Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Influence of a heat-shock pre-treatment on wound-induced phenolic biosynthesis as an alternative strategy towards fresh-cut carrot processing

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Abstract

In fresh-cut vegetables, plant tissues are often challenged by (a)biotic stresses that act in combination, and the response to combinatorial stresses differs from that triggered by each individually. Phenolic induction by wounding is a known response contributing to increase products phenolic content. Heat application is a promising treatment in minimal processing, and its interference on the wound-induced response is produce-dependent. In carrot, two-combined stress effects were evaluated: peel removal vs. shredding, and heat application (100 °C/45 s) vs. shredding, on changes in total phenolic content (TPC) during 10 days (5 °C). By applying the first stress combination, a decrease in TPC was verified on day 0 (~50%), ascribed to the high phenolic content of peels. Recovery of initial fresh carrot levels was achieved after 7 days owing to phenolic biosynthesis induced by shredding. For the second combination, changes in TPC, phenylalanine-ammonia-lyase (PAL), and peroxidase (POD) activity of untreated (Ctr) and heat-treated (HS) peeled shredded carrot samples were evaluated during 10 days. The heat-shock did not suppress phenolic biosynthesis promoted by PAL, although there was a two-day delay in TPC increments. Notwithstanding, phenolic accumulation after 10 days exceeded raw material TPC content. Also, the decrease in POD activity (30%) could influence quality degradation during storage.

Keywords

Daucus carota L., heat-shock, shredding, phenolic synthesis, PAL activity

Date received: 26 June 2020; accepted: 6 May 2021

INTRODUCTION

The health benefits of fruit and vegetables (F&V) have been attributed mainly to their nutritional and phytochemical profiles, in particular, their high natural antioxidant content (World Health Organization, 2014). Considering renewed consumer health-conscious and convenience-oriented attitudes, demand for fresh-cut F&V still increases due to their fresh-like properties (Velderrain-Rodríguez et al., 2015).

Food Science and Technology International 0(0) 1–9

DOI: 10.1177/10820132211020837
journals.sagepub.com/home/fst
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Shredded carrot is an excellent example of the success of fresh-cut F&V given the widespread market acceptance either *de per si* (e.g., sticks, sliced, shredded) or in salad mixes. Carrot (*Daucus carota* L.) has an interesting bioactive profile with high amounts of carotenoids, phenolic compounds, vitamins (A, B, C, D, E, and K), minerals (Ca, K, Na, Fe) and dietary fibre (Ülger et al., 2018). Carrots phenolic composition actively contributes to its antioxidant capacity and other health-promoting biological activities (e.g., anti-inflammatory and antihypertensive activities) (Santana-Gálvez et al., 2017).

As fresh-cut products, carrot undergoes a rapid microbial degradation and loses its characteristic colour and aroma having a limited shelf-life (Seljäsen et al., 2001). The fresh-like loss is attributed to both microbial and enzymatic degradation, and throughout minimal processing, there are no effective treatments to control them (Rico et al., 2007). One of the critical steps is the sanitation process in which sodium hypochlorite continues to be widely used despite the identified drawbacks such as the low microbial inactivation efficiency and loss of essential phytochemicals (carotenoids and phenolics) by leaching/oxidation (Ruiz-Cruz et al., 2007; Vandekinderen et al., 2008). The subsequent microbiological development during storage is responsible for the spoilage of the products fresh-like quality.

The development of alternative technological strategies to minimise the off-putting effects of processing and maintain their fresh-like properties is a constant challenge. The combined use of different low-intensity treatments based on the hurdle-technology has been a desirable option in the context of minimal processing of fruits and vegetables, regardless of the treatments nature. More effective preservation (i.e., synergistic effects of hurdles) is obtained if small stresses with different targets (multi-target preservation), instead of small stresses with the same target (i.e., additive effect of hurdles), are selected to inhibit microorganisms growth (Gómez et al., 2011).

