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Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot

Carla Alegria[a,b,c], Elsa M. Gonçalves[a], Margarida Moldão-Martins[b], Luis Cisneros-Zevallos[c,**], Marta Abreu[a,*]

[a] Unidade Estratégica de Investigação e Serviços de Tecnologia e Segurança Alimentar, Instituto Nacional de Investigação Agrária e Veterinária, I.P. Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal
[b] LEAF—Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
[c] Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133, United States

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ABSTRACT

In this study we evaluated the effect of abiotic stresses, peeling and shredding, in different carrot tissues as a phenolic synthesis elicitor to improve bioactive quality of shredded carrot as a fresh-cut. The phenolic content (TPC) present in carrot peels (2954 mg kg^{-1}) is up to ~6 times higher than that of inner tissues (762 and 510 mg kg^{-1} for cortical parenchyma and vascular tissues, respectively). However, the effect of peel removal is mitigated by the respective tissue proportion in the root (~11% for peel and ~89% for inner tissues). Phenolic biosynthesis was verified in all carrot tissues and even when peel was removed, inner tissues were able to significantly accumulate phenolics during low temperature storage (5 °C, 10 d), with increases of 155% (compared to day 0). As key enzyme of the phenylpropanoid pathway, phenolic biosynthesis, in inner tissues, was confirmed by the phenylalanine-ammonia lyase (PAL) activity increase ($p < 0.05$) after wounding (peeling and shredding). It was also shown that color changes in carrot peel tissues (browning), with high polyphenoloxidase activity levels (up to 2 times regarding inner tissues), were more pronounced than in inner tissues under a high intensity wounding (shredding), showing that carrot fresh-cut production can benefit from peel removal. The use of controlled wound stresses, by increasing pre-existing raw material antioxidants, creates an opportunity to guarantee the bioactive fresh-like quality, a major challenge for fresh-cuts.

1. Introduction

Fresh-cut carrot products, such as shredded, sliced, sticks and baby carrots, are one of the most highly consumed ready-to-eat vegetables worldwide. According to Lucier and Lin (2007), just in the United States, about 80% of fresh-cut carrot products are consumed at home. The shelf-life of this product, particularly the shredded format, is usually compromised by excessive microbial outgrowth reaching unacceptable limits (aerobic colony counts > 10^7 cfu/g, PHLS, 2000) after just a few days leading to pronounced changes in the sensory quality (Alegria et al., 2010; Klaiber et al., 2005).

Besides ensuring products shelf-life extension, there is also a growing interest of food technologists and consumers to keep and enhance produces' bioactive composition. This trend is related to the public awareness of the health benefits associated with fruit and vegetable consumption, which mostly arise from the presence of antioxidants, especially phenolic compounds (Du et al., 2012). It is therefore an essential aim to implement food-processing technologies with minimal impact over the phenolic composition.

Phenolic content and profile varies significantly among the different carrot tissues (peel, cortical parenchyma and vascular tissue), where peels provide about 50% of the carrots' total phenolic amount (Zhang and Hamauzu, 2004). Nonetheless, carrot peeling during minimal processing improves the appearance of the final product (Barry-Ryan and O'Beirne, 1998), since browning reactions are prevented. It is known that phenol oxidizing enzymes, such as polyphenol oxidase (PPO, EC 1.10.3.1.),

* Corresponding author at: UEISTSA/Instituto Nacional de Investigação Agrária e Veterinária, I.P. Av. da República, Quinta do Marquês, 2780-157 Oeiras, Portugal.
** Corresponding author at: Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133.
E-mail addresses: csmalegria@gmail.com, csmalegria@hotmail.com (C. Alegria), goncalves.melsa@gmail.com (E.M. Gonçalves), mmoldao@isa.ulisboa.pt (M. Moldão-Martins), lcisnero@tamu.edu (L. Cisneros-Zevallos), marta.abreu@iniav.pt, marta.mmn.abreu@gmail.com (M. Abreu).

