated water treatments, all samples were stored for 30 days at 2 °C and 95% relative humidity to simulate commercial practice. Pesticide residue contents were determined before ozonated water treatments (T_0) and 30 days after the cold storage (T_1). The comparison highlighted the different degradation rates as regards Fludioxonil and Fluxapyroxad. The best results were reached among the non-systemic pesticide such as Fludioxonil. Using 3 mg/L ozonated water to wash grapes for 10 min represented the optimal degradation conditions for the analyzed pesticides.

1 Introduction

Plant protection products (PPPs), or pesticides, are used against pathogens, insects or weeds to prevent crop damage. It was estimated that 30-40% of food is lost if adequate protection is not provided by PPPs [1]. Therefore, it is essential to provide the minimum level of pesticides that ensures food health. For this reason, the application of PPPs and their subsequent degradation have to be investigated. The degradation processes of PPPs are due to dissolution in the surrounding atmosphere, hydrolysis, microbial degradation, oxidation and photo-degradation [2]. Nevertheless, minimal amounts may remain as pesticide residues in food until harvest and reach consumers with possible chronic health effects [3]. Pesticide residues are subject to legal regulation and monitoring. For each active compound, the maximum residue limit (MRL) indicates the legal amount for placing on the market and allows national authorities to verify that PPPs have been used correctly. Unfortunately, table grapes are among the foods where MRLs are most frequently exceeded [4]. Residues in fresh and processed products are controlled not only by official monitoring by national authorities, but also independently by distributors, processing industries, importers and growers through the application of secondary requirements that are becoming more numerous and complex. Initially introduced in the European Union, they now play an increasingly important role in international trade. These requirements may be based on the limits set by law, but they go beyond the legal requirements: in fact, secondary requirements are generally based on a lower percentage of the MRLs set by law and, for certain crops, on a maximum number of detectable residues at the limit of quantification of the analytical method. For this reason, many distribution chains and food industries require growers to reduce residue levels to an even greater extent. Therefore, in addition to controlling the PPPs' pre-harvest interval time in the field, effective ways are being considered to preventively remove pesticide residues already present on vegetables to avoid adverse effects on human's health [5]. One of the latest methods involves the generation of ozone gas in water as a washing treatment to reduce pesticide residues in fruits and vegetables [6-9]. Ozone is a natural substance in the atmosphere that is generally recognized as safe (GRAS) for food contact applications [10]. Ozone is also an effective sanitizer against a wide range of microorganisms [11] and enables the elimination of mycotoxins [12]. In this context, the study was conducted to investigate the effectiveness of post-harvest washing with ozonated water on reducing pesticide residues in grapes. PPPs commonly used in pest control in table grapes were included in the study trial and detected as residues. In particular, the analyzed PPPs were Fludioxonil and Fluxapyroxad. Fludioxonil (phenylpyrroles) is a contact fungicide effective against gray mold while Fluxapyroxad (SDHI-fungicides) shows locally systemic properties and it is widely used against powdery mildew.

2 Materials and methods

2.1 Grape samples and ozonated water treatment

The study was performed on freshly harvested Melissa seedless table grapes collected from a commercial vineyard located in Southern Italy (Casamassima, Apulia Region, 223 m above sea level). Treatments against the most common diseases were simulated in the field using Geoxe (Fludioxonil) and Sercadis (Fluxapyroxad). At harvest, around 80 kg of grapes were collected along the rows. From the harvested grapes, a 60 kg grape sample was randomly collected and preliminary utilized for picking three replicates of 500 g each for residue determination before starting the cold storage period (T_0). The remaining grapes were utilized to assemble 500 g closed perforated plastic clamshell containers (12 for each treatment) to be destinated to ozonated water treatments and successively to cold storage period. The ozonated water treatments were performed at the beginning of the cold storage. A 70 L plastic box containing water was connected to an ozone generator and it was continuously alimented by ozonated water at different concentrations. The ozone generator was capable of producing ozone ranging from 18 to 65 Nm^3 and it was utilized for obtaining three ozone concentration levels in water: 3, 5 and 10 mg/L, which were monitored by an ozone analyser (Fig. 1). After the ozone concentration reached the fixed level in the water, the 500 g replicates plastic clamshell containers were immersed utilizing two immersion times: 5 and 10 minutes. Overall, six different ozonated water treatments were provided and immediately destinated for cold storage. Contemporarily, three SO_2 generating plastic bags each containing four 500 g grape samples in plastic clamshell containers were prepared and stored together with the ozonated samples. To compare all the treatments with the control and to test the effect of cold storage alone, four 500 g grape samples in plastic clamshell containers were prepared and stored without any treatments. All the operations were conducted at room temperature (around 17 °C) and all samples were stored for 30 days at 2 °C and 95% relative humidity to simulate commercial practice. After the cold storage period (T_1), pesticide residue contents were determined on three 500 g replicates for each treatment. Pesticide residues determination was performed by UNI EN 15662:2018 method with LC – MS/MS determination.

