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Effect of anti-browning solutions on quality of fresh-cut fennel during storage

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ABSTRACT

Fresh-cut fennel (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Orion*) is a very perishable crop due to the browning that affects the cut-surface, especially on the stem portion of the slices. The occurrence of browning is the main cause of quality loss and decrease of visual acceptance of this product. In the present work the effectiveness of different anti-browning solutions (0.5% ethanol, 1% L-ascorbic acid, 0.5% L-cysteine at pH 7, 1% citric acid and 0.5% 4-hexylresorcinol) on maintaining quality characteristics of fresh-cut fennel during 6 days at 5 °C in air conditions were investigated. Results showed that dipping in solutions of citric acid, ascorbic acid, cysteine and 4-hexylresorcinol, did not result in substantial improvement of the appearance of fresh-cut fennels cut-surfaces compared to water control. Among all applied anti-browning solutions, dipping in 1% citric acid and 0.5% 4-hexylresorcinol produced a more severe browning than control, in both stem and sheath cut-surfaces. Dipping for 2 min in 0.5% ethanol was effective in preserving visual quality of fresh-cut fennel stored in air for six days at 5 °C, significantly reducing the browning in both stem and sheath cut-surface. In addition ethanol is a 'generally recognized as a safe' (GRAS) product and did not negatively influence the aroma of fresh-cut fennel. Based on these considerations, dipping in 0.5% ethanol for 2 min could be a useful pretreatment for extending the shelf-life of fresh-cut fennel.

1. Introduction

Fennel (*Foeniculum vulgare* Mill. Subsp. *Vulgare* var. *azoricum*) belongs to the family Apiaceae and it is native to the Mediterranean region (Azeez, 2008). Italy is one of the largest producers of fennel in Europe, which is an autumn/winter vegetable and its edible leaf sheathes are particularly appreciated by consumers of South Europe for their fleshy, and crispy texture and aromatic flavour. The edible part is the swollen basal part called "*grumolo*", but more often referred to as "head" and it is eaten raw in salads or cooked. Processing fennel heads as a fresh-cut product would provide convenience due to the high percentage of discarded plant waste, and the complexity of preparation and trimming operations. The most important factor that limits the shelf life of fresh-cut fennel, is the browning of the cut surfaces (Albenzio et al., 1998; Artés et al., 2002a,b; Escalona et al., 2005a,b), as also for whole fennels which turn brown on the butt end zone. Browning of the cut surface of fresh-cut products is mainly caused by oxidation of phenolics to *o*-quinones, catalyzed by the oxidative enzymes, including polyphenol oxidase (PPO) and peroxidases (POD). Quinones then polymerize to form dark pigments, leading to browning appearance (Garcia and Barret, 2002). Browning may be prevented by inhibiting the activity of PPO by removing one of its necessary reaction components, O_2, enzyme, Cu^{2+} contained on its active site, or substrate (Richardson and Hyslop, 1985; Lambrecht, 1995), or by mechanical or chemical methods (Garcia and Barret, 2002). Among chemical, one of the most common approaches is dipping the slices in aqueous solutions containing anti-browning agents. The anti-browning agents are used to control surface discoloration and generally act directly on the enzyme (i.e. PPO), as enzyme inhibitors, others by rending the medium inadequate for the development of the browning reaction, while others by reacting with the products of the enzymes reaction before the formation of dark pigments (Garcia and Barret, 2002). Ascorbic acid is one of the most extensively used agent to avoid enzymatic browning and acts by reducing the quinone products to their original polyphenol compounds and, to a lesser extent, as an acidulant (Walker, 1977; Garcia and Barret, 2002). Citric acid is a strong acidulant and can inhibit the PPO

