Ozone applied to the nutrient solution and its impact on red chard baby leaves yield

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Abstract

Safety of fresh-cut products is largely affected by the microbial load of raw material. In this sense, a hydroponic production system allows to reduce microbial contamination, as long as the quality of the used water on the production system is ensured. This can be achieved through water sanitation. Moreover, treatments used to sanitize the nutrient solution can have an effect on its composition affecting subsequently the crop yields, especially if a strong oxidizing agent such as ozone (O_3) is used. In this work the effect of periodically gaseous O_3 application on nutrient solution composition and its impact on the yield of red chard baby leaves was evaluated. O_3 applications were performed every two days of culture at doses of 0 (control), 0.50, 1.0 and 2.0 mg L^{-1} for 3 min. Three harvests were performed on days 10, 17 and 26 after transplant. Macro- and micronutrients, dissolved oxygen (DO) and electrical conductivity (EC) of the nutrient solution were determined. Red chard yield was evaluated by fresh mass production and leaf area. Nutrient solution macronutrients were not affected by O_3, while micronutrients such as Fe and Mn contents decreased by 88 and 40%, respectively, on 0.50 mg L^{-1} dose. DO reached 2.0 mg L^{-1} increases up to 50% more than the control in each application. EC was not significantly affected by O_3 applications (ranges 2.46 and 2.82 dS m^{-1} at 25°C). However, all O_3 treatments increased red chard yield compared to the control. The dose of 0.50 mg L^{-1} produced the highest fresh matter and leaf area (53 and 22% more, respectively, than the control). It is concluded that O_3 applications to hydroponic red chard culture increase yield even though the availability some micronutrients as Fe and Mn could be affected.

Keywords: ozonation, oxygenation, hydroponic chard culture

INTRODUCTION

Fresh water scarcity is a global concern and legislation regarding the use of this resource in highly demanding sectors, such as agriculture, is increasingly strict in terms of protecting their availability (Pfister et al., 2011; Rosegrant et al., 2009). In this sense, some farmers dedicated to hydroponics cultures, aware of this problem, have implemented the nutrient solution reuse (Ikeda et al., 2002; Graham et al., 2009, 2011). However, recycling the nutrient solution has some limitations since loss of nutrients and microorganism growth, including human pathogens, can occur (Richard et al., 2006). The risk of pathogen proliferation is currently the highest impediment to water recycling systems (Daughtrey and Benson, 2005; Hong and Moorman, 2005; Johansson et al., 2002; Stewart-Wade, 2011; Van Os, 1999). However, it is possible to apply sanitization methods in order to ensure the microbiological quality of nutrient solutions.

Numerous technologies, including physical treatments as ultraviolet radiation, filtration, heat treatment and/or chemical treatments as chlorination, hydrogen peroxide, and ozone (O_3), etc., are commercially available (Ehret et al., 2001). Ozonation is particularly

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interesting because it is highly effective in controlling microorganisms, due to its strong oxidation potential and has also other advantages as it allows overcoming oxygen deficiencies, besides being easy to implement in production systems (Graham et al., 2011).

Although O_3 has many attractive features as sanitizer for recycled nutrient solutions, there are also certain disadvantages and lack of important knowledge that prevents farmers implement more massively. Therefore, further researches on O_3 use, are needed in order to use it as nutrient solution sanitizer method, without altering its chemical composition and consequently crop yield and quality.

With this in mind, the objectives of the present study were 1) to determine O_3 application effect on yield of baby red chard leaves produced in root floating hydroponic system and 2) to evaluate changes in the nutrient solution under O_3 application.

MATERIALS AND METHODS

Plant material and crop management

Red chard (*Beta vulgaris* L. var. *cicla* 'SCR 107') used in this experiment was grown in a closed root floating hydroponic system. The experiment was performed during Southern Hemisphere spring (October-November). The seedlings were irrigated daily with tap water until the state of expanded cotyledons, which was reached after 10 days of sowing. Subsequently, a modified Hoagland nutrient solution II (Hoagland and Arnon, 1950) diluted to 50% was applied. Once the plants reached 2 to 3 true leaves (25 days post seeding), they were transferred to the floating root system composed of 20 trays with 6 plants (0.20×0.16×0.07 m), and containing 1 L of Hoagland II 100% nutrient solution.