Heat treatments are commonly used to reduce microbial load and inhibit enzymatic activities effectively; however, not always compatible with minimal processing as it may lead to undesirable changes to the products fresh-like quality (colour, texture, flavour and nutritional value) (Maghoumi et al., 2013). At the same time, favourable physiological effects are promoted by heat treatments (Aghdam and Bodbodak, 2014), which could prove advantageous to minimal processing if treatments intensity is carefully chosen so as not to compromise the produce fresh-like quality. Heat-shock treatments may be considered under the high-temperature-short-time (HTST) concept, supported on the principle that microorganism inactivation mostly relies on the treatment temperature, while undesirable quality changes mainly depend on its duration (Ohlsson, 1980). The use of heat-shocks has already been proven as a practical methodology for quality maintenance in multiple fresh-cut F&V (Abreu et al., 2011; Aguayo et al., 2008; Martín-Diana et al., 2005). Regarding fresh-cut shredded carrot, previous studies have shown that the use of heat-shocks, as a pre-treatment, is important for effective control over microbial development and shelf-life extension (Alegria et al., 2010; Klaiber et al., 2005a). Further, multiple biochemical pathways are expected to participate in the stress responses set by heat, including heat-shock proteins synthesis, regular cellular protein synthesis interruption entailing the inhibition/inactivation of quality-related enzymes (e.g., peroxidase; polyphenol oxidase), and changes in the kinetic behaviour of previously synthesised enzymes (Campos-Vargas et al., 2005; Saltveit, 2000).

Wounding, as abiotic stress, triggers a signalling cascade that conveys signals to non-wounded tissues and induces an array of physiological responses, including the biosynthesis and accumulation of phenolic compounds during storage of fresh-cut F&V (Jacobo-Velázquez et al., 2015; Ke and Saltveit, 1989; Rakwal and Agrawal, 2003; Zhao et al., 2005). Wound-induced phenolic biosynthesis by the phenylpropanoid pathway is up-regulated by phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) and emerges as a major defence mechanism against wounding (Dixon and Paiva, 1995; Jacobo-Velázquez et al., 2015; Matern and Grimmig, 1994; Yoshikawa et al., 2018). PAL enzyme is regarded as a precursor of a substantial number of phenolics with distinct biological functions, including chlorogenic acid, the predominant phenolic compound found in carrots. Considering the phenolic compounds antioxidant properties, wounding presents itself as an opportunity to complement the vegetable tissue antioxidant capacity (Alvarado-Ramírez et al., 2018; Cisneros-Zevallos, 2003; Jacobo-Velaźquez et al., 2011; Santana-Gálvez et al., 2016). Taking into account the wound-induced phenolic synthesis during storage of fresh-cut products, respective phenolic accumulation could be sufficient to counteract the phenolic losses by leaching/oxidation phenomena in washing operations. The maintenance of the peel may also contribute to maximising the phenolic content of the fresh-cut product, given that these tissues gather higher phenolic contents (Alegria et al., 2016; Santana-Gálvez et al., 2019; Zhang & Hamauzu, 2004).

The present research aimed to: (1) evaluate the effects of a previously optimised heat-shock pre-treatment (100 °C/45 s), and of peel removal on the wound-induced phenolic accumulation in carrot, prompted by shredding, during storage (5 °C, 10 days).

A factorial design (2×2) was used for this purpose considering both abiotic stresses as independent factors, and; (2) assess the effects of the 100 °C/45 s heat-shock on the phenolic biosynthesis dynamic by measuring total phenolic content and PAL and POD enzyme activities during storage of peeled, shredded carrot (5 °C, 10 days).

MATERIAL AND METHODS

Plant material

A local fresh-cut processor (Campotec, S.A.; Torres Vedras, Portugal) provided 2 lots of 10 kg of cv. Nantes carrot (*Daucus carota* L.). At the laboratory, carrots were screened to remove damaged units, washed (50 mg . L^{-1} NaOCl), dried and kept at refrigeration conditions until use (16 h; 5 ± 1 °C; Cryocell RS600SE, Aralab – Equipamentos de Laboratório e Electromecânica Geral Lda., Portugal).