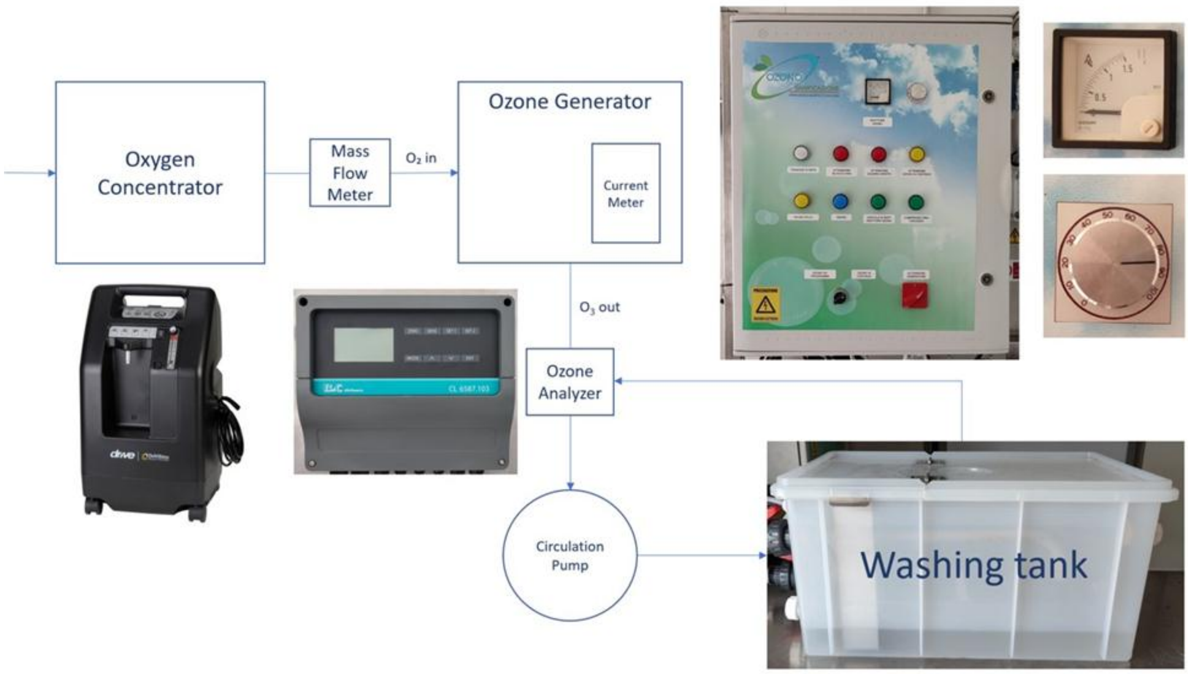

Figure 1. Scheme of the apparatus used for ozonated water washing of grapes.

2.2 Statistical analysis

On the collected data and for each pesticide residue, the analysis of variance (ANOVA) was carried out using a one-way model to evaluate the effect of cold storage after the harvesting and the effect of SO_2 with respect to the ozonated water treatments. Successively, for each molecule, a two-way model was performed to test the effect of washing time and ozone concentration in the water. F test was used to compare averages in the one-way models and to test the factors interaction in the two-way model. When interactions were significant means were separated with Tukey's HSD test ($p < 0.05$). ANOVA was performed by means of STATISTICA software v. 6.0 (StatSoft Inc., Tulsa, UK).

3 Results and Discussion

The pesticide residue rates showed a general decreasing trend between pre and post cold storage (T_0 vs. T_1, Fig. 2). Ozonated water treatments showed in general better results in terms of pesticides' degradation compared to SO_2 treatment (Fig. 3). Fludioxonil, which has a contact effect, showed the highest average rate of degradation, being reduced by 81.9% on average by treatments with ozonated water and by 60.7% on average by SO_2 treatment. Pesticide properties could be considered responsible for this behaviour given that the ozonated water could have degraded pesticide residues on vegetable surfaces by a greater efficiency as compared with the pesticides that are absorbed into tissues by the vegetables [7]. Despite its lower water solubility (3.4 mg/L), Fluxapyroxad showed the best results in terms of residues degradation for the ozone treatment compared to SO_2. Indeed, this systemic molecule was reduced by 67% on average, compared to the SO_2 treatment where a reduction of only 32% on average was observed. These results suggested that, in addition to the cleaning on the surface of vegetables, the ozonated water may gradually penetrate the first layers of the fruits and acts on the pesticides that are absorbed into the first layers of the fruits tissues, considering that impact of ozone is significantly limited once the active substance passed the cellular wall [12]. Molecular water solubility alone does not seem to govern the removal of a pesticide. On the other hand, the efficiency of pesticide residue removal is different in different fruits because their different types of surfaces (soft, coarse, smooth, glossy or hard) can affect the absorption and penetration properties [6]. Significant differences in residual Fludioxonil were found either due to ozone concentrations ($p < 0.01$) or washing time ($p < 0.05$), while significant differences in Fluxapyroxad residues were only due to ozone concentration ($p < 0.05$). Moreover, as regard Fludioxonil, a positive interaction between ozone concentration and washing time was observed ($p < 0.01$). The best Fludioxonil residues degradation was obtained with a minimum ozone dose (3 mg/L) combined with a maximum washing time (10 min), providing a 94.8% reduction, while the best Fluxapyroxad residues degradation was obtained with a minimum ozone dose (3 mg/L) independently from washing time, providing a reduction ranging from 68.2 to 78.2%. Higher residues of Fludioxonil and Fluxapyroxad at 5 and 10 mg/L ozone concentration could be attributed not to a lower efficiency of the ozone treatments with respect to 3 mg/L dose, but to the pesticide accumulation in washing water: indeed, these active substances have a moderate water solubility and DT50 in water. So, according to Sadlo et al. [13], the pesticides may have been transferred from contaminated water during grape clusters washing.