Experimental design and aqueous ozone application

The experiment was set up as a factorial design with two factors: O_3 dose and harvest time. O_3 dose consist of 4 levels, without O_3 (D0, control), (D1): 0.50 mg L^{-1}; (D2): 1.0 mg L^{-1} and (D3): 2.0 mg L^{-1} of O_3, respectively. The corresponding doses were applied every 2 days since transplant. Samples were taken at days 10, 17 and 26 after transplant.

Growth evaluation

- Leaf area was determined with the Sigma Scan Pro 5.0 software and results were expressed in cm^2.
- Aerial fresh weight: all aerial parts were separated from the roots and weighed on an analytical balance (Radwag, AS 100, Poland). Results were expressed in grams (g).

Nutrient solution evaluation

- Chemical composition: Cl^-, NO_3^- and NH_4^+ were determined by potentiometry, while Ca^{2+}, Mg^{2+}, K^+, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^{2+} were measured by atomic absorption spectrophotometry according to the methodology described by Sadzawka et al. (2007). B and SO_4^{2-} were determined by colorimetric method (Sadzawka and Apablaza, 1991), and HCO_3^- by titration before and after 20 min O_3 application. Macro- and micronutrient analysis were only performed on the lowest dose of 0.5 mg L^{-1}.
- Dissolved oxygen: was daily measured in the nutrient solution by an oxygen meter (Hanna Instruments, HI 9146, Romania). Results were expressed as mg L^{-1}.
- Electrical conductivity (EC): was recorded with a conductivity meter (Hanna Instruments, HI 99301, Romania) in each culture unit and expressed as dS m^{-1} at 25°C.

Statistical analysis

Results were analyzed using two-way analysis of variance (ANOVA) using the statistical program Infostat (version 2015, National University of Cordoba, Argentina). Significant differences of means values were detected by Tukey test ($p \leq 0.05$; 0.01; 0.001; 0.0001).

RESULTS AND DISCUSSION

Leaf area and fresh mass

The leaf area obtained values between 8.98 and 15.21 cm^2 for the three harvest times analyzed (Table 1). All O_3 treatments showed higher leaf area compared to the control. As shown on Table 2, differences among O_3 doses were found. O_3 treatments with 0.5 mg L^{-1} reached the highest leaf area.

Table 1. Effect of O_3 dose and harvest time on hydroponic baby red chard leaf area (cm^2).

O_3 dose (mg L^{-1})	Leaf area (cm^2)		
	Harvest time 1	Harvest time 2	Harvest time 3
0.0	11.44±0.20[1]	11.02±0.37	10.83±0.62
0.5	15.00±0.40	15.21±0.26	14.91±0.35
1.0	12.78±0.78	12.21±0.25	12.10±0.36
2.0	8.98±0.38	11.97±0.61	10.71±0.13
Significance level[2]			
Dose	(1.68)*		
Harvest	NS		
D×H	NS		

[1]The values correspond to mean (*n*=6) ± standard error of the mean.
[2]NS, *: Not significant or significant at p≤0.05.

Table 2. Effect of O_3 dose on hydroponic baby red chard leaf area (cm^2).

O_3 dose (mg L^{-1})	Leaf area (cm^2)
0.0	10.55 a[1]
0.5	15.04 c
1.0	11.09 a b
2.0	12.37 b

[1]Letters indicate significant differences by Tukey test (p≤0.05). Values are mean (*n*=6) ± standard error of the mean.

Fresh mass fluctuated between 10.25 and 15.14 g throughout the experiment (Table 3). O_3 treatments also affected this parameter and, as observed on leaf area, the dose of 0.5 mg L^{-1} differed positively from the remaining treatments, obtaining the highest average value, of 14.35 g as presented in Table 4.

Table 3. Effect of O_3 dose and harvest time on hydroponic baby red chard fresh mass (g).

O_3 dose (mg L^{-1})	Fresh mass (g)		
	Harvest time 1	Harvest time 2	Harvest time 3
0.0	14.79±0.49[1]	15.14±0.25	13.12±0.39
0.5	11.56 ±0.38	10.86±0.84	11.56±0.38
1.0	11.13±0.12	12.06±0.76	11.13±0.12
2.0	10.25±0.28	11.73±0.41	11.25±0.13
Significance level[2]			
Dose	(1.49)****		
Harvest	NS		
D×C	NS		

[1]The values correspond to mean (*n*=6) ± standard error of the mean.
[2]NS, *, **, ***, ****: Not significant or significant at p≤0.05, 0.01, 0.001 or 0.0001, respectively.