Sample handling and preparation

Impact of peel removal and heat-shock pre-treatment on the phenolic accumulation. A complete factorial design was designed to assess the effects of heat-shock pre-treatment (100 °C/45 s) and from peel removal on phenolic content and its accumulation in shredded carrot tissues during refrigerated storage (5 °C, 10 days). To conform with the design matrix, 10 kg of cv. Nantes carrot were used to set four sample types (independent triplicates; 125 g per sample replicate per storage day) identified as: *PR* – "peel removal" × "no heat-shock"; *PR_HS* – "peel removal" × "heat-shock"; *WP* – "no peel removal" × "no heat-shock"; and *WP_HS* – "no peel removal" × "heat-shock". Peel removal was achieved by using a manual stainless-steel vegetable peeler (*PR* samples). Heat-shock (HS) samples were treated at already optimised conditions of 100 °C/45 s and prepared as described in Alegria et al. (2012). Briefly, whole carrots (with or without peel) were immersed in a thermostatically controlled pilot-scale steriliser set to 100 °C (±1 °C; temperature continuously monitored with T-type thermocouples; Ellab) and, immediately after carrot submersion, treatment time (45 s) initiated. After heat treatment, carrots were placed in ice-cold water for 5 min, dried, and further processed. Carrots (with or without peel and/or thermal processing), were shredded on a pilot-scale vegetable slicer (Dito Sama model MV50 fitted with a CX21S and J7-8 knife, Dito Sama, Aubusson, France). Samples (125 g; triplicates) were set into 4-L clear glass jars and stored at 5 °C (±1 °C, Cryocell RS600SE). The total phenolic content (TPC) of the samples was determined on day 0 (within 2-h from sample preparation), 3, 7, and 10. The raw material (fresh carrot with peel) phenolic content was also determined to assess the minimal processing impact (day 0) on this bioactive response and estimate its recovery during storage.

Changes in wound-induced phenolic synthesis promoted by heat-shock pre-treatment. To investigate the phenolic biosynthesis dynamics, namely the effects of the heat-shock pre-treatment (100 °C/45 s) on the total phenolic content (TPC) and phenylalanine ammonia-lyase (PAL) and peroxidase (POD) enzymatic activities of peeled-shredded carrot during storage (5 °C, 10 days), another trial was implemented. For that purpose and from a lot of 10 kg of cv. Nantes carrot, two sample types (set by independent triplicates of 125 g), peeled heat-treated (sample id.: *HS*), and peeled untreated samples (sample id.: *Ctr*), were prepared. Heat-shock and further processing were carried out as previously described, and samples stored at 5 °C (±1 °C, Cryocell RS600SE). All samples were analysed at day 0, 3, 5, 7, and 10 of storage. The same responses were also measured in fresh carrot.

Analytical procedures

Total phenolic content. The total phenolic content was evaluated, as described in Heredia and Cisneros-Zevallos (2009). Briefly, a methanolic extract of each sample replicate (n = 3) was obtained (1:4, w:v, 100% methanol) and, after reaction with the Folin-Ciocalteu reagent (0.25 N) and Na_2CO_3 (1 N), the spectrophotometric readings (725 nm) were recorded (ATI Unicam UV/VIS 4 spectrophotometer; Unicam Sistemas Analíticos, Lisboa, Portugal). Quantification was achieved by the standard curve obtained from the absorbance readings of known concentrations of chlorogenic acid (0–350 mg . L^{-1}), and results were expressed as mg chlorogenic acid equivalents per 100 g of fresh tissue (mg CAE . 100 g^{-1}).

