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Garlic volatile compounds mimic nitric oxide (NO) effects on ripening of sweet pepper (*Capsicum annuum* L.) fruits and improve their commercial and nutritional properties†

Verónica Codesido[1,2], Carmelo Ruiz-Torres[1], Francisco J. Corpas[1] and José M. Palma[1,*]

[1] Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, Spanish National Research Council (CSIC), Granada, Spain; carmelo.ruiz@eez.csic.es (C.R.-T.); javier.corpas@eez.csic.es (F.J.C.); josemanuel.palma@eez.csic.es (J.M.P.)

[2] Present address: MIFCO Biosciences, Antequera, Málaga, Spain; v.codesido@mifcobs.com (V.C.)

* Correspondence: Correspondence: josemanuel.palma@eez.csic.es

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Abstract: Pepper (*Capsicum annuum* L.) fruits are one of the most consumed vegetables worldwide. Ripening of pepper fruits has been well characterized both phenologically and metabolically. Recently, nitric oxide (NO) has been proved to delay pepper fruit ripening and to enhance the ascorbate concentration about 40%. Recent research carried out in our laboratory found that garlic (*Allium sativum* L.) clove samples release nitrogen-containing volatile compounds containing significant amounts of NO gas. In this work, incubation of pepper fruits in the presence of garlic preparations at 6ºC and room temperature was achieved. Diverse biochemical, nutritional and commercial parameters such as brix, ascorbate, glutathione, carotenoids, flavonoids, and lipid peroxidation were determined in treated pepper fruits and were compared to fruit subjected to exogenous NO treatment. Our data indicate that garlic preparations exerted similar effects as NO in pepper fruits, delaying ripening and increasing some trait values such as ascorbate, glutathione and flavonoids. These results suggest that this experimental design could be up-scaled for agro-biotechnological purposes with the circular economy being promoted.

Keywords: *Allium sativum*; ascorbate; carotenoids; flavonoids; garlic clove; glutathione; nitric oxide; pepper fruits; ripening

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1. Introduction

Pepper (*Capsicum annuum* L.) fruits are one of the most consumed vegetables worldwide what confers to this crop a high nutritional/economic interest. The ripening of the non-climacteric pepper fruits is characterized by a shift in the fruit color from green to red, yellow, orange or purple depending on the variety. This event implies chlorophyll breakdown and synthesis of new carotenoids and anthocyanins, emission of organic volatiles, new protein synthesis and cleavage of existing ones and cell wall softening, among others [1]. From a metabolic point of view, this physiological process is accompanied by an increase of the lipid peroxidation, the activity of some enzymes such the superoxide-generating respiratory burst oxidative homolog (Rboh) and NADP-dehydrogenases, as well as by higher NADPH levels and some post-translational modification of proteins. Conversely, during ripening nitric oxide (NO) content and catalase activity lower [2]. NO has been proved to delay pepper fruit ripening and to enhance the ascorbate concentration about 40% [3,4].

Garlic (*Allium sativum* L.) has been thoroughly investigated from a physiological and metabolic point of view [5-7], but the major number of references are related to its pharmaceutical and medicinal properties [8-10]. However, the direct effect of garlic (cloves) on

the metabolism of other plant species has not been addressed so far. In this work, incubation of pepper fruits in the presence of nitrogen- and sulfur containing volatile compounds from garlic preparations at 6ºC and room temperature was achieved. The content of ascorbate, glutathione, carotenoids, and flavonoids was determined in treated pepper fruits and was compared to fruit subjected to exogenous NO treatment.

2. Materials and Methods

2.1. Experimental design

Sweet pepper fruits (6, about 1.5 kg) at breaking point were incubated for 1 h in a hermetic methacrylate box in the presence of nitrogen- and sulfur-containing volatile compounds emitted from 300 g garlic cloves as shown in Fig. 1. In parallel, 6 fruits at the same ripening stage were also subjected to exogenous NO treatment for 1 h as reported earlier [3,11]. Then, fruits from both treatments were left at 6ºC and room temperature for 2 weeks. Sampling was carried out at 1 and 2 weeks and stored at -80ºC until further assay. In all fruits, the percentage of fresh weight loss was calculated with respect to the fresh weight of fruits at the onset of the experiment. Dry weight was calculated after fruits were maintained at 80ºC for 1d. The Brix index was determined by an Atago refractometer.

