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Response of carrot roots to wounding stress induced by processing – changes in chemical composition and enzyme activity

Resposta de raízes de cenoura à injúria induzida por processamento – alterações na composição química e na atividade enzimática

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Summary

The production of Cenourete®, a product similar to 'baby-carrot', consists of polishing cylindrical carrot root segments by abrasion against an abrasive surface. The processing operations can result in oxidative stress, which in turn can result in changes in the content and composition of the bioactive compounds. In the present work both whole carrot roots and Cenouretes® from the cultivars Esplanada and Sugar Snax 54, were stored for 17 days at 5 ± 1.5 °C under dark or light conditions and evaluated after 2, 5, 8, 11, 14 and 17 days of storage. Processing induced a decrease in the carotenoid content as compared to the intact carrot root for both cultivars. The total phenol content decreased during storage for both the intact and processed roots and the differences amongst the cultivars were not relevant. The changes in phenol content were not correlated with the activity of either peroxidase (POD) or polifenoloxidase (PPO), while a small but significant correlation was found between the carotenoid content and POD activity. POD activity was inhibited in the processed roots as compared to the intact roots for both cultivars, while for PPO the differences between cultivars were more important, with the Esplanada cultivar showing higher activity than Sugar Snax 54. None of the factors studied was influenced by the presence of light.

Key words: *Daucus carota L.; Minimal processing; Fresh-cut carrot; Cultivar; Cenourete®.*

Resumo

A produção de Cenourete®, um produto similar à 'baby-carrot', consiste no polimento de pedaços de cenoura por abrasão contra uma superficie abrasiva. Estas operações podem acelerar processos oxidativos que, por sua vez, podem causar alterações no teor e na composição de compostos bioativos. No presente trabalho, raízes inteiras e Cenouretes® das cultivares Esplanada e Sugar Snax 54 foram armazenadas por 17 dias a 5 ± 1,5 °C e avaliadas aos 2, 5, 8, 11, 14 e 17 dias de armazenamento. O teor de carotenoides totais foi reduzido em cenoura processada, comparativamente ao teor em cenoura inteira, para ambas as cultivares. O teor de fenóis totais decresceu durante o armazenamento tanto em cenoura inteira como em processada, e a diferença entre cultivares, apesar de significativa, não foi relevante. Não foi observada correlação entre os teores de fenóis totais e a atividade das enzimas peroxidase (POD) e polifenoloxidase (PPO), enquanto houve correlação pequena, mas significativa, ente o teor de carotenoides totais e a atividade de POD. A atividade de POD foi inibida em cenoura cortada comparativamente à da cenoura inteira para ambas as cultivares, enquanto para PPO as diferenças entre cultivares foram mais importantes, com Esplanada apresentando maior atividade dessa enzima do que Sugar Snax 54. Nenhum dos fatores estudados foi influenciado pela presença de luz.

Palavras-chaves: *Daucus carota L.; Processamento mínimo; Cenoura minimamente processada; Cultivar; Cenourete®.*

1 Introduction

Cenourete® is a minimally processed carrot product similar to the American 'baby carrot'. Its production consists of polishing cylindrical carrot root segments by abrasion against an abrasive surface. After abrasion, the segments take the shape of small carrots.

Minimally processed vegetables undergo oxidative stress that can result in changes in the content and composition of the bioactive compounds (LINDLEY, 1998; CHEN and DJURIC, 2001). Due to its importance as a source of pro-vitamin A in the diet, there is particular interest in the effect of processing on the carotenoid content of Cenouretes®. Carotenoids are unstable when exposed to oxygen or light (SHI and MAGUER, 2000), all of which may occur when cells are disrupted and the internal tissues are exposed by cutting. Studies on carotenoid retention in cut carrot are not conclusive (CARLIN et al., 1990; HOWARD and DEWI, 1996; LI and BARTH, 1998; ALASALVAR et al., 2005).

