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Antibrowning effect of antioxidants on extract, precipitate, and fresh-cut tissue of artichokes

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ABSTRACT

The effect of antioxidants controlling enzymatic browning of artichokes cv. 'Blanca de Tudela' was studied in extracts and fresh-cut tissue. Initially, the effect of ascorbic acid (AA), citric acid (CA), peracetic acid (PA), calcium chloride ($CaCl_2$), cyclodextrin (CD), cysteine (Cys), hexametaphosphate (HMP), and 4-hexylresorcinol (Hexyl) at different concentrations was studied in extracts and precipitates. Absorbance at 450 nm of artichoke extract and color of the pellets were measured, as a preliminary screening of antioxidants controlling browning. AA at 10 mol/m^3 was the most effective controlling browning in the extract and pellet; whereas, Cys and 4-Hexyl were effective at a higher concentration (50 mol/m^3) and CA was only effective in the extract. Application of AA, CA, Cys, and Hexyl at different concentrations was studied on fresh-cut artichokes during storage at 5 °C. Samples treated with Cys (0.1, 0.3, 0.5, 1%) showed the highest L^* and lowest a^* values. An increase in Cys concentration decreased a^* and increased b^* values, which correlated with a decrease in browning and an increase in yellowness of the tissue. Application of CA (1, 2.7, 5.3%), AA (0.5, 1, 1.5, 2%) and Hexyl (0.002, 0.005%) did not inhibit enzymatic browning. Visual evaluation confirmed these results.

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1. Introduction

Artichoke (*Cynara scolymus* L.) is a typical vegetable of the Mediterranean area widely known for its high dietary fiber, and healthy protective actions as diuretic, hypocholesterolemic, hepatoprotective, anti-inflammatory and anti-microbial properties (Gebhardt, 1997). Due to the nutritional benefits and gastronomic properties artichoke demand is increasing among consumers. Minimally processing steps such as washing, removal of external leaves, slicing and packaging can bring important advantages for artichoke commercialization, reducing transport costs, storage space, and preparation time for consumers. However, these steps induce enzymatic browning, in addition to a quality deterioration associated with water loss, softening, microbial contamination, increase of respiration and ethylene production, that result in a reduced shelf life (Ahvenainen, 1996). Among all the factors reducing shelf-life of fresh-cut artichoke, enzymatic browning caused by oxidation of phenolic compounds by polyphenol oxidase enzyme (PPO) is the major problem. Browning might be prevented by chemical and physical methods, including reduction of temperature and/or oxygen, use of modified atmosphere (MA) packaging, and application of antioxidant agents that act to inhibit enzyme, remove its substrates or function as preferred substrate (Garcia & Barrett, 2002).

Antioxidant treatments used in several fresh-cut fruits and vegetables include acidulant and reducing agents such as citric and ascorbic acid (CA and AA) (Lattanzio, Linsalata, Palmieri, & Van Sumere, 1989; Son, Moon, & Lee, 2001), thiol containing compounds such as cysteine (Cys) (Amodio, Cabezas-Serrano, Peri, & Colelli, 2011; Oms-Oliu, Aguiló-Aguayo, & Martín-Belloso, 2006; Pérez-Gago, Serra, & del Río, 2006), chelating and complexing agents such as hexametaphosphate (HMP) and cyclodextrin (CD) (Pilizota & Sapers, 2004), or compounds that directly inhibit PPO such as 4-hexylresorcinol (Hexyl) and calcium chloride ($CaCl_2$) (Arias, González, Peiró, Oria, & López-Buesa, 2007; Luo & Barbosa-Cánovas, 1997; Monsalve-Gonzalez, Barbosa-Cánovas, Cavalieri, McEvily, & Iyengar, 1993; Rosen & Kader, 1989).

Effectiveness of an antibrowning agent depends on many factors such as cultivar, concentration, synergy with other antioxidants, pH,

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application system, etc. In artichoke, the degree of browning has been correlated with the nature and amount of its phenolic compounds that may vary according to the genotype (Cabezas-Serrano, Amodio, Cornacchia, Rinaldi, & Colelli, 2009; Doğan, Turan, Ertürk, & Arslan, 2005; Todaro, Peluso, Catalano, Mauromicale, & Spagna, 2010). Artichoke cv. Blanca de Tudela is one of the main cultivars grown in Spain. A few works have studied the effect of packaging and washing disinfection on spoilage and microbiological quality of minimally processed artichokes 'Blanca de Tudela' (Giménez et al., 2003; Sanz, Giménez, & Olarte, 2003; Sanz, Giménez, Olarte, Lomas, & Portu, 2002). However, enzymatic browning is still the main limitation for the development as a minimally processed produce, and no works have been published with the aim of finding an effective anti-browning treatment. Few approaches have been conducted to inhibit browning in fresh-cut artichokes, mainly using CA and AA (Giménez et al., 2003; Lattanzio et al., 1989). In a recent work, Amodio et al. (2011) reported that only Cys had some effect controlling browning of fresh-cut 'Catanese' artichokes, whereas AA, CA, $CaCl_2$, and Hexyl had little or not effect.

