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Physiological basis of sensitivity to enzymatic browning in 'lettuce', 'escarole' and 'rocket salad' when stored as fresh-cut products

Elena Degl'Innocenti [a], Alberto Pardossi [b], Franco Tognoni [b], Lucia Guidi [a,*]

[a] *Dipartimento di Chimica e Biotecnologie Agrarie, Università di Pisa, 56124 Pisa, Italy*
[b] *Dipartimento di Biologia delle Piante Agrarie, Università di Pisa, 56124 Pisa, Italy*

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Abstract

In fresh-cut leafy vegetables, the operation of cutting may stimulate enzymatic browning, with important commercial consequences. In this work, a number of physiological and biochemical parameters, including the activities of key enzymes involved in the metabolism of phenols (such as PAL, PPO, and PODs) and ascorbic acid (ASA), were measured in three species: lettuce (*Lactuca sativa* var. *capitata* L.), escarole (*Cichorium indivia* var. *latifolium*) and rocket salad (*Eruca sativa*), upon cold storage as fresh cuts. The first two species are quite sensitive to leaf browning, which does not affect rocket salad.

The resistance of rocket salad to browning was associated with a much higher ASA content and a decrease in this compound upon storage, compared to other species in which ASA remained either constant (lettuce) or increased (escarole). It is proposed that the resistance of rocket salad to this disorder, which markedly affects other leafy species, e.g., lettuce and escarole, is a result of the inhibition of PPO activity and/or the reduction of quinones to phenols, which may both be induced by ASA.

Keywords: Ascorbic acid; Enzymatic browning; Peroxidases; Phenylalanine ammonia lyase; Polyphenol oxidase; Visual quality

1. Introduction

Fresh-cut (minimally-processed) horticultural products are subjected to simple operations soon after harvest, such as cleaning, washing, cutting and packaging, which make them ready-to-use. Cutting is the main factor responsible for the deterioration of these products during storage which, as a consequence, is more rapid than whole products.

Enzymatic browning in leafy vegetables is considered one of the most important disorders, since it is easily detected by consumers, evident consequences on marketing. Plant species react in different ways to cut-induced leaf browning; lettuce is one of the most studied species from this point of view as it shows a great sensitivity to this disorder (Saltveit, 2000). Altered phenol metabolism is thought to be involved in leaf browning of lettuce (Saltveit, 2000). The first step in phenol metabolism is the conversion of the amino acid L-phenylalanine to *trans*-cinnamic acid by the enzyme phenylalanine ammonia lyase (PAL, EC 4.3.1.5). The phenol compounds synthesized by PAL activity can be oxidized, by polyphenoloxidase (PPO, EC 1.10.32), to quinones, which spontaneously polymerize to brown pigments responsible for tissue browning (Ke & Saltveit, 1986, 1989). For this reason, some authors have suggested that the activity of PAL may be a marker for shelf-life in some fresh-cut products (e.g., Degl'Innocenti, Guidi, Pardossi, & Tognoni, 2005; Hyodo & Fujinami, 1989; Ke & Saltveit, 1986; Lopez-Galvez, Saltveit, & Cantwell, 1996; Ritenour, Ahrens, & Saltveit, 1995; Tavarini, Degl'Innocenti, Guidi, & Paradossi, in press). Indeed, an increased PAL activity was found to be correlated with the susceptibility to browning in lettuce fresh-cuts (Couture, Cantwell, Ke, & Saltveit, 1993). Moreover, the content of phenols is associated with the sensitivity to

* Corresponding author. Tel.: +39 50 2216613; fax: +39 50 2216630.
E-mail address: guidilu@agr.unipi.it (L. Guidi).

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enzymatic browning in several vegetables, such as artichoke (Brecht et al., 2004).

In addition to the oxidation of phenols carried out by PPO, peroxidases (POD; E.C. 1.11.1.7) contribute to tissue browning. Indeed, mono- and di-phenol compounds may be substrates for POD activity (Degl'Innocenti et al., 2005; Nicolas, Cheynier, Fleuriet, & Rouet-Mayer, 1993; Robinson, 1991; Tavarini et al., in press). These enzymes catalyze the oxidation of phenols by the H_2O_2 produced during respiration or as a consequence of wounding (Amiot, Fleuriet, Veronique, & Nicolas, 1997).

