Postharvest Biology and Technology 55 (2010) 45–52

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Anatomical and physiological evidence of white blush on baby carrot surfaces

Adriano do N. Simões [a], Marília C. Ventrella [a], Celso L. Moretti [b], Marcelo A.G. Carnelossi [c], Rolf Puschmann [a,*]

[a] Plant Biology Department, Federal University of Vicosa, 36571-000 Minas Gerais, Brazil
[b] Postharvest Laboratory, Embrapa Vegetables, 70.359-970 Federal District, Brazil
[c] Chemical Engineering Department, Federal University of Sergipe, 49.100-000 Sergipe, Brazil

ARTICLE INFO

Article history:
Received 9 December 2008
Accepted 19 July 2009

Keywords:
Daucus carota
Minimally processed
Cell structure
White blush
Dehydration
Lignification
Suberization

ABSTRACT

This study characterizes dehydration and white blush processes, structural alterations and synthesis of phenolic compounds (lignin and suberin), in relation to development of white blush on baby carrot surfaces. Carrots were minimally processed as baby carrots and kept on polypropylene trays with or without polyvinyl chloride (PVC) film at 5 ± 2 °C, 90 ± 5% RH. During storage, baby carrots that were not wrapped with PVC film were rehydrated 1, 1.5, 15 and 17 h after minimal processing. Fresh-cut baby carrots were evaluated for white blush index, sensory analysis (visual scores), fresh matter loss, phenylalanine ammonia-lyase (PAL) activity and structural and histochemical changes. Increases in white blush index and subjective visual scores on the carrot surface occurred in the first hours, when the material was kept on trays without PVC film and after 3 and 6 d, when stored on trays covered with PVC film. Visual assessment of white blush resulted in a more accurate assessment than instrumental evaluation because it allowed the perception of minor differences between distinct white blush levels, especially at the tips. Hydrating baby carrot surfaces for 1 and 1.5 h after processing allowed partial absorption of water by tissues and the orange color was reestablished on the surface. Fifteen hours after processing, even after rehydration, the color did not return to the original orange standard. The rapid increase in PAL activity in the secondary phloem of baby carrots, compared to that of whole carrots, suggested a physiological response as a result of abrasion. Dehydration and structural alterations of the more superficial cell layers were the main causes of white blush in baby carrots that was not related to lignin accumulation, but rather to synthesis of non-structural phenolic compounds.

1. Introduction

Baby carrots are processed raw carrots that are selected, rinsed, cut into cylindrical pieces 5–6 cm long, put through a lathe, washed in cold chlorinated water, centrifuged and packed (Bolin and Huxoll, 1991; Avena-Bustillos et al., 1994). Technological problems during storage, such as white blush on the surface, can reduce acceptance and consequently commercialization.

Early studies by Tatsumi et al. (1991, 1993) suggested that white blush occurred as result of superficial dehydration and therefore was a physical phenomenon. Later, Cisneros-Zevallos et al. (1995) confirmed the close relationship between dehydration and white blush. The same authors concluded that, in the short term, white blush was reversible, that is, when baby carrots were hydrated they returned to their original orange color. However, in the long term, this process would not happen, suggesting that there was a physiological event taking place.

Further research by Bolin and Huxoll (1991), Bolin (1992), Howard and Griffin (1993) and Howard et al. (1994), suggested that the superficial whitening of peeled carrots was related to alteration in phenolic metabolism and resulted in lignin deposition on the surface, involving key enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase (POD) and others (Howard and Griffin, 1993). On the other hand, Avena-Bustillos et al. (1994) suggested that white blush in peeled carrots resulted essentially from dehydration and they reported that other authors could have misunderstood the phenomenon, since dehydration and lignification can occur independently and would not result in white blush.

Although white blush in fresh-cut baby carrots has been extensively studied, on most occasions physico-chemical, biochemical and physiological tools have been used. Few studies have considered anatomical issues. In some studies reddish purple spots have been reported on the surface of mini carrots, characterized by the reaction between lignin and phloroglucinol without showing the anatomical cut (Bolin and Huxoll, 1991; Howard and Griffin, 1993). Furthermore, recent results obtained by scanning electronic microscopy have shown lignin in minimally processed carrot tissues (Rico et al., 2007). Histochemical studies allow identification of

* Corresponding author. Tel.: +55 31 3899 2591.
E-mail address: rolf@ufv.br (R. Puschmann).

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doi:10.1016/j.postharvbio.2009.07.007

chemical substances and their location in plant cells and tissues and have been used in potatoes and carrots to identify lignin (Walter and Schadel, 1983). Fifteen years after the first studies on white blush, few studies currently have focussed on this subject, and the contribution of lignin to white blush is still doubtful, because this event occurs rapidly, with almost no time for the induction of a physiological response. In considering the aspects mentioned above, the objective of the present study was to characterize the dehydration process, white blush and the histological location of suberin, phenolic compounds and lignin and to assess their contribution to white blush on baby carrot surfaces.

2. Materials and methods

2.1. Raw material

Carrot plants (*Daucus carota* L. cv. Esplanada) were cultivated at the Federal University of Viçosa and collected 90 d after planting. Carrots were washed in running water, with one batch of carrots kept whole and another minimally processed.

2.2. Minimal processing

Carrots up to 2 cm in diameter were cut into pieces (5–6 cm long), selected and rounded using a PCE SKYMSEN® ETERNA® lathe, in two phases. The first phase worked with abrasive sandpapers (60 mesh) and the second with small mesh files (100 mesh) both for 60 s to obtain baby carrots (Lana et al., 2001).

