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Effect of antioxidants in controlling enzymatic browning of minimally processed persimmon 'Rojo Brillante'

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ABSTRACT

'Rojo Brillante' is an important variety of persimmon that after removal of the astringency with high levels of CO_2, maintains firmness and sweetness, making possible its commercialization as a fresh-cut commodity. However, the commercial success of the product is limited mainly by enzymatic browning. This work presents the effect of a wide range of antioxidants on enzymatic browning of 'Rojo Brillante' persimmon combining in vitro (extracts and precipitates) and in vivo (cut tissue) studies. Preliminary screening of the antioxidants, determined by absorbance and color measurements of persimmon extracts and pellets, showed that 4-hexylresorcinol (Hexyl), citric acid (CA) and calcium chloride ($CaCl_2$) were effective at controlling browning at 10 mM; whereas, ascorbic acid (AA) required a higher concentration (25 mM). Peracetic acid, cyclodextrin, cysteine, and hexametaphosphate were not effective at controlling browning, even at a concentration of 50 mM. In in vivo studies, AA (1.12%) and CA (0.21%) were the most effective treatments to control enzymatic browning of fresh-cut material, reaching the limit of marketability in 5–7 days, whereas, Hexyl and $CaCl_2$ did not reach 1 day of storage. The results showed that optimum concentrations in cut tissue did not always correlate with the in vitro studies, indicating that antioxidants have an effect not only in browning reactions, but also in metabolic activity and cell wall changes during wound-induced reactions. The results provide relevant information for further development of minimally processed 'Rojo Brillante' persimmon during storage at 5 °C.

1. Introduction

'Rojo Brillante' is an important persimmon cultivar in the zone Ribera Xuquer (Valencia, Spain). In the last decade, its production has significantly increased and the fruit is now considered as an alternative to other crops, with an important presence in European markets. At harvest, this cultivar has excellent sensory quality and firmness; however, the presence of high concentrations of soluble tannins makes the fruit inedible because of its astringency. Exposure to high levels of carbon dioxide (95% for 24 h at 20 °C) has proven to be the most effective way to remove astringency while maintaining fruit firmness (Arnal and del Río, 2003). The effectiveness of this technology makes possible the commercialization of 'Rojo Brillante' persimmon as fresh-cut fruit. However, the high phenolic content of persimmons increases the susceptibility of oxidation by the enzyme polyphenol oxidase (PPO) in the presence of O_2, leading to the formation of brown pigments on the cut surface.

Common technologies used to prevent browning include reduction of temperature, use of modified atmosphere (MA) packaging, and application of antioxidants (Garcia and Barrett, 2002). In persimmon, very few studies have been carried out to control browning and extend the quality of the fresh-cut fruit. Wright and Kader (1997) showed that controlled atmosphere storage with 12% CO_2 slightly increased shelf-life of sliced persimmon fruit, delaying the appearance of black areas on the surface. The application of honey solution dips extended the shelf-life of fresh-cut persimmon fruit by delaying off-aroma development, firmness loss and jelling (Ergun and Ergun, 2010), whereas, persimmon cubes subjected to vacuum impregnation with sucrose did not avoid browning, suggesting the need for antioxidants (Igual et al., 2008).

The antioxidants used with fresh-cut fruit and vegetables include acidulants such as citric, ascorbic, and peracetic acid (CA, AA, and PA), reducing agents such as cysteine (Cys) and AA, chelating and complexing agents such as hexametaphosphate (HMP) and cyclodextrin (CD), or enzymatic inhibitors such as 4-hexylresorcinol (Hexyl) and calcium chloride ($CaCl_2$) (Garcia and

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Table 1
Effect of antioxidant type and concentrations on browning of 'Rojo Brillante' persimmon extracts and precipitates.

Treatment[a]	Concentration (mM)	% (w/v)	pH	Extract effectiveness		Precipitate effectiveness		Global effectiveness
				Abs_{450}	Visual[b]	ΔE[c]	Visual[c]	
AA	10	0.18	4.56	0.223 b	1	3.94 a	2	NO
	25	0.45	3.97	0.126 a	3	8.64 b	3	YES
CA	2	0.04	4.83	0.322 b	0	4.74 a	3	NO
	10	0.21	3.77	0.121 a	3	6.01 a	3	YES
PA	10	0.08	4.90	0.234 b	1	2.19 a	0	NO
	25	0.20	4.57	0.203 b	1	3.28 a	0	NO
	50	0.62	4.32	0.146 a	2	7.02 b	2	NO
$CaCl_2$	2	0.02	5.92	0.175 b	3	2.88 a	2	NO
	10	0.12	5.74	0.080 a	3	8.06 b	3	YES
CD	10	1.14	5.68	0.351 b	0	3.74 a	2	NO
	25	2.84	5.95	0.205 a	1	4.22 a	2	NO
	50	5.69	6.02	0.264 a	1	9.55 b	3	NO
Cys	10	0.12	5.50	0.317 a	0	3.59 ab	0	NO
	25	0.31	5.92	0.300 a	0	5.47 b	2	NO
	50	0.62	5.74	0.286 a	0	4.56 b	2	NO
	75	0.92	5.41	0.281 a	0	2.06 a	2	NO
HMP	10	0.10	6.27	0.403 a	0	2.95 a	1	NO
	25	0.26	6.19	0.437 a	0	5.35 b	2	NO
	50	0.52	5.88	0.491 b	0	4.09 ab	2	NO
Hexyl	2	0.04	5.87	0.172 b	3	7.11 a	2	NO
	10	0.20	5.97	0.048 a	3	13.54 b	3	YES
Control	0	0	5.88	0.300	0	–	0	NO
Blank	113	2.00	3.20	0.117	3	8.23	3	YES