Wounding was shown to promote the production of phenolic compounds such as chlorogenic acid and p-hydroxy-benzoic acid in carrot roots. (AUBERT et al., 1993). Phenolic compounds are related to many sensory attributes (HOWARD and GRIFFIN, 1993; TALCOTT and HOWARD, 1999; ALASALVAR et al., 2001) and appear to be good indicators of varietal suitability for the cold storage of carrots as well as for industrial transformation into fresh-cut vegetables (AUBERT et al., 1993).

The oxidation of phenolic compounds by oxyreductases, a group that includes peroxidases and polyphenoloxidases, is related to browning of cut tissues (VERMERRIS and NICHOLSON, 2006) and surface discoloration in cut carrot (HOWARD et al., 1994). In addition, it can result in the formation of metabolites that can account for spoilage of the foods after processing. Phenols can also be oxidized due to auto-oxidation as a result of exposure to light and oxygen (VERMERRIS and NICHOLSON, 2006).

Equally important indicators of post processing quality are the activity of specific enzymes. Peroxidase is a key enzyme in the lignification process in carrot and in the enzymatic oxidation of carotene, being considered as an indicator of oxidative stress (HOWARD and GRIFFIN, 1993; LI and BARTH, 1998; LAMIKANRA and WATSON, 2001). Peroxidases can also interact with phenolics in reactions related to deteriorative changes (BARZ et al., 1985).

The content and composition of bioactive compounds is highly dependent on the environment – genetic interaction (KIDMOSE et al., 2004). In addition, carrot genotypes seem to vary in their capacity for membrane restructuring following wounding (PICCHIONI and WATADA, 1998), which has direct implications on their post processing shelf life. Brazilian carrot cultivars, especially those from the Brasília group, present a genetic background quite distinct from the cultivars used for baby-carrot production in the Northern Hemisphere. The cultivar Esplanada introduced onto the Brazilian market in 2005 (VIEIRA et al., 2005) is a cultivar from the Brasilia cultivar group, especially developed for processing. Imported hybrids from the Imperator group, or similar, present roots with very good characteristics for processing but they are not well adapted to the Brazilian summer conditions and are very susceptible to foliar diseases. The response of Brazilian cultivars to wounding stress has not been studied in detail. This information is important both for the selection of cultivars with longer shelf-life after processing, and for the selection of storage conditions which result in better quality maintenance.

The response to stress is also modulated by environmental conditions. In the present work, the effect of light is of particular interest. The exposure to light resulted in a significant cultivar-dependent increase in chlorogenic acid content in potato tubers (ZUCKER, 1968), and had distinct effects on the chemical composition of a variety of fresh-cut fruits (GIL et al., 2006).

The objective of the present work was to determine the changes that occur in the carotenoid and phenol contents in cut carrots during refrigerated storage under light and dark conditions. In addition, the activity of oxidative enzymes that have been previously implicated in deteriorative changes in carrot were investigated. The performance of the recently released Brazilian cultivar Esplanada was compared with that of the imported hybrid Sugar Snax 54, both recommended for the production of 'baby-carrot' like products.

2 Material and methods

Carrots from the cultivars Esplanada and Sugar Snax 54 were sowed in April 2007 and harvested 90 days after sowing in Brasília-DF, Brazil. The cultural practices included fertilization with 4-30-16 (200 $g.m^{-2}$) followed by two applications with ammonium sulphate during the cycle (50 $g.m^{-2}$ each). Chemical weed control was achieved by applying Afalon, Ronstar and Fusilade.

After harvesting, the roots were washed and transported to the Postharvest Laboratory at Embrapa Vegetables, Brasília-DF. The following day, half the roots were processed into Cenourete® and the other half were left intact. For processing, the roots were cut using the mechanized cutter (Cortadora Horizontal) and then shaped in the Shaper (Processadora de Cenourete e Catetinho) (SILVA et al., 2009).

The Cenouretes and the carrot roots were sanitized with a 0.66% solution of sodium dichloro-s-triazinetrione dehydrate (3% active chlorine) and packed in LDPE 20 µm plastic bags containing 7 roots or about 250 g

of Cenourete®. Each bag represented one experimental unit. Care was taken to close the bag such that the free headspace was proportionally the same in both treatments, namely for the intact and processed carrots. All the processing operations were performed at room temperature.