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by lowering the pH below the optimal value for the enzyme activity; in addition citric acid can inhibit PPO working through a non-competitive mechanism, by chelating copper at the enzyme active site (Ibrahim et al., 2004; Altunkaya and Gökmen, 2008; Ali et al., 2015). L-cysteine can inhibit the browning by trapping *o*-quinones through the formation of cysteinyl adducts or reducing *o*-quinones to their polyphenols precursors (Cilliers and Singleton 1990; Richard-Forget et al., 1992). However the effectiveness of L-cysteine as anti-browning is largely influenced by pH. In a study on fresh-cut artichoke Cabezas-Serrano et al. (2013) used L-cysteine solutions at the same concentration but at different pH (from 2.2 to 7) and results revealed that L-cysteine at pH 7 was most effective in control browning than low pH cysteine solutions. Similar observations were previously reported by Gorny et al. (2002) on fresh-cut pears and these authors explained that nucleophilic attack of quinones by cysteine may be more effective at a neutral pH since the thiol group of cysteine has a pK_a of 8.33. Thus sulfites act as a reducing agent at a pH below 4 (Cheynier et al., 1989) while at pH above 4, quinones will form colorless adduct products with sulfites or cysteine (Richard et al., 1991). 4-hexylresorcinol is another compound used as anti-browning agent since it is a competitive inhibitors of PPO: it interacts with PPO to render an inactive complex incapable of catalyzing the browning reaction (Lambrecht, 1995; Whitaker and Lee, 1995). A wide range of studies have evaluated the efficacy of different anti-browning agents, alone or in combinations, on fresh-cut fruits and vegetables, (Monsalve-González et al., 1993; Luo and Barbosa-Cánovas, 1995; Sapers and Miller, 1998; Moline et al., 1999; Dong et al., 2000; Chiesa et al., 2001; Gorny et al., 2002; Ibrahim et al., 2004; Amodio et al., 2011; Pace et al., 2015; Wang et al., 2014). Regarding the application of anti-browning reagents to reduce the browning on fennels limited investigations are available. Artés et al. (2002b) reported that treatments with 1% ascorbic acid and 5% citric acid for 1 min at room temperature, stored for 14 d at 0 °C followed by 4 d in air at 15 °C, did not control browning of butt-end cut surface of whole fennels. On the other hand Albenzio et al. (1998) investigated the effectiveness of citric acid solution at different concentrations applied for 15 or 30 min, concluding that dipping fresh-cut fennels for 15 min in 0.1% citric acid was effective in delaying the occurrence of browning during 5 d at 4 °C. In other studies controlled atmosphere (CA) or modified atmosphere packaging (MAP) were applied to preserve quality of fresh-cut fennel, finding that low oxygen (from 4 to 6 O_2 kPa) and high CO_2 levels (from 10 to 15 CO_2 kPa) can reduce the browning of the cut surface (Escalona et al., 2005a,b, 2006; Rinaldi et al., 2010). Starting from these results, it is of interest to further study the efficacy of anti-browning agents on maintaining quality characteristics of fresh-cut fennel during storage at 5 °C in air conditions.

2. Materials and methods

2.1. Sample preparation and experimental design

Fennel heads (*Foeniculum vulgare* Mill. subsp. *vulgare* var. *azoricum* cv. *Orion*) were harvested on December 2013 in Puglia (Italy), transported under passive refrigeration to the Postharvest laboratory of the University of Foggia and kept at 0 °C until processing. After trimming operations, fennel heads were washed in chlorine solution (0.01% v/v) for 2 min, rinsed in tap water for 1 min and dried. Samples were divided in 3 groups of replicates consisting of 13 fennel heads each. The 3 groups were processed one after another in order to avoid the occurrence of browning due to cutting operations. Each of the 13 fennel heads was cut into 8 slices of approximately 1 cm thickness by cutting with a sharp knife perpendicularly to the longitudinal axis, obtaining about 104 slices which were then randomly divided into 13 batches of 8 slices each. One batch was used for initial determinations. The remaining 12 batches were separated in 6 groups (1 for each treatment) containing 2 batches each (one for each sampling day). Fennel slices were immersed for 2 min into water (control) or in one of the following solutions: 0.5% (v/v) ethanol (ET), 1% (w/v) L-ascorbic acid (ASC), 0.5% (w/v) L-cysteine, adjusted to pH 7.0 with NaOH 1 mol L^{-1} (CYS), 1% (w/v) citric acid (CIT), 0.5% (w/v) 4-hexylresorcinol (HR). After drying the slices with 2 layers of cheesecloth, 8 fennel slices per sample were placed in macro-perforated polyethylene clam-packs (119 × 189 × 90 mm; capacity 500 g; CL1/90 Carton Pack®), and stored at 5 °C. Initially, and after 2 and 6 d of storage, three replicates per samples were evaluated for sensorial, physical, chemical and microbiological quality attributes, as following described.