Phenylalanine-Ammonia lyase (PAL) activity. The phenylalanine-ammonia lyase (PAL) activity assay was adapted from Ke and Saltveit (1986) with the modifications described in Alegria et al. (2016). The extraction (1:4, w:v) was carried out using borate buffer (50 mM, pH 8.5, with 400 µL L^{-1} β-mercaptoethanol). Activity determination was performed using L-phenylalanine (100 mM) as substrate and spectrophotometric readings (290 nm; ATI Unicam UV/VIS 4 spectrophotometer) taken after 1 h of incubation at 40 °C. Each sample replicate was extracted, and PAL activity expressed as the amount (µmol) of t-cinnamic acid synthesised per hour per 100 g of fresh tissue (U.100 g^{-1}). Per sample replicate, three independent measures were taken.

Peroxidase (POD) activity. Peroxidase (POD) activity was determined spectrophotometrically as described by Alegria et al. (2010), using H_2O_2 and guaiacol as substrate. One unit of POD activity was defined as an increase of 1.0 in absorbance (470 nm; ATI Unicam UV/VIS 4 spectrophotometer) per min per mL enzyme crude extract and results expressed as enzyme units per gram fresh weight ($U.g^{-1}$). Per sample replicate, three independent measures were taken.

Statistical analysis

Statistical analysis was carried out using Statistica^{TM}v.8 Software from Statsoft (StatSoft Inc., 2007). The data were analysed by analysis of variance (ANOVA) and mean separation determined using the Tukey Honestly Significant Difference (HSD) test with a significance level of 0.05.

RESULTS AND DISCUSSION

Impact of peel removal and heat-shock pre-treatment on the phenolic accumulation

From the statistical analysis, its was found that at day 0 (after processing), the interaction effect *"Peel removal" × "Heat-shock"* ($F = 1.254$) was non-significant ($p > 0.05$) enabling the discussion of the *"Peel removal"* ($F = 54.354$; $p < 0.001$) and *"Heat-shock"* ($F = 2.106$; $p > 0.05$) as independent effects. From these results and observing the initial impact (after processing, day 0) of *"Peel removal"* and *"Heat-shock"* on shredded carrot phenolic levels, shown in table 1, it was possible to confirm that only *"Peel removal"* had a significant effect over this response. Similar TPC levels ($p > 0.05$) were determined between untreated (*WP* and *PR*) and heat-treated (*WP_HS* and *PR_HS*) samples (Table 1), showing that the 100 °C/45 s treatment did not promote any further phenolic changes than those imposed by peel removal. The TPC losses of ~26% concerning the raw material (52.1 ± 2.66 mg.100 g^{-1}) were similar in the two samples with peel (*WP_HS* and *WP*, $p > 0.05$), which is in line with previous results obtained by our group (Alegria et al., 2016) and others (Santana-Gálvez et al., 2019). In both peeled samples (*PR_HS* and *PR*) and regardless of thermal processing, the losses of 46–54% were also similar ($p > 0.05$) and attributed to peel removal.

Changes in phenolic content of the four carrot samples during storage are shown in Figure 1. Again, the interaction effect (*"Peel removal" × "Heat-shock"*; $F = 0.0356$) was non-significant, and only the "*Heat-shock*" effect ($F = 4.3200$) proved to be significant ($p < 0.05$) over sample phenolic contents during storage. Significant increases in TPC levels were observed in all samples during storage (Figure 1), indicating that phenolic biosynthesis and accumulation were not compromised by applying the heat-shock treatment (100 °C/45 s) before the shredding procedure, irrespective of peel removal. It is worth noting that the initial significant TPC increase was recorded from day 3 to 7 of storage ($p < 0.05$) in all samples. The exception to this behaviour was observed in sample *WP_HS* ("with peel" × "heat-shock" sample), where the increase (~30%) from day 3 to 7 was not significant.