Different treatments have been evaluated to reduce browning in fresh-cut products, such as the application of antioxidant compounds (e.g., sulfites), calcium salts to maintain membrane integrity, chemical inhibitors of PPO and/or POD, or the use of modified atmosphere packaging to exclude oxygen (Saltveit, 2000). Ascorbic acid (ASA) is a highly effective inhibitor of enzymatic browning in many tissues, primarily because it is able to reduce quinones to phenolic compounds, thus preventing the synthesis of the brown pigments (Walker, 1995). Moreover, at high concentration, ASA may inhibit PPO by decreasing the cytosol pH (Vamos-Vigyazo, 1981).

The aim of this work was to study the main biochemical processes involved in the enzymatic browning of minimally processed leafy vegetables; in particular, we tested the hypothesis developed in previous papers (Degl'Innocenti et al., 2005; Reyes, Villareal, & Cisneros-Zevallos, 2007) that a high constitutive ASA content is associated with the resistance of fresh-cut leaves to colour alteration upon storage. Three species were selected for their different sensitivities to this disorder, as assessed in preliminary work: lettuce (*Lactuca sativa* var. *capitata* L.), escarole (*Cichorium indivia* var. *latifolium*), and rocket salad (*Eruca sativa*); these species also differed with respect to their leaf constitutive content of ASA. Lettuce and escarole, in which browning is evident within few hours after cutting, had a much lower ASA content (less than 4 $\mu g\ g^{-1}$ FW) than had rocket salad, which contained up to 80 $\mu g\ g^{-1}$ FW and did not show any browning up to 8–10 days of cold storage.

2. Materials and methods

2.1. Plant material

Plant material was taken from a local market and processed in the laboratory within a few minutes. The plants had been cultivated in commercial greenhouses in the period between January and May 2005. The leaves were gently washed with chlorinated drinking water and excess water was removed with a manual salad spinner. Afterwards, leaves were cut (approximately 1 cm × 2 cm) perpendicular to the midrib with stainless steel scissors and stored for up to three days at 4 °C in plastic boxes (1.5 l). Whole heads of lettuce and escarole or intact leaves of rocket salad were stored under the same conditions as the fresh-cuts. Determinations were made of both fresh-cuts and intact leaves every day during storage and, for some parameters, also at different times during the first 24 h from processing.

2.2. Leaf browning assessment

To evaluate the occurrence of leaf browning, Image Tool 3.00 for Windows (University of Texas Health Science Center, San Antonio, USA) scanning software was used. A picture of each sample was captured with a digital camera Nikon Coolpix E4500, with a resolution of 118 pixel cm^{-2}. The images were imported by Image Tool, which created a grey copy to identify the brown spots on the leaves. The incidence of browning was determined as the percentage of colour-altered area.

2.3. Enzyme assay

2.3.1. PAL activity

PAL activity was measured as described by Degl'Innocenti et al. (2005). One unit of PAL activity equals the amount of PAL required to deaminate 1 μmol of L-phenylalanine to *trans*-cinnamic acid and NH_3 in 1 h/g fresh weight.

2.3.2. PAL inactivating factor

To measure PAL-Inactivating Factor (IF), 10 g of vegetable tissue were placed in tubes containing 16 ml of HEPES (hydroxyethylpiperazine ethanesulfonic acid) buffer, 0.1 M (pH 7.5), with 400 $\mu l\ l^{-1}$ of β-MeSH (mercaptoethanol) and 1 g PVPP (polyvinylpolyrrolidone) (Ritenour & Saltveit, 1996). The vegetable tissue was homogenized and centrifuged at 10 000*g* for 15 min. The supernatant was discarded and the pellet resuspended in 1 ml of HEPES buffer, 0.1 M (pH 6.0–8.8). Incubation of sample preparations and PAL enzyme were carried out by mixing 32 μU PAL from *Rhodotorula glutinis* (Sigma) with 1 ml of resuspended pellet and holding it at 30 °C. After 1 h of incubation, 0.3 ml of the incubation solution was microcentrifuged for 2 min and 0.2 ml of the resulting supernatant was used to measure PAL activity, as described in Degl'Innocenti et al. (2005).

2.3.3. PPO enzyme

PPO activity was determined according to the method reported by Degl'Innocenti et al. (2005). One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (480 nm) per min per mg of soluble protein. Protein determinations were performed using the Protein Assay Kit II (Bio Rad).

2.3.4. Peroxidases (POD)

The determination of POD activity was carried out as reported in Degl'Innocenti et al. (2005). Absorbance was recorded at different wavelengths, depending on the substrate utilized: 470 nm for guaiacol substrate, 654 nm for tetrametylbenzidine (TMB), 410 nm for chlorogenic acid and 450 nm for caffeic acid. POD activity was expressed as Δ_{ABS} per min per g fresh weight.