Both types of carrot were stored at 5 ± 1.5 °C for 17 days under dark or light conditions. For this, the same storage room was split into two with a black curtain, so that half of it was in the dark and the other half in the light. Light was provided by 4 fluorescent lamps in an area of 3.5 x 1.5 m, with light intensity at the height at whitch the samples were stored of 400 lux. Evaluations were performed 2, 5, 8, 11, 14 and 17 days after processing. The experiment was set up in a complete randomized design with two cultivars x two types of carrot x two light conditions x seven storage times with 5 replicates.

2.1 Preparation of samples for chemical analyses

The samples were obtained by removing the top of the roots and Cenouretes® and taking an equal amount of transversal slices from all the Cenourete®/roots in the replicate in order to obtain about 20 g of fresh weight. After that, the carrot tissue was immediately frozen in liquid nitrogen and stored at –20 °C until freeze dried. Freeze dried samples were transported to the Federal University of Viçosa – Department of Plant Science, Viçosa-MG where the chemical analysis were carried out.

2.2 Carotenoid extraction and determination

The extraction of total carotenoids was carried out according to Pereira (2002) with modifications. Approximately 125 mg of freeze dried root was mixed with 10 mL cold acetone and maintained at –20 °C for a week. The samples were then homogenized in a polytron (Ultra Turrax, Brazil) and filtered through a fast filter paper with cold acetone. The residue was washed several times until a clear residue was obtained and the volume of the filtrate was completed to 50 mL and transferred to a separation funnel with 25 mL of petroleum ether. Quantification of the total carotenoids was carried out at 449 nm in a Shimadzu UV1601 spectrophotometer (Japan). The coefficient of molar extinction for carotenoid was 2.592 (RODRIGUEZ-AMAYA, 1989). The total carotenoid content was expressed in $\mu g.g^{-1}$ dry weight.

2.3 Total phenol extraction and determination

The soluble phenolic compounds were quantified as described by Prince and Butler (1977). A 0.5 g sample of freeze dried roots was extracted by mixing the sample with 10 mL methanol and homogenizing in a polytron, followed by centrifugation at 14.000 g for 15 min. A 0.5 mL aliquot of clear supernatant was mixed with 2.5 mL Folin-Ciocaltieu reagent (diluted 1:3, v/v, in water) and 2 mL of a 10% anhydrous sodium carbonate solution. After one hour, the absorbance was determined at 700 nm in a Shimadzu UV1601 spectrophotometer (Japan). D-catechin was used as the standard and the content expressed in µg D-catechin.g^{-1} dry weight.

2.4 Peroxidase activity

Peroxidase activity was determined as described by Menolli et al. (2008). For the enzyme extraction, 0.5 g of freeze dried sample was homogenized in 10 mL 0.1 M phosphate buffer pH 6.5, 0.1% sodium bisulfite and 0.15 M sodium chloride using a polytron. The homogenate was centrifuged at 13.000 g for 30 min at 4 °C and the activity determined after adding 300 µL of enzyme preparation to the 0.05 M phosphate buffer pH 6.0, 0.3% hydrogen peroxide and 0.3% guaiacol. The rate of guaiacol oxidation was determined at 470 nm for 2.5 min at 25 °C. The activity was expressed in AU/min/mg protein. The total protein was determined by the dye binding method (BRADFORD, 1976), using BSA as the standard.

2.5 Polyphenoloxidase activty

The enzyme was extracted as described by Thipyapong et al. (1995) with modifications. A sample weighing 0.5 g of freeze dried root was homogenized in a polytron with 7 mL of 100 mM Tris-HCl, pH 7.0, 100 mM KCl, 1 mM PMSF, 1% Triton X-100 and 1% PVPP. The homogenate was passed through four layers of cheese cloth and the filtrate centrifuged at 13.000 g for 30 min at 4 °C. The activity was determined according to Söderhäll (1995) with modifications. The enzyme extract was added to a previously mixed reaction buffer containing 50 mM Tris-HCl pH 8.0, 2 mM $CaCl_2$ and 5 mM catechol. The change in absorbance was followed for 2.5 min at 490 nm and 25 °C in a Shimadzu UV1601 spectrophometer. The activity was expressed as AU/min/mg protein. Total protein was determined by the dye binding method (BRADFORD, 1976), using BSA as the standard.