Both non-thermally treated samples (*WP* and *PR*) had comparable ($p > 0.05$) TPC increments during

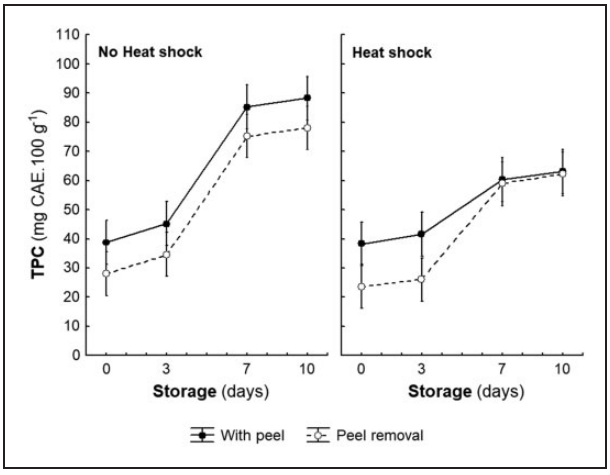

Figure 1. Peel removal and heat-shock (100 °C/45 s) stress effects on the total phenolic content (TPC) during storage of shredded carrot (5 °C, 10 days). Error bars correspond to the confidence interval at 95%.

Table 1. "*Peel removal*" and "*Heat-shock*" stress effects on the total phenolic content (TPC) of shredded carrot after processing (day 0).

Peel removal	Heat-shock (100 °C/45 s)	Sample ID	TPC (mg . 100 g^{-1})	Stress impact* (%)
With peel	No heat-shock	WP	$38.83^{b} \pm 4.75$	$25.57^{a} \pm 9.16$
	Heat-shock	WP_HS	$38.27^{b} \pm 3.50$	$26.67^{a} \pm 6.73$
Peel removed	No heat-shock	PR	$28.13^{a} \pm 0.50$	$46.10^{b} \pm 0.95$
	Heat-shock	PR_HS	$23.73^{a} \pm 0.21$	$54.53^{b} \pm 0.42$

*Stress impact is shown as relative TPC decrease (%) against the raw material (TPC = 52.1 ± 2.66 mg CAE.100 g^{-1}). Values are mean of three replicates ± SD. In the same column, different letters represent significant differences at $p = 0.05$ (Tukey HSD test).

storage, and after 7 days, the attained phenolic accumulation allowed to overcome the raw material phenolic content ($52.1 \pm 2.66\,mg\,.\,100\,g^{-1}$). Similarly, the heat-treated samples, registering increases ($p > 0.05$) of 1.7 and 2.6 times initial contents (*WP_HS* and *PR_HS* samples, respectively) by day 7, had also achieved the raw material TPC. Therefore, the maintenance of peel in shredded carrot proved not to be influential to respective phenolic accumulation. Furthermore, other studies have also shown that peel removal is beneficial to control microbial development (Garg et al., 1990) as well as to prevent colour changes during product storage (Alegria et al., 2016).

Changes in wound-induced phenolic synthesis promoted by heat-shock pre-treatment

Total phenolic content and phenylalanine (PAL) activity. The induction of the phenylpropanoid metabolism, mediated by PAL enzyme, leading to phenolic accumulation in stressed plant tissues, is well documented and is considered as a primary stress response (Yoshikawa et al., 2018). The heat-shock stress treatment (100 °C/45 s) effects on TPC and PAL activity of shredded carrot samples during storage are shown in Figure 2(a) and (b), respectively. Initial contents of $67.1 \pm 1.8\,mg\ \ CAE\,.\,100\,g^{-1}$ and $4.0 \pm 1.9\,U\,.\,100\,g^{-1}$ for TPC and PAL were determined in the raw material.