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are present at higher concentrations in peels compared to more inner carrot tissues, which are correlated with the respective browning potential (Chubey and Nylund, 1969). Besides, from the microbiological stand point, carrot peeling provides significant microflora reduction (~2 Log) to the fresh-cut product (Garg et al., 1990) which is of utmost importance to ensure that microbial limits are kept under control to prevent quality loss.

In general, during industrial food processing, phenolic degradation is induced by chemical or enzymatic oxidation, which can also lead to changes in bioavailability or biological activity (Tomás-Barberán and Espín, 2001). However, fresh-cut products consist of living tissues that are still physiologically active and able to synthetize phenolic compounds during the products shelf-life, and thus lessen this degradation trend. The industrial preparation of fresh-cut shredded carrot includes wounding operations (e.g., peeling/cutting), which are known mechanisms of enhancing the phenolic content and consequently the antioxidant capacity (Simões et al., 2011; Reyes et al., 2007; Ruiz-Cruz et al., 2007; Toivonen and DeEll, 2002). The accumulation of phenolic compounds is a stress response caused by a change in phenylalanine ammonia lyase activity (PAL, EC 4.3.1.5), a key enzyme in the phenylpropanoid pathway (Ke and Saltveit, 1986). This mechanism is however dependent on several factors, including wounding intensity and/or cut methods (Surjadinata and Cisneros-Zevallos, 2012; Kenny and O'Berine, 2010; Heredia and Cisneros-Zevallos, 2009), initial phenolic concentration (Reyes et al., 2007), atmospheric gas composition during storage (Jacobo-Velázquez and Cisneros-Zevallos, 2009) and storage temperature (Padda and Picha, 2008). More recently, Amodio et al. (2014), proposed a kinetic model to describe the occurring phenolic changes during fresh-cut fruits and vegetables shelf-life. These changes can be characterized as an initial increase in pre-existing phenolic amounts, triggered by the increase in PAL activity, followed by phenolic oxidation reactions, namely those involving PPO.

In this study, we address an important issue of concern for the fresh-cut industry related to the influence of peel removal on the overall biosynthesis of phenolic antioxidants in carrot tissue. Although several studies can be found about the effects of wounding intensity over phenolic accumulation in carrot, information about the effect of peel removal over the wound-induced stress responses has not, to our knowledge, been addressed before. Thus, the aim of this research was to assess the contribution of peeling to the wound-induced phenolic biosynthesis under different storage studies of wounding intensities using different carrot tissues and measuring PAL and PPO enzyme activities, phenolic metabolites and color parameters.

2. Materials and methods

2.1. Materials

Carrots (*Daucus carota* L. cv. Nantes, 30 kg) were obtained from a local fresh-cut processor (Campotec, Torres Vedras, Portugal) and transported to the lab in a refrigerated truck. Upon arrival to the laboratory, carrots were hand-sorted to select undamaged units, washed (50 mg L^{-1} NaOCl), dried and maintained at 5 °C (±1 °C) until use (16 h).

Commercial standards of chlorogenic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and ferulic acid and p-hydroxybenzoic acid from Fluka Analytical (Sigma Chemical Co., St. Louis, MO, USA). Analytical grade solvents were obtained from Panreac (Barcelona, Spain) and Sigma (St. Louis, MO, USA). HPLC grade solvents used for HPLC analysis were also from Panreac (Barcelona, Spain).

2.2. Sample handling and preparation

2.2.1. Characterization of carrot peel, cortical parenchyma and vascular tissue

To evaluate the total phenolic content (TPC) and phenylalanine-ammonia lyase (PAL) activity of different carrot anatomical tissues, a batch of 30 carrots was divided into two groups and weighed. The first group was evaluated for both determinations using carrot as a whole (raw material). From the second group, samples were collected according to the visually defined tissues: peel (0.7 ± 0.1 mm in thickness; measured using a Kuhnke video seam monitor VSM III (v4.2), Manfred Kuhnke, Berlin, Germany), cortical parenchyma and vascular tissue (phloem and xylem) and the resulting tissues were individually weighed to estimate the respective tissue proportion (%) and used separately for TPC and PAL activity evaluation.