Table 4. Effect of O_3 dose on hydroponic baby red chard fresh mass (g).

Dose	Fresh mass (g)
0.0	11.63 b[1]
0.5	14.35 a
1.0	11.32 b
2.0	11.44 b

[1] Letters indicate significant differences by Tukey test ($p \leq 0.0001$). Values are mean (n=6) ± standard error of the mean.

The dose of 0.5 mg L^{-1} increased yield expressed as fresh mass and leaf area. This increase could be explained by the antimicrobial power of O_3 controlling root diseases (Ehret et al., 2001; Graham et al., 2011). Previous studies in spinach, with moderate deficiencies of Fe, showed increased biomass as well as sugars, proteins and nitrates contents (Jin et al., 2013). Similar finding were reported by Ohashi-Kaneko et al. (2009) who did not observe low yield in tomato plants subjected to O_3.

It is noteworthy that doses of 1 and 2 mg L^{-1} of O_3 determined appreciable leaves damages on the leaves, therefore these doses could be considered phytotoxic for this crop. For this reason, the dose of 0.5 mg L^{-1} of O_3 was selected for the following analysis.

Dissolved oxygen and electrical conductivity

Dissolved oxygen fluctuated between 5.68 and 10.86 mg L^{-1} (data not shown). All O_3 treatments had increased dissolved oxygen compared to control even when all treatments received oxygenation for 3 min every 3 h throughout the experiment by using an aquarium pump. The highest values were observed in treatment with 2 mg L^{-1} of O_3. Obtained results indicate that O_3 application largely contributes to increase dissolved oxygen. Studies in tomato plants shown that enhanced oxygenation have benefits in terms of improved productivity and pathogen control in greenhouse production (Zheng et al., 2007), so this is considered as a positive aspect. EC was not significantly affected by O_3 applications, varying between 2.46 and 2.82 dS m^{-1} at 25°C (data not shown).

Nutrient solution composition

Application of O_3 at 0.5 mg L^{-1} reduced strongly iron (Fe) and manganese (Mn) in 39.6 and 88.2%, respectively, as shown in Figure 1. Besides, boron (B) and zinc (Zn) showing a reduction of less impact (2.9 and 10%, respectively), while copper (Cu) showed a slight increase of 2.1%.

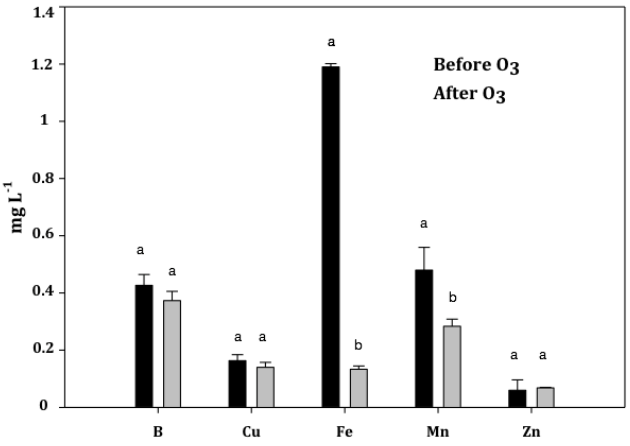

Figure 1. Micronutrients content in the nutrient solution before and after application of 0.5 mg L^{-1} of O_3. Values are means ± standard error of the means (n=3).

Fe acts as a cofactor in the photosynthetic electron transport chain and is essential for chlorophyll biosynthesis. In fact, chloroplasts contain up to 90% of Fe in the leaf cells, with about half in the stroma and thylakoid membranes (Shingles et al., 2002). Fe deficiency can result in a strong chlorosis usually showed in younger leaves (Kosegarten et al., 1998). While Mn deficiency is associated with is chlorosis and necrotic spots that occurs in both old and young leaves depending on species and growth rate (Taiz and Zeiger, 2010). The main symptom of Mn deficiency is the internerval chlorosis associated with the development of necrotic spots observed in old and young leaves according to species and growth rate (Taiz and Zeiger, 2010). The normal range in the nutrient solution for the Mn is 0.25-0.5 mg L^{-1} and for the Fe of 0.5-1.5 mg L^{-1} (Virgili, 1996). Considering these values, the Mn maintained an acceptable range after ozonization (0.28 mg L^{-1}), while Fe decreased to deficiency ranges (0.13 mg L^{-1}). In this way, if the loss of this element is a cause of ozonation is high, it is recommended to consider a replacement of this element subsequent to the application of O_3 as long as damage to the crop to justify.