In the bibliography, browning evaluation is based on reflectance measurement (L^*, a^*, b^*) on fresh-cut surface of fruits and vegetables during storage (*in vivo* studies). Nevertheless *in vitro* studies, involving extraction of soluble browning products and measurement of absorbance at particular wavelengths, can be performed as pre-screening to determine the potential effect of antioxidant agents controlling enzymatic browning of fruit and vegetable tissues (Garcia & Barrett, 2002). Considering that not all PPO products are water soluble, Amiot, Tacchini, Aubert, and Nicolas (1992) suggested that the degree of browning of a tissue can be estimated by measuring the maximum optical absorbance of the supernatant and the reflectance of the pellet (L^*, a^* and b^* values), obtained after centrifugation during the preparation of the soluble pigment extract, as values of soluble and insoluble brown pigments, respectively. Therefore, the aim of this work was to study the effectiveness of a wide range of antibrowning agents at different concentrations controlling browning in extracts and precipitates of artichoke (*C. scolymus* L., cv. Blanca de Tudela) (*in vitro* studies). Then the most effective antioxidant agents were studied on fresh-cut 'Blanca de Tudela' artichokes during storage at 5 °C (*in vivo* studies).

2. Materials and methods

The study was divided in two steps. In the first part enzymatic browning was determined in artichoke extracts and precipitates, meanwhile the second part was carried out in fresh-cut artichoke cv. Blanca de Tudela.

2.1. Plant material and antioxidants

Artichoke (*C. scolymus* L., cv. Blanca de Tudela) were purchased in a local market (Valencia, Spain) and stored at 5 °C for 24 h until processing. 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. Louise, MO; USA).

2.2. Determination of enzymatic browning in artichoke extracts and precipitates

Samples were washed to eliminate soil and dirt. External bracts, leaves and stalk were removed and artichoke heads were cut in small sections, frozen with liquid nitrogen and crushed with a blender (Braun, Model MR350, Kronberg im Taunus, Germany). Ground samples were stored at −20 °C till analysis to avoid browning of the tissue.

For the analysis, 3 g of frozen samples were introduced in a centrifuge tube containing 30 ml of the antibrowning agent. An initial concentration of 10 mol/m^3 was tested for all the antioxidants. Concentrations were either increased or decreased depending on absorbance and reflectance measurements obtained for each antioxidant. Table 1 shows the antioxidant concentrations tested. A reference sample or 'blank' was prepared with 113 mol/m^3 AA. This concentration of AA provided a complete inhibition of -soluble and insoluble brown pigments. Water was used as untreated control. Samples were homogenized with a high-shear probe mixer (PolyTron, Model PT 2100, Kinematica AG Inc., Lucerne, Switzerland), left 1 h at 20 °C and then centrifuged for 10 min at 12,000 rpm at 5 °C. Absorbance of extracts was determined at 450 nm with a UV spectrophotometer (Thermo Electron Corporation, Auchtermuchty, Fife, UK). This absorbance corresponded to the maximum difference observed among samples in the range 360–500 nm. The precipitate was poured into a petri dish and L^* (lightness), a^* (red to green), and b^* (yellow to blue) values were measured with a Minolta (Model CR-300, Ramsey, N.Y., USA) on the bottom of the dish. A standard white calibration plate was employed to calibrate the equipment. Data were reported as the total color difference with the control sample (c) with no antioxidant as:

$$\Delta E = \left(\left(L^* - L_c^*\right)^2 + \left(a^* - a_c^*\right)^2 + \left(b^* - b_c^*\right)^2\right)^{1/2}$$

Both extracts and precipitate were evaluated visually by three judges using an enzymatic browning scale, where: 0 = totally browned, 1 = partially browned, 2 = slightly browned, 3 = no browned. Treatment was considered effective when both extracts and precipitate were visually scored as 3.

2.3. Determination of enzymatic browning in fresh-cut artichokes

After washing, artichoke external bracts, leaves and stalk were removed. Artichoke hearts were cut in slices (5 mm approximately) using a sharp stainless-steel knife. Pieces were dipped in the antioxidant solutions for 3 min, drained and dried under cold conditions. In the first experiment with fresh-cut artichokes, antioxidants were AA, CA, Cys, and Hexyl at concentrations reported in Table 2. A second experiment was conducted with AA (0.5 g/100 g, 1 g/100 g, and 1.5 g/100 g), Cys (0.1 g/100 g, 0.3 g/100 g, 0.5 g/100 g, and 1 g/100 g) and Hexyl (0.002 g/100 g, and 0.005 g/100 g) at different concentrations from those studied in experiment 1 to conclude the effect of the antioxidants in fresh-cut artichokes. Once dried, 4 pieces were placed in polypropylene trays that were heat-sealed with microperforated films (35 μm thickness) (35 PA 200, Amcor Flexibles, Barcelona, Spain). To ensure no modification of the atmosphere in the tray and study only the effect of the antibrowning agents, the polypropylene films were additionally perforated with a needle. Finally, samples were stored 7 days at 5 °C. A maximum of 15 artichokes were processed at the same time to minimize their exposure to oxygen and the whole process was carried out in a temperature-controlled room at 10 ± 1 °C under suitable hygienic conditions.

Color measurements were made periodically with a Minolta (Model CR-300, Ramsey, N.Y., USA) on 12 artichoke pieces per treatment using the CIELAB color parameters, L^*, a^*, and b^*. Each measurement was taken randomly at three different locations of each sample piece. A standard white calibration plate was employed to calibrate the equipment. The results were expressed as the means of the 12 measured samples per each sampling day.

During storage, artichoke pieces were also evaluated visually by 10 judges. Each treatment was coded, presented in random order

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