Figure 1. Sweet pepper fruits at breaking point were incubated in a hermetic methacrylate box in the presence of 30 g garlic cloves for 1 h at room temperature.

2.2. Determination of ascorbate and glutathione content

The method reported earlier was followed [1,12]. Basically, 0.4 g fruits were homogenized with 1.3 mL 0.1 MN HCl. Then, samples were centrifuged at 15,000 × g for 15 min at 4°C and the extract was kept on ice, in the dark. The quantification of ascorbate, reduced glutathione (GSH) and oxidised glutathione (GSSG) was obtained after HPLC coupled to MS.

2.3. Measurment of total carotenoids and β-carotene

Fresh samples of pepper fruit were homogenized using a pestle and mortar in the presence of liquid N_2. Obtained powder (0.5 g) was added to a mix of 50 ml containing acetone:hexane:ethanol (25:50:25) and centrifuged at 5,000 x g for 5 min at 4 °C . Two phases were separated and an aliquot was taken from the upper solution for measurement of optical density at 450, 453, 505, 645 and 663 nm. Total carotenoids were estimated from the absorbance at 450 nm according to [13]. For β-carotene, the following equation was used: $0.216 \times A_{663} - 1.22 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$ [14].

2.4. Phenolic and flavonoids content and lipid peroxidation

Samples (0.5 g) were powdered under liquid N_2 and then extracted in 1.33 ml of 0.1 M HCl. The homogenate was centrifuged a 15,000 g for 20 min at 4ºC and supernatants were used for assays.

Total phenolic content in extracts was determined using the Folin-Ciocalteau reagent (FCR, dilution 1:10) and gallic acid (GA) as standard. Pepper extracts (50 µl) were mixed with 400 µl FCR and incubated at room temperature for 10 min. Then, 950 µl sodium bicarbonate solution (80%, w/w) were added and the mixture was incubated at room temperature for 45 min in darkness. The absorbance of the solution was determined at 760 nm and compared with a GA equivalents calibration curve. For flavonoids assay, extracts (100 µl) were mixed with 400 µl of Milli-Q water and 30 µl of 5% (w/v) $NaNO_2$ for 6 min. Then, 60 µl of 10% (w/v) $AlCl_3$ were added, and after 5 minutes, 200 µl of 1 M NaOH were also added. The absorbance of the mix was determined at 510 nm. Lipid peroxidation was calculated by determining the concentration of MDA through the thiobarbituric acid reacting substances (TBARS) method [15].

2.5. Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard error of the mean. Phenotypic correlations were estimated as Pearson's correlation coefficients taking into account data from all samples (from control to treated fruits). Differences among means were considered statistically significant at $p < 0.05$.

3. Results and Discussion

As shown in **Fig. 2,** the volatile compounds from garlic cloves delayed the ripening of pepper fruits in a manner that could be detected 4 d after the incubation. This same effect on the ripening process was also provoked by the treatment of pepper fruits with exogenous NO [3,11]. And this was also observed in a climacteric fruit such as tomato [16].

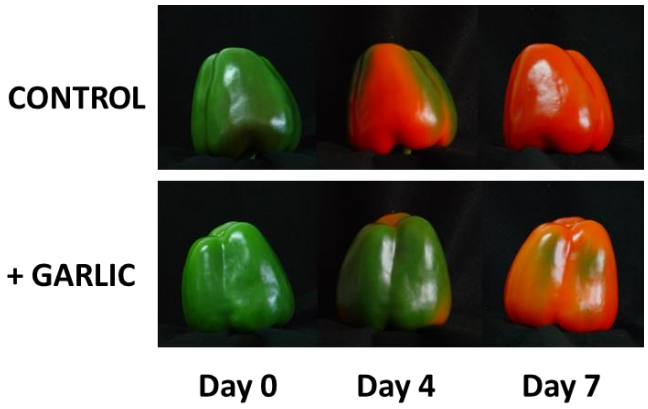

Figure 2. Phenotype of sweet pepper fruits at day 4 and 7 after the incubation with garlic cloves and further storage at room temperature.