Baby carrots were rinsed (rapid immersion in water at 5 °C) followed by immersion in water containing 200 mg L^{-1} active chlorine (dehydrated sodium dichloride-S-triazinatrion), at 5 ± 2 °C, for 600 s. The final rinse was carried out by immersing baby carrots in water containing 3 mg L^{-1} active chlorine at 5 ± 2 °C for 600 s. Baby carrots were then centrifuged for 15 s in a 251.33 rad s^{-1} angular speed centrifuge without load.

2.3. Refrigerated storage without PVC film

Whole carrots and baby carrots were stored without PVC film in display cases at 5 ± 2 °C and 90 ± 5% RH for 36 h. White blush index (WI), visual white blush, fresh matter loss, PAL activity and structural and histochemical alterations were assessed at 0, 2, 4, 6, 12, 24, and 36 h.

2.4. Rehydration of baby carrots storage without PVC film

Baby carrots 5 cm long, 1.5 ± 0.1 cm in diameter, were selected and stored at 5 ± 2 °C and 90 ± 5% RH, without PVC film. The surface was hydrated 1.0, 1.5, 15 and 17 h after processing, using 3 mL distilled water on each baby carrot (the volume was defined in a preliminary test, using a 5 mL syringe). White blush index (WI), visual white blush and fresh matter were determined.

2.5. Cold storage with PVC film

Baby carrots were stored on 90 mm × 170 mm × 25 mm (width × length × height) polypropylene trays wrapped in PVC film (12 μm thick) with 300 g m^{-2} d^{-1} atm^{-1} water vapor permeability, oxygen permeability of 15.68 mL m^{-2} d^{-1} atm^{-1}, and carbon dioxide permeability of 80.52 mL m^{-2} d^{-1} atm^{-1}. Wrapped baby carrots were kept at 5 ± 2 °C and 90 ± 5% RH for 21 d. White blush index (WI), fresh matter loss, PAL activity and structural and histochemical variations in whole carrots and baby carrots were assessed every 3 d.

2.6. White blush index (WI)

WI was determined according to Bolin and Huxoll (1991), using the parameters "*L*", "*a*" and "*b*" and calculated indirectly by the formula $WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$ given by a digital colorimeter (*Color reader* CR-10 Minolta).

2.7. Visual white blush

Visual sensory assessments used scores ranging from 1 to 4. Score 1 represented the surface with the original orange coloring, without white blush; 2, the start of white blush, 1–15% of the surface area and the extremities, without quality damage; 2.5, moderate white blush (16–50%); 3, advanced white blush (51–70%), acceptance limit; 4, extremely whitened surface (71–100%).

2.8. Fresh matter

Fresh matter was determined by gravimetry on a semi-analytical scale.

2.9. PAL (EC 4.3.1.5) analysis

Samples (2.5 mm × 2.5 mm × 2.5 mm; width × length × height) of the carrot surface, without the periderm, were obtained. Secondary phloem extraction and analyses were carried out according to Ke and Saltveit (1986), with adaptations. The enzyme extract was obtained by homogenizing 2.0 g of plant material with 5 mL sodium borate buffer (0.1 M) pH 8.8, containing β-mercaptanol (5 mM), EDTA (2 mM) and 1% insoluble polyvinyl pyrrolidone (PVPP) (p/v). The extract was then filtered through two layers of gauze and centrifuged at 25,000 × *g*, for 1200 s at 4 °C.

In the experiment 1.5 mL L-phenylalanine (60 mM) in borate buffer (0.1 M) pH 8.8 was kept before the reaction at 40 °C, for 900 s. Then 0.5 mL of the enzyme extract was added and after 1 h incubation at 40 °C, absorbance was measured at 290 nm. Previously boiled extract was used as the control.

2.10. Structural and histochemical assessment

Surface tissues were collected from whole carrots and recently processed baby carrots, stored without wrapping for 1 and 36 h after processing under conditions of 5 ± 2 °C and 90 ± 5% RH, without PVC film. Surface tissues were also collected from recently processed whole carrots and baby carrots stored for 10, 25 and 30 d after minimal processing at 5 ± 2 °C and 90 ± 5% RH, with PVC film.

All plant material was fixed in FAA_{50} for 48 h and kept in 70% ethanol (Johansen, 1940) until samples were processed. For structural analysis, 0.125 cm^3 sections of the surface region of whole carrots and baby carrots were blocked in methacrylate (Historesin-Leica) according to the manufacturer's recommendations, and cross cut 8 μm thick in a rotating microtome. The material was stained with toluidine blue (O'Brein et al., 1964) for metachromasia or with toluidine blue and lugol (Johansen, 1940), to show the phenolic compounds and starch, and mounted in synthetic resin (Permount).

For histochemical analysis, 1 cm^3 tissue pieces from the surface region of whole and fresh-cut baby carrots after 30 d of storage were sectioned with a table microtome and submitted to histochemical tests: acid phloroglucin (Johansen, 1940) to detect lignin and sudan scarlet (Brundrett et al., 1991) to detect lipid compounds, and mounted in glycerinated gelatin (Sass, 1958). Sudan scarlet was also used in the material blocked in methacrylate and mounted in glycerinated gelatin (O'Brien and McCully, 1981). Images were obtained by photomicroscopy (Olympus AX 70) using the U-Photo system.