2.6 Statistical analysis

The data analysis was carried out using PROC GLM from SAS (SAS Institute, 9.1 for Windows) at a significance level for the analysis of variance tables of $p > 0.05$. The value for R^2 was calculated according to Hatcher and Stepanski (1994), indicating the proportion of variance in the criterion variable that is accounted for by the predictor variable (s) of the study. The values for R^2 ranged from 0.00 to 1.00, with larger values indicating a greater treatment effect. The correlation between the chemical composition and the enzyme activity was calculated using PROC CORR (Pearson Correlation) from SAS (SAS Institute, 9.1 for Windows) at a significance level of $p > 0.05$.

3 Results and discussion

3.1 Total carotenoid content

The minimal processing operations significantly induced a decrease in the total carotenoid content ($Pr > F < 0.0001$) as compared to the intact roots (Figure 1). The differences between the intact and processed roots were significant from the beginning of the storage time and remained practically constant thereafter. The effect of processing was not significantly modulated by the light conditions ($Pr > F = 0.8998$) or cultivar ($Pr > F = 0.0781$). The individual effects of the light conditions ($Pr > F = 0.0540$) and cultivar ($Pr > F = 0.2080$) were also not significant.

The effect of the absence of light on carotenoid degradation in the intact carrot root was in agreement with a previous report by Kopas-Lane and Warthesen (1995). On the other hand, the effect on the cut tissues was shown to be tissue dependent when various fruits were analysed (GIL et al., 2006).

The lower carotenoid content in the processed carrot was most likely due to the removal of the external tissues than to a higher rate of carotenoid degradation. The external carrot tissues are known to have a higher carotenoid content than the inner ones (BARANSKA et al., 2006) and since the samples were obtained from transversal slices of both root and Cenourete®, a higher proportion of external tissue was present in the intact root samples. Rocha et al. (2007) reported a decrease in carotenoid content in grated carrot during storage at 2 °C. However, a close examination of the data presented in the paper indicated that this decrease was of a very small magnitude and within the range of variation observed for each evaluation day.

The irregular trend in carotenoid concentration of sliced carrot, as also the limited degradation throughout storage, as shown in Figure 1, were also reported