The heat-treated (*HS*) and control (*Ctr*) samples TPC contents (Figure 2(a)) were similar ($p > 0.05$), showing a loss of ~30% ($p < 0.05$) regarding the raw material content. The decreases in TPC contents can be attributed to losses during peeling and shredding operations, uninfluenced by the heat-shock (100 °C/45 s) treatment, as previously shown. Significant phenolic accumulation was found in both sample types during storage (Figure 2(a)), with TPC increases of 2.3 and 2.5 times the initial contents for *HS* samples and *Ctr* samples, respectively. In heat-treated samples (*HS*), the first significant TPC increase was again observed from day 3 to 5 ($p < 0.05$), while the same was expressed earlier in *Ctr* samples, from day 0 to 3 ($p < 0.05$). These results point out that the significant TPC increase in HS samples occurred with a 2-day delay compared to untreated samples showing the heat-shock effect on the dynamics of the wound-induced phenolic synthesis. In the first trial, and regardless of differences in samples TPC levels, after 7 days of storage, both heat-treated (*HS*, $71.0 \pm 1.4\,mg\ CAE\,.\,100\,g^{-1}$) and control (*Ctr*, $111.6 \pm 1.0\,mg\ CAE\,.\,100\,g^{-1}$) samples reached the raw material TPC levels ($67.1 \pm 1.8\,mg\ \ CAE\,.\,100\,g^{-1}$). Control (*Ctr*) samples had exceeded by 66% the fresh carrots phenolic content while a similar outcome ($p > 0.05$) was observed at day 10 in heat-treated samples (*HS*, 60%).

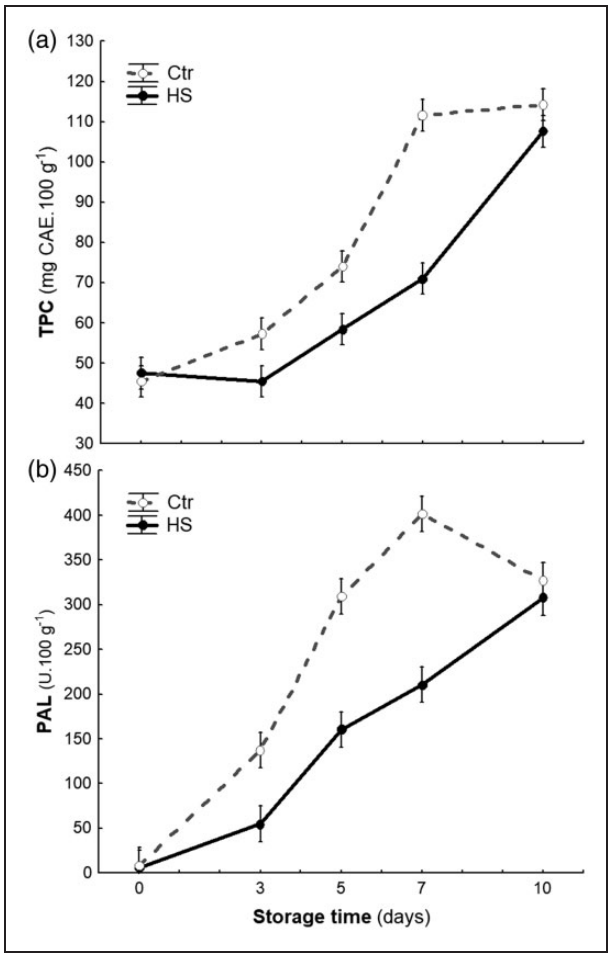

Figure 2. Heat-shock stress effects on (a) total phenolic content (TPC) and (b) phenylalanine ammonia-lyase activity (PAL) during storage of peeled shredded carrot (5 °C, 10 days). Error bars correspond to the confidence interval at 95%.

Alegria et al. (2016) observed a consistent and significant correlation between TPC increase and chlorogenic acid synthesis, proven to be the prevalent synthesised phenolic compound in stressed carrot tissues, as also supported by the findings of Formica-Oliveira et al. (2016) and Santana-Gálvez et al. (2019). The work by Alegria et al. (2016) reports high correlations between TPC and chlorogenic acid during storage of shredded carrot leading to an overall increase in the antioxidant activity of the product. It is thus fair to assume that the determined significant increases in both sample types are related to increased chlorogenic synthesis, closely related to increases in PAL activity (Alegria et al., 2016; Formica-Oliveira et al., 2016; Payyavula et al., 2015).