Although micronutrients were affected, no deficiency symptoms were observed, so it is considered that the remaining levels were sufficient to supply the crop requirements.

An interesting aspect to consider is the fact that macronutrients were not significantly affected by ozonation. Decreasing between 4.4 and 7.6% were registered which does not affect crop requirements (Figure 2). These results were consistent with the literature, since no significant losses have been reported by application O_3 (Ehret et al., 2001; Graham et al., 2011; Runia, 1995).

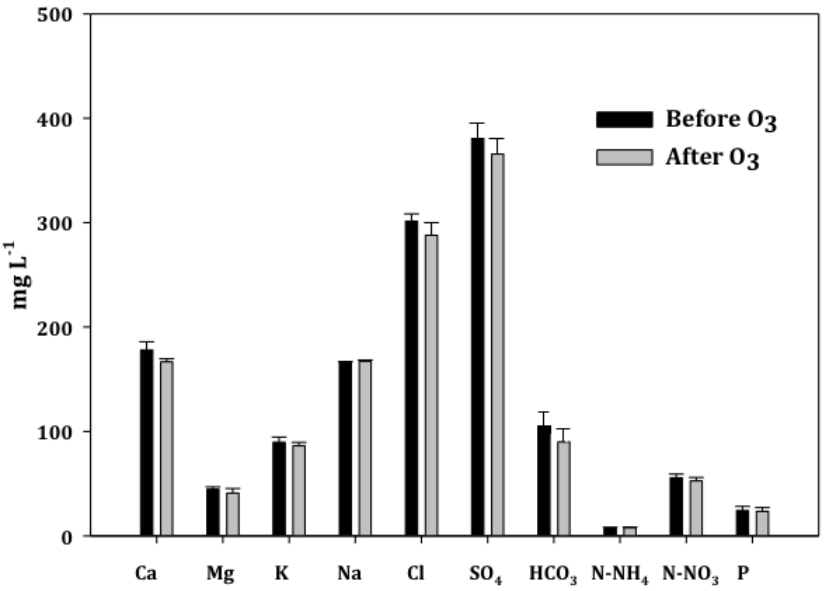

Figure 2. Macronutrients content in the nutrient solution before and after application of 0.5 mg L^{-1} of O_3. Values are means ± standard error of the means (*n*=3).

CONCLUSIONS

Based on the results of the current study:

- It is possible to applied 0.5 mg L^{-1} O_3 every 2 days directly to the nutrient solution without causing damage to the crop.
- Despite of the decreasing of some micronutrients, yield crop, under this crop condition (20-25°C and in a 26 days cycle) was not affected.
- On the contrary, O_3 application proved to be positive because an increased on the production was recorded probably linked to growth condition improved.

Further research is required to validate the obtained results with particular attention on the impact of O_3 on micronutrients.

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Literature cited

Daughtrey, M.L., and Benson, D.M. (2005). Principles of plant health management for ornamental plants. Annu Rev Phytopathol *43* (*1*), 141–169 https://doi.org/10.1146/annurev.phyto.43.040204.140007. PubMed

Ehret, D.L., Alsanius, B.W., Wohanka, W., Menzies, J., and Utkhede, R.S. (2001). Disinfestation of recirculating nutrient solutions in greenhouse horticulture. Agronomie *21* (*4*), 323–339 https://hal.archives-ouvertes.fr/hal-00886118/document https://doi.org/10.1051/agro:2001127.

Graham, T., Zhang, P., Zheng, Y., and Dixon, M. (2009). Phytotoxicity of aqueous ozone on five container-grown nursery species. HortScience *44*, 774–780 http://hortsci.ashspublications.org/content/44/3/774.full.

Graham, T., Zhang, P., Woyzbun, E., and Dixon, M. (2011). Response of hydroponic tomato to daily applications of aqueous ozone via drip irrigation. Sci. Hortic. (Amsterdam) *129* (*3*), 464–471 https://doi.org/10.1016/j.scienta.2011.04.019.

Hoagland, D., and Arnon, D. (1950). The Water-Culture Method for Growing Plants without Soil. Circular Nº347 (USA: California Agricultural Experiment Station, The College of Agricultural University of California), pp.32.