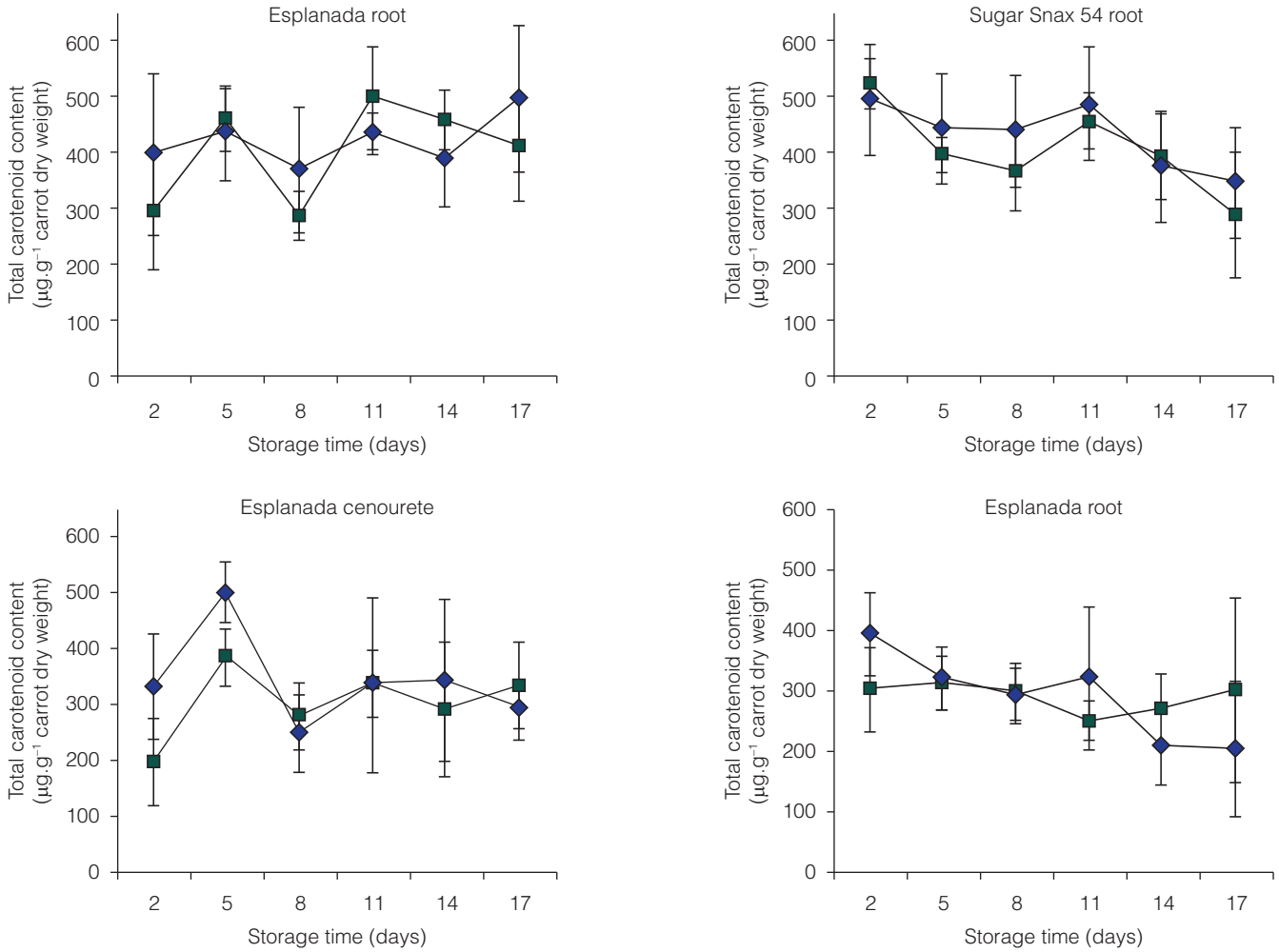

Figure 1. Total carotenoid content (µg.g^{-1} carrot dry weight) of carrot cultivars Esplanada and Sugar Snax 54, stored at 5 °C + 1,5 °C in the dark (blue symbols) or under light (green symbols), as intact root or minimally processed in the form of mini carrot (Cenourete®). Values are the average of 5 replicates + standard deviation.

by Lavelli et al. 2006. These authors considered that the irregular trend was due to carotenoid synthesis in response to stress, followed by degradation. However, the effect of biological variation is also a possible reason for this (DEKKER et al., 2000) and deserves further evaluation.

3.2 Total phenolic content

An increase in phenolic content of the Cenourete® was expected as a response to wounding stress as previously reported for minimally processed carrot (REYES et al., 2007). However, with the exception of the intact root of Sugar Snax 54, the total phenolic content decreased sharply from day 2 to day 5. As from day 5 and up to the end of storage, the total phenolic content remained practically constant for all treatments (Figure 2). About 40% of the variation in phenolic content was accounted for by the effect of time ($P > F < 0.0001$) alone.

The phenolic content was significantly affected by the interaction cultivar*time ($P > F < 0.0001$) and marginally affected by processing and cultivar* processing interaction ($Pr > F = 0.0247$), both most likely reflecting the particular behaviour of the Sugar Snax 54 intact root treatment.

The effect of light was not significant ($Pr > F = 0.1688$), similar to that reported for cut potato (REYES and CISNEROS-ZEVALLOS, 2003). The reaction to light was shown to be tissue dependent since it induced an increase in total phenolic content in mango cubes, but had no effect on cut pineapple, cantaloupe and strawberry (GIL et al., 2006).

The effect of processing was marginally significant ($P > F = 0.0025$) and explained less than 1% of the variation in the data set ($R^2 = 0.0078$). The limited effect of processing was not expected since the phenolic content is higher in the peel and phloem, totally and partially removed during processing, respectively, than in the xylem (ZHANG and HAMAUZU, 2004).