Regarding PAL activity (Figure 2(b)), heat-treated (*HS*) samples always had lower activity levels regarding *Ctr* samples during storage. Both samples registered a

significant increase in PAL activity from day 0 to 3; however, higher activity levels ($p < 0.05$) were recorded in *Ctr* samples. This significant PAL activity difference between *HS* and *Ctr* samples at day 3 (Figure 2(b)) supports the TPC maintenance registered in *HS* samples from day 0 to 3 ($p > 0.05$, Figure 2(a)). Moreover, the positive correlation ($r = 0.9$, $p < 0.05$) between these responses during storage emphasises the interdependence between TPC levels and PAL enzyme activity, as previously reported (Alegria et al., 2016; Formica-Oliveira et al., 2016). Thus, the lower PAL activity increments registered in *HS* samples during storage could be a result of the 100 °C/45 s stress treatment effect over the PAL *de novo* synthesis, as it could induce a metabolic shift to synthesise heat-shock proteins (Campos-Vargas et al., 2005; Saltveit, 2000). Stress treatments could either lead to a reduction in PAL synthesis and/or increasing its inactivation (Choi et al., 2005). Given our results, it is thus possible that the 100 °C/45 s heat-shock stress treatment, instead of inhibiting PAL activity, could have delayed tissues ability to synthesise PAL enzyme as a response to wounding by, at least, 2 days. Also supporting this hypothesis is the study by Campos-Vargas et al. (2005), which states that heat-shocks, either by decreasing PAL mRNA translation or by enhancing the induced PAL protein turnover, limit the wound-induced PAL response leading to a lowered enzyme activity.

Heat-treated (*HS*) samples (Figure 2(b)) still observed an increase in PAL activity ($p > 0.05$) between days 7 and 10 of storage, which correlates with the observed significant phenolic accumulation (Figure 2 (a)) in these samples. In contrast, a significant decrease in PAL activity was registered in control (*Ctr*) samples (Figure 2(b)) within this period (7–10 days), simultaneous with TPC maintenance (Figure 2(a)). This activity decrease could be a result of PAL activity retro-inhibition by reached phenolic concentration as previously proposed (Alegria et al. 2016; Boerjan et al., 2003). It is suggested that due to auto-regulation mechanisms or to transcriptional or post-translational PAL expression inhibition by cinnamic acid (feedback modulation), PAL activity is decreased. Several authors have already reported this decrease behaviour during storage of fresh-cut products: shredded carrot (Klaiber et al., 2005b), lettuce (Choi et al., 2005) and jicama (Aquino-Bolaños et al., 2000).

Peroxidase (POD) activity. Peroxidase (POD) is a ubiquitous enzyme in plant cells and its activity linked to oxidative degradation reactions, which lead to changes in, e.g., aroma and colour, impairing the quality of the products. Also, the increase in POD activity as a response to injury and storage was considered an indicator of the level of oxidative stress

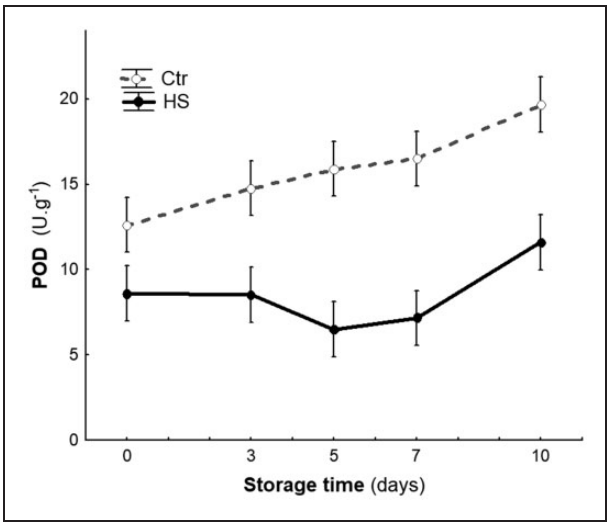

Figure 3. Heat-shock stress effects on peroxidase activity (POD) during storage of peeled shredded carrot (5 °C, 10 days). Error bars correspond to the confidence interval at 95%.