[a] AA: ascorbic acid; CA: citric acid; PA: peracetic acid; $CaCl_2$: calcium chloride CD: cyclodextrin; Cys: cysteine: HMP: hexametaphosphate; Hexyl: 4-hexylresorcinol; Blank: reference sample prepared with AA at 113 mM, which provided complete inhibition of browning in extract and precipitate.
[b] Visual evaluation: 0 = totally browned, 3 = not presence of browning.
[c] Color difference with control sample $\Delta E = ((L^* - L_c^*)^2 + (a^* - a_c^*)^2 + (b^* - b_c^*)^2)^{1/2}$.
For each antioxidant, means values with the same letter are not different ($p \leq 0.05$).

Barrett, 2002). In general, the effect of these antioxidants in controlling enzymatic browning of fresh-cut fruit depends on many factors such as commodity, cultivar, concentration, synergy with other antioxidants, pH, application system, etc. In persimmon, the effect of some antioxidants as natural inhibitors of PPO enzyme purified from the fruit has been studied (Núñez-Delicado et al., 2003; Özen et al., 2004), and preliminary work from our group showed some improvement in delaying browning of fresh-cut tissue (Pérez-Gago et al., 2009).

In the literature, browning evaluation is generally based on reflectance measurement (L^*, a^*, b^*) on fresh-cut surface of fruit and vegetables during storage (in vivo studies). Nevertheless, in vitro studies, involving extraction of soluble browning products and measurement of absorbance at particular wavelengths, have also been suggested as pre-screening to determine the potential effect of antioxidant agents controlling enzymatic browning of fruit and vegetables tissues, such as apples and pears (Eissa et al., 2006; Arias et al., 2008; Chiabrando and Giacalone, 2012). Because not all PPO products are water soluble, Amiot et al. (1992) suggested that to estimate the susceptibility of apple to browning, the absorbance at 400 nm of the supernatant and lightness (L^*) of the pellets obtained after centrifugation should be measured as a value of soluble and insoluble brown pigments, respectively. Therefore, the aim of this work was to study the potential to control enzymatic browning of a wide range of antioxidant agents at different concentrations in the extracts and precipitates of 'Rojo Brillante' persimmon (in vitro studies). Then the most effective antioxidants were studied on fresh-cut material during storage at 5 °C (in vivo studies).

2. Materials and methods

The study was divided into two parts. In the first part, enzymatic browning was determined in persimmon extracts and precipitates, and the second part was carried out with fresh-cut persimmon material.

2.1. Plant material and antioxidants

'Rojo Brillante' persimmons were provided by the Cooperative 'Nuestra Señora de Oreto' (l'Alcudia, Valencia, Spain). Astringency was removed by maintaining the fruit at 20 °C in closed containers with 95% CO_2 for 24 h. After removal from the containers, the fruit were stored in air at 15 °C for 1 day until processing. The antibrowning agents tested included ascorbic acid (AA) and citric acid (CA) from Quimivita (Barcelona, Spain), peracetic acid (PA) from Fluka (Sigma Co., Barcelona, Spain), calcium chloride ($CaCl_2$), hexametaphosphate (HMP), cyclodextrin (CD), cysteine (Cys), and 4-hexylresorcinol (Hexyl) from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Determination of enzymatic browning in persimmon extracts and precipitates (in vitro studies)

Persimmons were cleaned, peeled, cut into small pieces, frozen with liquid nitrogen, and crushed with a blender (Braun, Model MR350, Kronberg im Taunus, Germany). The ground samples were stored at −20 °C until analysis, which was done within 2 weeks, to avoid browning of the tissue.

For the analysis, 3 g of frozen samples were introduced into a centrifuge tube that contained 30 mL of the antioxidant solution. An initial concentration of 10 mM was tested for all the antioxidants and concentrations were increased or decreased depending on absorbance and reflectance measurements. The study was concluded when a concentration provided the complete inhibition of soluble and insoluble brown pigments or when an increase in the concentration of the antioxidant did not show a significant improvement to control browning. Table 1 shows the antioxidant