Simões et al. (2009) and Klaiber et al. (2005) showed that the accumulation of phenolic compounds in cut carrot was greatly influenced by the storage

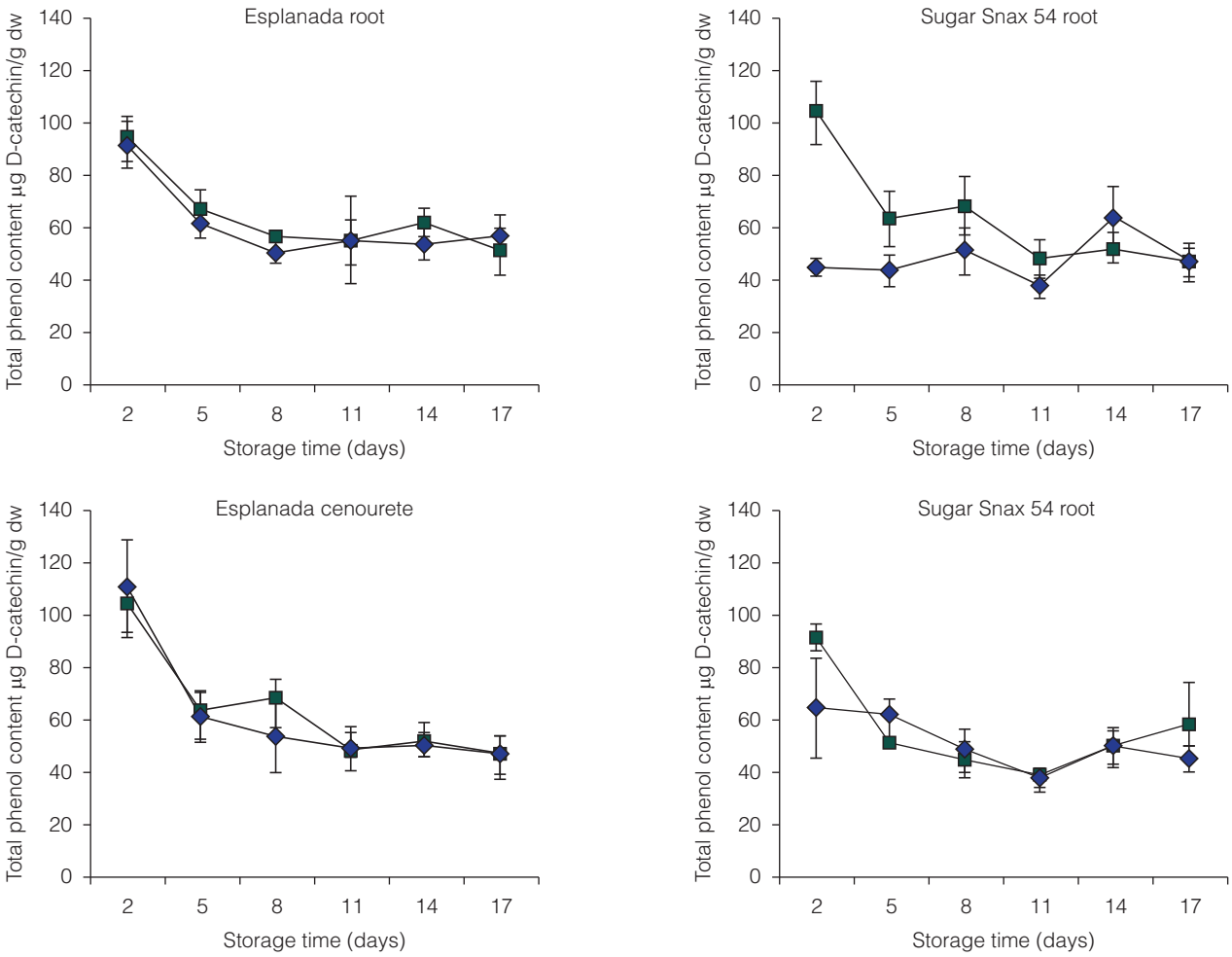

Figure 2. Total phenols content (µg D-catechin/g carrot dry weight) of carrot cultivars Esplanada and Sugar Snax 54, stored at 5 °C + 1,5 °C in the dark (blue symbols) or under light (green symbols), as intact root or minimally processed in the form of mini carrot (Cenourete®). Values are the average of 5 replicates + standard deviation.