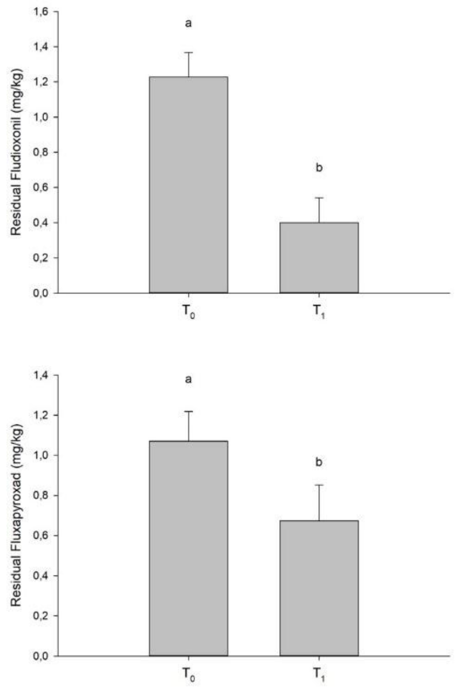

Figure 2. The residual pesticide (mg/kg) values in grapes before (T_0) and after 30 dd cold storage (T_1). Bars labeled by different letters are significantly different according to the Tukey's test ($p < 0.05$).

4 Conclusions

Based on these results and considering time, cost and treatment efficiency, it could be concluded that using 3 mg/L ozonated water to wash grapes for 10 min represents the optimal degradation condition for the analyzed pesticides. Consequently, the results could be considered interesting and fitting the usual commercial practices. Finally, given that high ozone concentrations could likely affect human health and cause corrosion [14] it has to be taken into account that a concentration of 3 mg/L could be relatively more safe for humans as well as for the vegetables. A potential health risk could be linked to pesticide degradation byproducts caused by ozonated water treatments given that they may be more toxic than the pesticides themselves. In this regard, earlier studies have already indicated that just a trace of unstable degradation byproducts could be found and that no bioaccumulation and toxicity were detected when ozone was used to degrade pesticides [5,15]. However, further research should be undertaken to investigate the toxicity of by-products resulting from ozonated water treatment of the tested pesticides.

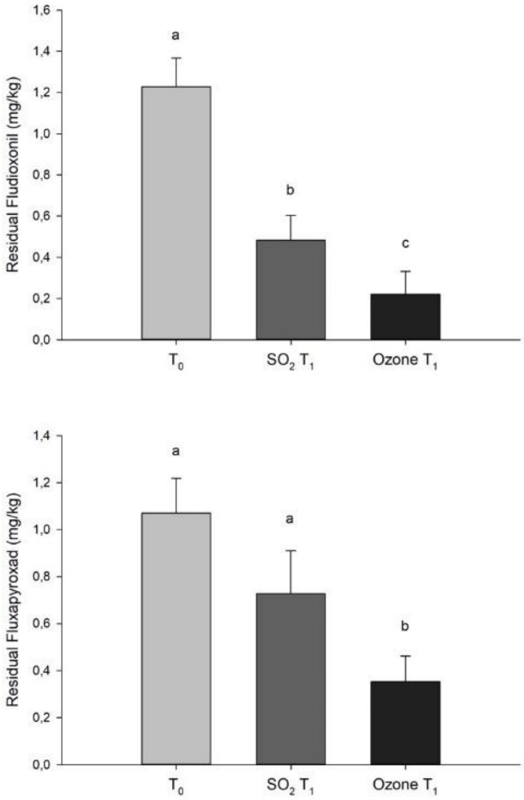

Figure 3. The residual pesticide (mg/kg) values in grapes before (T_0) and after 30 dd cold storage with sulfur dioxide (SO_2 T_1) and ozone (Ozone T_1) treatment. Bars labeled by different letters are significantly different according to the Tukey's test ($p < 0.05$).

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