Hong, C.X., and Moorman, G.W. (2005). Plant pathogens in irrigation water: challenges and opportunities. Crit. Rev. Plant Sci. *24* (*3*), 189–208 https://doi.org/10.1080/07352680591005838.

Ikeda, H., Koohakan, P., and Jaenaksorn, T. (2002). Problems and countermeasures in the re-use of the nutrient solution in soilless production. Acta Hortic. *578*, 213–219 https://doi.org/10.17660/ActaHortic.2002.578.26.

Jin, C.W., Liu, Y., Mao, Q.Q., Wang, Q., and Du, S.T. (2013). Mild Fe-deficiency improves biomass production and quality of hydroponic-cultivated spinach plants (*Spinacia oleracea* L.). Food Chem *138* (*4*), 2188–2194 https://doi.org/10.1016/j.foodchem.2012.12.025. PubMed

Johansson, R.C., Tsur, Y., Roe, T., Doukkali, R., and Dinar, A. (2002). Pricing irrigation water: a review of theory and practice. Water Policy *4* (*2*), 173–199 https://doi.org/10.1016/S1366-7017(02)00026-0.

Kosegarten, H., Wilson, G.H., and Esch, A. (1998). The effect of nitrate nutrition on iron chlorosis and leaf growth in sunflower (*Helianthus annuus* L.). Eur. J. Agron. *8* (*3-4*), 283–292 https://doi.org/10.1016/S1161-0301(98)00021-5.

Ohashi-Kaneko, K., Yoshii, M., Isobe, T., Park, J.S., Kurata, K., and Fujiwara, K. (2009). Nutrient solution prepared with ozonated water does not damage early growth of hydroponically grown tomatoes. Ozone Sci. Eng. *31* (*1*), 21–27 https://doi.org/10.1080/01919510802587523.

Pfister, S., Bayer, P., Koehler, A., and Hellweg, S. (2011). Projected water consumption in future global agriculture: scenarios and related impacts. Sci. Total Environ. *409* (*20*), 4206–4216 https://doi.org/10.1016/j.scitotenv.2011.07.019. PubMed

Richard, S., Zheng, Y., and Dixon, M. (2006). To Recycle or Not to Recycle? (Greenhouse Canada), p.22–24.

Rosegrant, M.W., Ringler, C., and Zhu, T. (2009). Water for agriculture: maintaining food security under growing scarcity. Annu. Rev. Environ. Resour. *34* (*1*), 205–222 https://doi.org/10.1146/annurev.environ.030308.090351.

Runia, W. (1995). A review of possibilities for disinfection of recirculation water from soilless cultures. Acta Hortic. *382* (*382*), 221–229 https://doi.org/10.17660/ActaHortic.1995.382.25.

Sadzawka, A., and Apablaza, N. (1991). Determinación colorimétrica de sulfato en aguas y extractos acuosos de suelo. Agric. Téc. (Chillán) *51* (*1*), 81–82.

Sadzawka, A., Carrasco, M.A., Demanet, R., Flores, H., Grez, R., Mora, M.L., et al. (2007). Métodos de Análisis de Tejidos Vegetales, 2nd edn (Instituto de Investigaciones Agropecuarias (INIA)), pp.140.

Shingles, R., North, M., and McCarty, R.E. (2002). Ferrous ion transport across chloroplast inner envelope membranes. Plant Physiol. *128* (*3*), 1022–1030 https://doi.org/10.1104/pp.010858. PubMed

Stewart-Wade, S.M. (2011). Plant pathogens in recycled irrigation water in commercial plant nurseries and greenhouses: their detection and management. Irrig. Sci. *29* (*4*), 267–297 https://doi.org/10.1007/s00271-011-0285-1.

Taiz, L., and Zeiger, E. (2010). Plant Physiology, 5th edn (Sunderland, MA: Sinauer Associates), pp.764.

Van Os, E.A. (1999). Closed soilless growing systems: a sustainable solution for Dutch greenhouse horticulture.

Water Sci. Technol. *39*, 105–112.

Virgili, A. (1996). Introducción a la Fertilización con Microelementos, 2nd edn (Barcelona, Spain: Welgro Micromix), pp.25.

Zheng, Y., Wang, L., and Dixon, M. (2007). An upper limit for elevated root zone dissolved oxygen concentration for tomato. Sci. Hortic. (Amsterdam) *113* (*2*), 162–165 https://doi.org/10.1016/j.scienta.2007.03.011.