atmosphere. Significant changes in the atmosphere can occur even at 5 °C, depending on the film package used (KLAIBER et al., 2005). In the present work, the internal atmosphere inside the packages was not measured. No visible signs of microbial growth that could contribute to an increase in internal CO_2 or the development of off-odours and off-flavours as a result of anaerobiosis, were observed.

Reyes et al. (2007) reported that changes in the phenolics content in response to wounding ranged from a 26% decrease to a 191% increase, depending on the type of vegetable or fruit tissue considered. Although they reported an increase in phenolic content for cut carrot, it is not known whether this change would differ amongst cultivars or maturity at harvest. In the present work, the carrots were harvested about 10-20 days before the recommended time for carrot intended for the fresh-market, in order to obtain thinner roots for processing, whitch could result in a different phenolic content and enzyme activity as compared to previous reports.

What remains to be investigated is whether the increase in phenolic content as a response to wounding, occurred in a time frame of a few minutes or of several hours after processing. If the latter case, what was measured here would be the degradation occurred after a previous increase in phenolic compound production. However, this possibility, as much as that of modified atmosphere, still does not explain why intact and cut roots of the Esplanada cultivar presented the same pattern of change with time.

3.3 Peroxidase (POD) activity

The peroxidase activity was significantly influenced by both processing ($P > F < 0.0001$) and storage time ($P > F < 0.0001$). There was no significant effect for cultivar ($P > F = 0.8749$) or light ($P > F = 0.7103$). Intact roots of both cultivars presented higher POD activity as compared to Cenouretes® (Figure 3). The POD activity in the carrot root was shown to be higher in the superficial tissue and to decrease towards the core region (VORA et al., 1999; LEPEDUŠ et al., 2004). The production of Cenourete® involves the removal of superficial tissue where the POD activity is higher, which is the most likely reason why the intact roots presented higher activity than the Cenourete® for both cultivars.