Results on commercial parameters (fresh and dry weights and Brix) are little affected by either NO or garlic treatment, and only temperature exerted some effects on these parameters (**Fig. 3**).

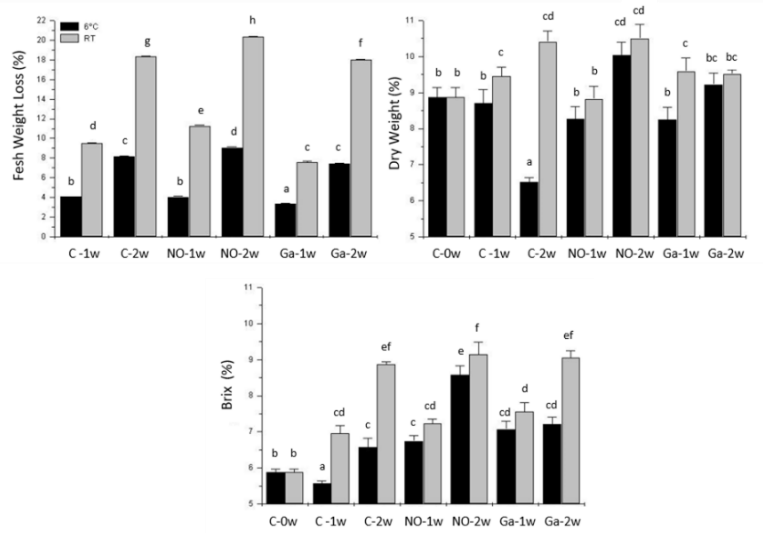

Figure 3. Effect of NO and garlic on fresh weight, dry weight and Brix of sweet pepper fruits at different times and storage conditions. Columns with different letters above depict means are significantly different at $p<0.05$. RT, room temperature.

Both treatments, NO and garlic also influenced the ascorbate and glutathione contents in fruits, and it was dependent on the storage conditions (**Fig. 4**). These results on the effect of NO on the ascorbate content corroborates those reported earlier [4] and are also in agreement with those reported recently in tomato fruits [16]. However, this is the first report on the direct effect of garlic treatment in the ascorbate and glutathione contents of fruits from a higher plant.

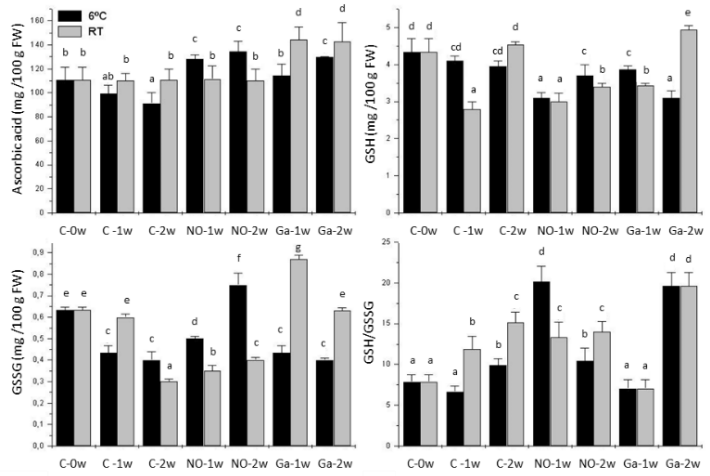

Figure 4. Effect of NO and garlic on ascorbate and glutathione contents in sweet pepper fruits at different times and storage conditions. Columns with different letters above depict means are significantly different at $p<0.05$. RT, room temperature.

The treatment with garlic also influenced the profile of total carotenoids and β-carotene, and this was especially relevant under room temperature conditions (**Fig. 5**).