(Lamikanra & Watson, 2001). The initial peroxidase activity (POD) determined in the raw material was 19.7 ± 0.6 U.g^{-1}, and the effects of the heat-shock on POD changes during storage are shown in Figure 3.

From the comparison of mean POD activity levels of *HS* and *Ctr* samples after processing (day 0), it is possible to conclude that the heat-shock led to a significant decrease in POD activity, of ~60% ($p < 0.05$) regarding the raw material, and of ~30% ($p < 0.05$) regarding *Ctr* samples, confirming previous studies (Alegria et al., 2010). Contrary to the POD activity increase ($p > 0.05$) in untreated shredded carrot (*Ctr*) samples during storage (Figure 3), heat-treated (*HS*) samples registered low activity levels during storage. Similar results were also reported in steam-treated fresh-cut lettuce (Martín-Diana et al., 2007) and carrot sticks (Howard et al., 1994), and water heat treated fresh tomato (Pinheiro et al., 2016).

The reduction of POD synthesis resulting from heat treatments had already been reported by Martín-Diana et al. (2005), proposing two mechanisms: (i) by feedback inhibition from the scarcity of phenolic substrates; (ii) by an immediate effect on an unidentified receptor involved in POD synthesis. Either way, from our results, it seems more likely that the 100 °C/45 s heat-shock stress treatment could have induced a diversion of protein synthesis favouring heat-shock protein production as suggested by Saltveit (2000) for PAL enzyme. Considering the study by Lamikanra and Watson (2001), the attained low POD activity levels also contribute to reducing the oxidative degradation rate during storage. This result is expected to hold a

significant effect over phenolic degradation during the storage of shredded carrot as POD is associated with phenolic oxidation. Moreover, maintenance of reduced POD activity levels promoted by heat treatments proves beneficial to minimise carrot irreversible superficial discolouration, white blush, due to stimulation of the phenolic metabolism and lignin production (Alegria et al., 2010; Howard et al., 1994). As such, it is important to note that no observable colour changes were perceived after the application of the heat-shock or during storage, confirming the effect of reduced POD activity levels to colour maintenance.

CONCLUSIONS

The combined abiotic stress effects (heat-shock vs. shredding and peeling vs shredding) modulate the acclimation response of carrot tissues, by interfering with the phenolic synthesis/oxidation dynamics, delaying PAL *de novo* synthesis and reducing POD activity. Despite the delay induced by the application of the 100 °C/45 s heat-shock treatment before the shredding procedure and regardless of the presence of peels, the phenol accumulation exceeded the phenolic content of the raw material during ten days of storage. The reduction of POD activity levels is also essential to prevent oxidative reactions and white blush during product-storage. These findings can be presented as valuable information in the context of fresh-cut processing of shredded carrot, taking into account the potential for higher antioxidant capacity and fresh-like quality of the product.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Campotec, S.A., for supplying used raw material (carrots).

AUTHOR'S NOTE

Carla Alegria is now affiliated with SFCOLAB - Associação Smart Farm COLAB Laboratório Colaborativo para a Inovação Digital na Agricultura, Torres Vedras, Portugal.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Author Carla Alegria was financially supported by Fundação para a Ciência e a Tecnologia (Ministério da Ciência e do Ensino Superior, Portugal) through a Doctoral fellowship (SFRH/BD/62211/2009).

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