Title Carrots as biofactories of nutraceuticals: using extreme postharvest stress conditions to intensify the production of antioxidants via a mechanism mediated by reactive oxygen species

Author Daniel A. Jacobo-Velázquez, Ginés B. Hernández-Martínez, Silvia del C. Rodríguez and Luis Cisneros-Zevallos

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Abstract

The application of postharvest abiotic stresses in horticultural crops induces the accumulation of antioxidants. This approach can be exploited as an alternative to genetic manipulation. Understanding the physiological basis for the synthesis of antioxidants as a postharvest stress response is crucial for the effective use of this technology. Phenylalanine ammonia-lyase (PAL) is activated by reactive oxygen species (ROS, i.e. O_2- and H_2O_2) and other stress signaling molecules. PAL catalyzes the liming step in phenolics biosynthesis. Superoxide radical (O_2-) is produced via a diphenyleneiodonium chloride (DPI) sensitive Ca2+-dependent NADPH oxidase and converted into O_2 and H_2O_2 by superoxide dismutase (SOD). Ascorbate peroxidase (APX) and catalase (CAT) are key enzymes in ROS signaling since they finely modulate low nontoxic H_2O_2 levels. The present project objectives were: (i) to evaluate the use of extreme postharvest stress conditions of wounding and hyperoxia to produce high amounts of phenolics in carrots, and (ii) to determine the role of ROS as signal for the stress response. Whole carrots were wounded to obtain shreds. The shredded-carrots were stored at 20°C in jars and ventilated with humidified flows of either air or 80% O_2 (hyperoxia) for 48 h. Prior to storage, a portion of the shredded-carrots were submerged for 3 min in a DPI solution (to inhibit ROS production). Before and during storage, total phenolics content (PC) and total antioxidant activity (AOX) were evaluated. Likewise, the wounding and hyperoxia effects on PAL, NADPH oxidase, SOD, APX and CAT activities were determined. Shredded-carrots stored under hyperoxia induced a larger fold increase in PC~4.3, AOX~4.38 and PAL activity~47 than air stored shreds (PC~3.7, AOX~3.34, PAL~37) compared with day 0 samples. Hyperoxia induced a higher NADPH oxidase and SOD and a lower APX and CAT activation in the wounded carrots suggesting higher ROS accumulation. Treating shredded-carrots with DPI inactivated NADPH oxidase and PAL as well as PC accumulation confirming the ROS mediated synthesis of phenolics. Carrots can be used as biofactories of antioxidants when subjected to extreme postharvest stresS conditions. Combining hyperoxia and wounding is an effective strategy to increase the wounded induced-ROS accumulation thus triggering a higher production of phenolic antioxidants.