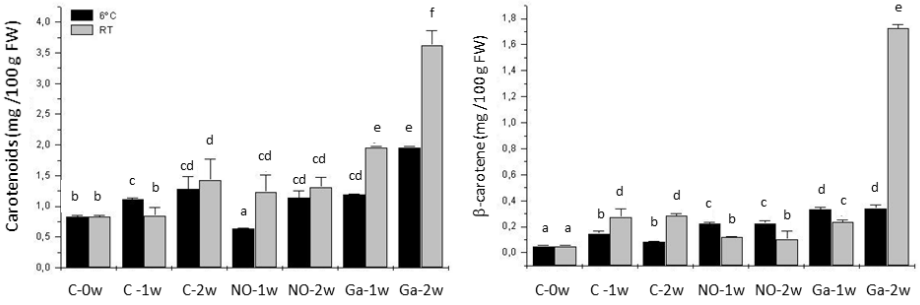

Figure 5. Effect of NO and garlic on carotenoids and β-carotene contents in sweet pepper fruits at different times and storage conditions. Columns with different letters above depict means are significantly different at $p<0.05$. RT, room temperature.

Likewise, it was remarkable the increase of the flavonoid levels favored by NO and garlic, and this was also dependent on the storage conditions with little influence on the content of MDA (**Fig. 6**), a marker of lipid peroxidation and associated with oxidative stress [2,17].

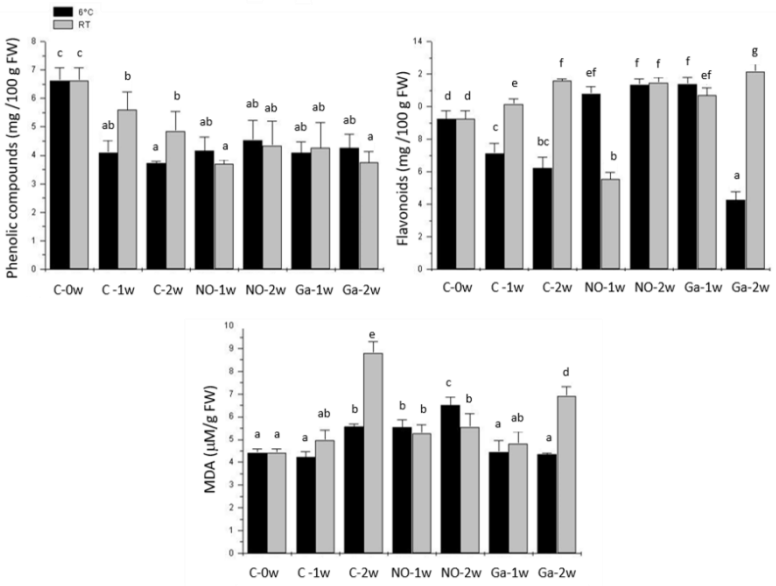

Figure 6. Effect of NO and garlic on phenolic compounds and flavonoid contents and lipid peroxidation level (MDA) in sweet pepper fruits at different times and storage conditions. Columns with different letters above depict means are significantly different at $p<0.05$. RT, room temperature. MDA, malondialdehyde.

4. Conclusions

Our data indicate that garlic preparations mostly exerted similar effects as NO in sweet pepper fruits, delaying ripening and increasing some commercial traits such as ascorbate, glutathione and flavonoids, thus improving the added value of this horticultural product. This is the first report on the direct influence of garlic on the metabolism and nutritional properties of a crop fruit. These results suggest that this experimental design could be up-scaled for agro-biotechnological purposes with the circular economy being promoted.

Author Contributions: J.M.P., F.C.A. and C.R.-T. conceived the experimental design. V.C. and C-R.-T. achieved the experimental work and V.C. prepared the figures. J.M.P. and F.C.A. wrote and