2.2. Sensory and physical attributes

A group of 5 laboratory-trained panelists subjectively assessed samples for appearance, stem and sheath browning, aroma, crunchiness, dehydration and overall quality on a 5-point scale. Appearance was scored using a scale from 5 to 1, where 5 = excellent (fresh appearance, bright white color), 4 = good (slight dehydration on the cut surfaces, very mild signs of deterioration of the stem and absence of defects in the sheath, slight yellowing), 3 = fair (noticeable dehydration on the cut surfaces and yellowing, appreciable signs of deterioration on the stem and mild on the sheath), 2 = poor (severe symptom of dehydration and perceptible deterioration on stem and on the sheath, appreciable browning of the stem), 1 = very poor (severe dehydration on stem and sheath, severe browning of the stem, possible microbial spoilage). A score of 3 was considered as a limit of marketability while a score of 2 was considered as a limit of edibility. Browning of the cut surfaces was scored separately on stem and sheath parts of sliced fennel using a scale from 1 to 5, where 1 = absence of browning, 3 = slight browning, 5 = complete browning. The same 5 point scale structure was used to evaluate subjectively the other attributes such as aroma (1 = absent, 3 = moderate, 5 = full characteristic), crunchiness (1 = not crunchy, 3 = fairly crunchy, 5 = very crunchy), and dehydration (1 = fresh-like, 3 = slightly dehydrated, 5 = very dehydrated). Finally, on the base of all these sensorial parameters, panelists attributed an overall evaluation using a scale from 1 to 5, where 1 = very poor, 3 = fair, and 5 = excellent.

Color of the fennel slices was measured elaborating the images acquired with a Spectral scanner (version 1.4, DV s.r.l., Padova, Italia) equipped with a Spectral Imaging spectrometer V10 type (400–1000 nm, 25 μm slit, resolution 5 nm). One scan of 8 slices per replicate was acquired with a speed of 0.05 mm s^{-1} in a dark room with a stabilized halogen light source (150 W). On each fennel slice, regions of interest (ROI), were manually selected, one on the stem and one on the sheath, as the maximum subscribed rectangle, allowing to calculate in the reflectance mode, the CIE L*, a* b* scale color parameters. Hue angle ($h^\circ = \arctan \frac{b^*}{a^*}$) and saturation ($\text{Chroma} = \sqrt{a^{*2} + b^{*2}}$) were calculated from a* and b* values.

2.3. Total soluble solids, and pH

For the measurement of total soluble solid (TSS), and pH, 20 g of fennel tissues were transferred in a falcon tube, homogenized in an Ultra-Turrax (IKA T18 basic, Wilmington, NC, USA) and filtered with two layers of cheesecloth. Few drops of the fennel juice obtained were used to measure TSS content with a digital refractometer (Atago PR32-Palette, Tokyo, Japan) and another fraction of fennel juice was used to measure the pH with a pH meter (Titrator T50, Mettler Toledo).

2.4. Total phenolic content and antioxidant activity determinations

The same extraction was carried out for analyses of total phenolic content and antioxidant activity, following the procedure described by Amodio et al. (2014) with slight modifications. Fresh fennel tissue (5 g) were homogenized in 2 mmol L^{-1} sodium fluoride (NaF) methanol:-water solution (80:20) for 1 min, using an Ultraturrax (IKA, T18 Basic;