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Rapid communication

Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity

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Abstract

The interest in the consumption of pepper fruits (*Capsicum annuum* L.) is, to a large extent, due to its content of bioactive nutrients and their importance as dietary antioxidants. A greenhouse experiment was carried out to determine the effects of salinity and different ripening states of pepper fruits on several compounds with antioxidant properties. Fruits from plants grown under three saline treatments (0, 15, and 30 mM NaCl) were collected at three maturity states (green, turning, and red). Antioxidant activity in the hydrophilic (HAA) and lipophilic (LAA) fractions, lycopene, β-carotene, ascorbic acid, total phenolic compounds and reducing sugars were determined. From the nutritional point of view, the red state was the most appropriate state of maturation, since red peppers had the highest levels of lycopene, β-carotene, and sugars and the highest antioxidant activity for both hydrophilic and lipophilic fractions. The effect of salinity depended on the maturity state of the peppers: it had no effect on HAA, β-carotene or sugars, but decreased ascorbic acid and total phenolic compounds, and increased LAA and lycopene. The use of a moderately-saline water was beneficial when peppers were harvested in the red state, by increasing HAA and LAA in fruits, with no significant effects on other parameters.

Keywords: *Capsicum annuum* L.; Lycopene; β-carotene; Antioxidant activity; Ascorbic acid; Phenolic content; Ripening state; Salinity

1. Introduction

Pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colours and antioxidant compounds (Howard, Talcott, Brenes, & Villalon, 2000; Lee, Howard, & Villalon, 1995). The intake of these compounds in food is an important health-protecting factor. They have been recognized as being beneficial for prevention of widespread human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts (Bramley, 2000; Sies, 1991).

A wide spectrum of antioxidant compounds is present in pepper fruits. Phenolic compounds retard or inhibit lipid autoxidation by acting as radical scavengers (Namiki, 1990) and, consequently, are essential antioxidants that protect against propagation of the oxidative chain. It is also known that vitamin C, an important compound of pepper fruits, chelates heavy metal ions (Namiki, 1990), reacts with singlet oxygen and other free radicals, and suppresses peroxidation (Bielski, Richter, & Chan, 1975), reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Harris, 1996). Carotenoids play an important role in fruit colouring and act as antioxidants, reacting with free radicals, mainly peroxide radicals and singlet molecular oxygen (Namiki, 1990). Lycopene is a powerful natural

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antioxidant that acts as the most efficient singlet oxygen quencher in vitro among common carotenoids (Di Mascio, Kaiser, & Sies, 1989) and as a determinant factor in reducing the mortality from several cancers (Gerster, 1997; Tsugane, Tsuda, Gey, & Watanabe, 1992; Zhang et al., 1997). For another major carotenoid in pepper, β-carotene, there is much in vitro evidence of its interaction with free radicals, acting as a chain-breaking antioxidant and as a scavenger and quencher of singlet oxygen (Conn, Lambert, Land, Schalch, & Truscott, 1992; Palozza & Krinsky, 1992).

From the agronomic point of view, for optimum production, pepper plants require environmental (temperature and light) conditions typical of arid and semi-arid regions, where irrigation water is usually limited, and saline. Salinity decreases pepper yield (Chartzoulakis & Klapaki, 2000; Navarro, Garrido, Carvajal, & Martinez, 2002) and imposes stress conditions on crop plants. Plants subject to harmful stress conditions produce cytotoxic activated oxygen that can seriously disrupt normal metabolism, through oxidative damage of lipids, proteins, and nucleic acids. In order to defend themselves against oxidants, plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes that protect against the potentially-cytotoxic species of activated oxygen. Plants with high levels of antioxidants, either constitutive or induced, have been reported as having greater resistance to this oxidative damage (Dhindsa & Matowe, 1981; Foyer, 1993).

Pepper fruits can be consumed at different ripening stages (green, red or not fully-ripe). Free sugars play an important role in the flavour characteristics of fruits and ripening physiology has a considerable implication for the pattern of sugar accumulation of the fruits (Bognar, Bohling, & Forty, 1990; Schaffer, Rylski, & Fogelman, 1989). Apart from changes in carbohydrates, other events of nutritional importance for pepper take place during ripening. The scarcity of good-quality water in areas where peppers are grown makes necessary the use of saline waters for irrigation. Since hyperosmotic stress activates a physiological antioxidative response (Smirnoff, 1995), in the present work we have studied this response in pepper grown at different salinity levels, as well as the changes that take place during different maturity stages, in order to improve the management and harvesting of this crop and obtain fruits of a higher nutritional value.

2. Material and methods

2.1. Plant material and chemicals

The experiment was carried out in a greenhouse equipped with an automatic regulated computer system for drip irrigation. Pepper plants (*Capsicum annuum* L. cv. Orlando, a "California"-type pepper) obtained from a commercial nursery, were transplanted (13th December) into 1.2 m length perlite sacks. The base nutrient solutions used for irrigation (pH 5.6) had the following macronutrient composition (mM): NO_3^-, 14; $H_2PO_4^-$, 1.5; SO_4^{2-}, 1; Ca^{2+}, 4; K^+, 7.5; Mg^{2+}, 1. Micronutrient concentrations were (mg l^{-1}): Fe, 1.0; Mn, 0.5; B, 0.25; Cu, 0.02; Mo, 0.01. The plants were irrigated according to the demand detected in the appropriate trays.

Salinity treatments consisted of three NaCl levels (0, 15, and 30 mM NaCl), that constituted S1, S2, and S3 treatments, respectively. Salt was added on three consecutive days to the base nutrient solution to avoid osmotic shock. Each treatment was replicated three times and consisted of two sacks of perlite, each containing three plants and three 2-l h^{-1} emitters, about 40 cm apart. The pH and the conductivity of the nutrient solution were controlled during each irrigation period, while the amount of nutrient solution applied depended on the demand detected in the appropriate trays.

2.2. Samples

Three ripening stages were considered: green (fully developed fruit just before the onset of maturation), turning (approximately one-half green skin and the other half red) and red (completely red skin). Overmature and damaged fruits were discarded. During the middle of the harvest period, six uniform fruits in the green, turning, and red states were selected from each replicate. The six fruits of each replicate were divided into two subgroups, there being a total of six replicates per treatment and three fruits per replicate. Each fruit was weighed fresh, after being washed with deionized water, rinsed free of seeds and cut into two halves. One of them was liquefied, centrifuged and frozen at −20 °C. The three fruit extracts from the same replicate were combined; this constituted the water-soluble fraction for the sugars, ascorbic acid and phenolic acid contents and the antioxidant activity in hydrophilic fraction (HAA) determinations. The other half was cut into small pieces. A sample of these pieces was weighed fresh and then oven-dried for water content determination. The rest of the fruit pieces (frozen at −20 °C) from the same replicate were combined for determination of carotenoids, chlorophyll, and antioxidant activity of the lipophilic fraction (LAA).

2.3. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) was obtained from Fluka Chemical Co. and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) from Sigma–Aldrich Chemical Co. Other reagents were of analytical grade.