discussed the manuscript. Globally, all authors have made a collaborative, direct and intellectual contribution to the work, and have approved it for publication.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Palma JM, Terán F, Contreras-Ruiz A, Rodríguez-Ruiz M, Corpas FJ. Antioxidant profile of pepper (*Capsicum annuum* L.) fruits containing diverse levels of capsaicinoids. *Antioxidants* **2020**, *9*, 878.
2. Chu-Puga A, González-Gordo S, Rodríguez-Ruiz M, Palma JM, Corpas FJ. NADPH oxidase (Rboh) activity is up regulated during sweet pepper (*Capsicum annuum* L.) fruit ripening. *Antioxidants* **2019**, *8*, 9.
3. Chaki M, Álvarez de Morales P, Ruiz C, Begara-Morales JC, Barroso JB, Corpas FJ, Palma JM. Ripening of pepper (*Capsicum annuum*) fruit is characterized by an enhancement of protein tyrosine nitration. *Ann. Bot.* **2015**, *116*, 637-647.
4. Rodríguez-Ruiz M, Mateos RM, Codesido V, Corpas FJ, Palma JM. Characterization of the galactono-1,4-lactone dehydrogenase from pepper fruits and its modulation in the ascorbate biosynthesis. Role of nitric oxide. *Redox Biol.* **2017**, *12*, 171-181.
5. Wang GL, Ren XQ, Liu JX, Yang F, Wang YP, Xiong AS. Transcript profiling reveals an important role of cell wall remodeling and hormone signaling under salt stress in garlic. *Plant Physiol. Biochem*. **2019**, *135*, 87-98.
6. Ding HY, Ali A, Cheng ZH. An allelopathic role for garlic root exudates in the regulation of carbohydrate metabolism in cucumber in a hydroponic co-culture system. *Plants-Basel* **2020**, *9*, 45.
7. Kong QS, Mostafa HHA, Yang WL, Wang JL, Nuerawuti M, Wang Y, Song JP, Zhang XH, Ma LC, Wang HP, Li XX. Comparative transcriptome profiling reveals that brassinosteroid-mediated lignification plays an important role in garlic adaption to salt stress. *Plant Physiol. Biochem*. **2021**, *158*, 34-42.
8. Singh RN, Kumar P, Kumar N, Singh DK. Garlic (*Allium sativum*): pharmaceutical uses for human health. *Int. J. Pharmaceut. Sci. Res.* **2020**, *11*, 4214-4228.
9. Netzel ME. Garlic: much more than a common spice. *Foods* **2020**, *9*, 1544.
10. Farhat Z, Hershberger PA, Freudenheim JL, Mammen MJ, Blair RH, Aga DS, Mu LN. Types of garlic and their anticancer and antioxidant activity: a review of the epidemiologic and experimental evidence *Eur. J. Nutr*. **2021**, DOI: 10.1007/s00394-021-02482-7.
11. Palma JM, Ruiz-Torres C, Corpas FJ (2018) A simple and useful method to apply exogenous NO gas to plant systems: bell pepper fruits as a model. In: *Nitric Oxide: Methods and Protocols, Methods in Molecular Biology* (Mengel A, Lindermayr C, ed,) **2018**, *1747*, 1-11.
12. Airaki M, Sánchez-Moreno L, Leterrier M, Barroso JB, Palma JM, Corpas FJ. Detection and quantification of S-nitrosoglutathione (GSNO) in pepper (*Capsicum annuum* L.) plant organs by LC-ES/MS. *Plant Cell Physiol*. **2011**, *52*, 2006–2015.
13. Deepa N, Kaur C, George B, Singh B, Kapoor HC. Antioxidant constituents in some sweet pepper (*Capsicum annuum* L.) genotypes during maturity. *Lebensmit. Wissensch. Technol*. **2007**, *40*, 121–129.
14. Masayasu N, Ichiji Y. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shok. Kogyo Gakk*. **1992**, *39*, 925−928.
15. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol*. **1978**, *52*, 302–310.
16. Zuccarelli R, Rodríguez-Ruiz M, Lopes-Oliveira PJ, Pascoal GB, Andrade CSC, Furlan CM, Purgatto E, Palma JM, Corpas FJ, Rossi M, Freschi L. Multifaceted roles of nitric oxide in tomato fruit ripening: NO-induced metabolic rewiring and consequences for fruit quality traits. *J. Exp. Bot*. **2021**, *72*, 941–958.
17. Solórzano S, Corpas FJ, González-Gordo S, Palma JM. Reactive oxygen species (ROS) metabolism and nitric oxide (NO) content in roots and shoots of rice (*Oryza sativa* L.) plants under arsenic-induced stress. *Agronomy* **2020**, *10*, 1014.