2.2.2. Peel and inner tissues potential to induce phenolic biosynthesis under wounding stress

To evaluate peel and inner carrot tissues' (cortical parenchyma and vascular tissue) ability to accumulate phenolic compounds as a stress response, four sample types were prepared: peels and inner tissues with low intensity (peeling) and high intensity (shredding) wounding stress. The wound intensity value (A/W) of each sample type was calculated by dividing the resulting cut/wound area (m^2) over the weight (kg). Low intensity wounding samples were set as follows: Peel tissue (0.7 ± 0.1 mm thick) was removed in 152.0 ± 10.9 × 10.3 ± 1.7 mm strips from the length of the roots by means of a sharp stainless steel vegetable peeler (sample id.: *Peel*; A/W = 1.79 m^2 kg^{-1}). The A/W of the remaining inner tissues, ø 40.9 ± 2.0 × 204.7 ± 3.9 mm, was 0.12 m^2 kg^{-1} (sample id.: *Inner tissue*).

High intensity wounding samples were obtained using a Grindomix GM200 (3000 rpm × 1 s), which provided an A/W of 2.10 m^2 kg^{-1} for peels (12.1 ± 2.6 × 9.6 ± 1.1 × 0.7 ± 0.1 mm; sample id.: $Peel_{shreds}$) and of 2.27 m^2 kg^{-1} for inner tissues (8.1 ± 1.6 × 5.1 ± 1.6 × 3.3 ± 1.1 mm; sample id.: $Inner\ tissue_{shreds}$). Three independent replicates (three carrots for inner tissues and 125 g for peels, stressed peels and stressed inner tissue samples) were prepared for each sample type placing the product in 4-L clear glass jars (closed and vented every 8 h to avoid CO_2 accumulation as described by Reyes et al., 2007) and stored for 14 d at 5 °C (±1 °C) (Cryocell RS600SE, Aralab—Equipamentos de Laboratório e Electromecânica Geral Lda., Portugal). Total phenolic content, PAL and polyphenol oxidase (PPO) activity levels and CIELab color were measured on five sampling dates, *i.e.*, 0 (within 2 h from sample preparation), 3, 7, 10 and 14 d.

2.2.3. Peel removal impact in shredded carrot: phenolic synthesis under peel removal, shredding and low temperature storage

To evaluate the effects of the minimal processing operations of peeling and shredding, on the induced phenolic synthesis at low temperature storage, carrots (10 kg) were equally divided into four sample types: Whole unpeeled carrots (sample id.: *Whole*; A/W = 0 m^2 kg^{-1}); Whole peeled carrots (sample id.: *Inner tissue*; A/W = 0.12 m^2 kg^{-1}); Unpeeled shredded carrots (sample id.: $Whole_{shreds}$; 5.4 ± 0.5 × 5.4 ± 0.8 × 59.1 ± 8.6 mm; A/W = 2.65 m^2 kg^{-1}) and; Peeled shredded carrots (sample id.: $Inner\ tissue_{shreds}$; 5.4 ± 0.5 × 5.4 ± 0.8 × 59.1 ± 8.6 mm; A/W = 2.77 m^2 kg^{-1}). Peel removal was performed using a sharp stainless steel vegetable peeler and the shredding operation was done using a Dito Sama MV-50 equipped with a CX.21 (knife J7-8) vegetable slicer (Dito Sama, Aubusson, France). Five independent replicates (of three carrots for whole and 125 g for shredded samples) were prepared for each sample type and stored as previously described for 10 d at 5 °C (±1 °C). Total phenolic content, PAL activity,