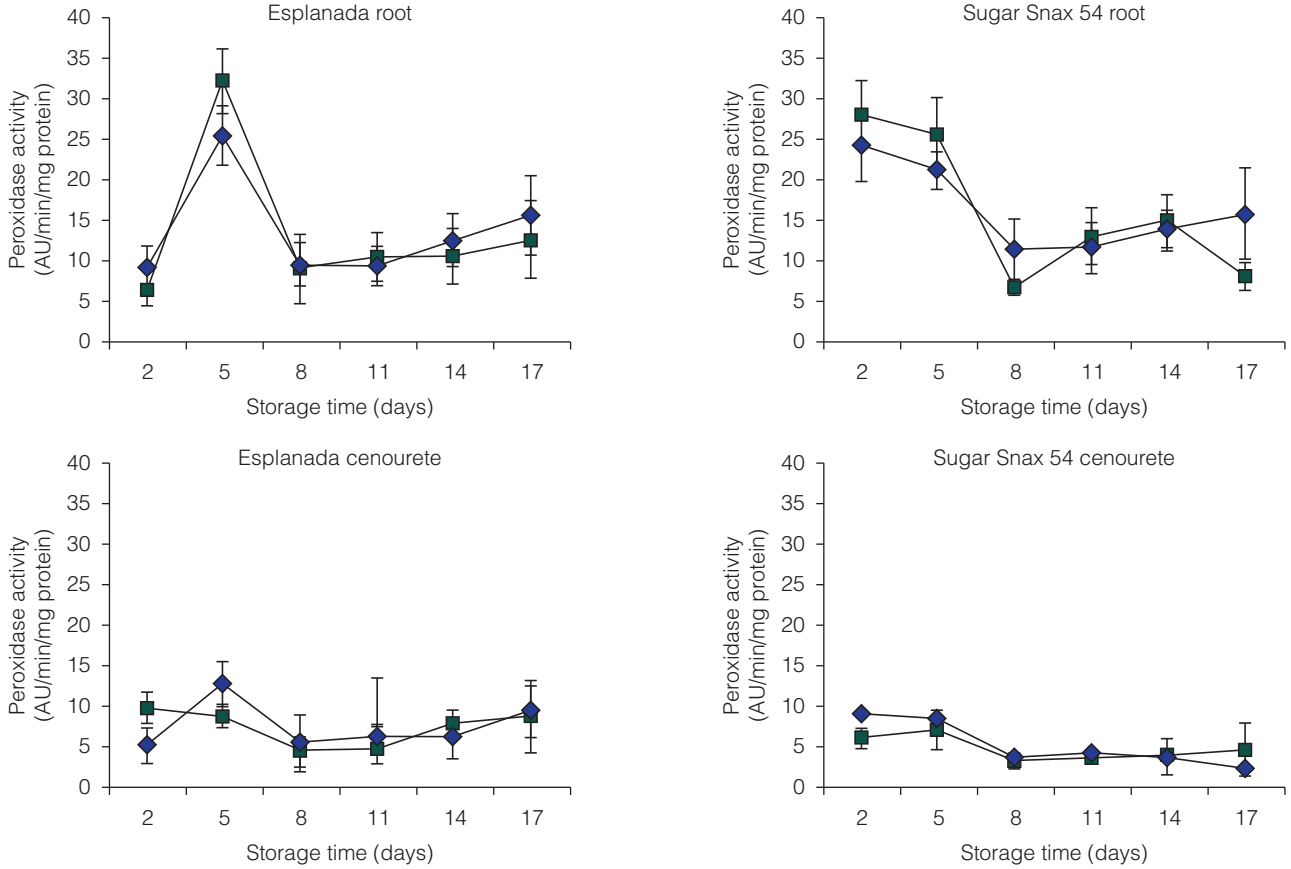

Figure 3. Peroxidase activity (AU/min/mg protein) of carrot cultivars Esplanada and Sugar Snax 54, stored at 5 °C + 1,5 °C in the dark (blue symbols) or under light (green symbols), as intact root or minimally processed in the form of mini carrot (Cenourete®). Values are the average of 5 replicates + standard deviation.

In shredded carrot, stored at 5 °C, the POD activity maintained the initial level until day 4, reaching its highest activity by day 7, followed by a decrease up to the end of the storage period (ALEGRIA et al., 2010). The peeled carrot sticks stored at 2 °C also showed an increase in POD activity, but only by day 3 (HOWARD et al., 1994). In both cases, the POD activity was related to deteriorative changes in the colour.

The correlation between total phenol content and peroxidase activity, when all the treatments were considered, was not significant (Pr > F = 0.7073). When the data from Cenourete® and the intact root were analysed separately, the correlation between the phenolic content and peroxidase activity in the intact root remained non-significant (Pr > F = 0.1891) while for Cenourete® it presented a significant (Pr > F < 0.0001) but very small correlation (03349). Higher correlation was observed between the carotenoid content and peroxidase activity, respectively 0.3349 (Pr > F = 0.0002) and 0.5467 (Pr > F < 0.0001) for the intact and processed root.

3.4 Polyphenol oxidase (PPO) activity

The PPO activity was present at much lower rates as compared to POD activity. The PPO activity was significantly affected by the cultivar (P > F < 0.0001), by storage time (P > F < 0.0001) and by their interaction (P > F < 0.0001). The higher PPO activity of the Esplanada cultivar had no remarkable effects on the carrot appearance and shelf-life when compared with Sugar Snax 54. Both the Esplanada variety intact and cut roots showed an increase in activity, reaching their maximum by day 5, followed by an intense drop by day 8 (Figure 4). The PPO activity in the cultivar Sugar Snax 54 was much lower and it seems unlikely that it had a major role in the oxidation of the soluble phenols. The correlation between total phenolic content and PPO was significant (P > F < 0.0001) but rather small (0.3595).

The reasons for the effect of cultivar could not be determined in this work. Marked differences in both the level of PPO activity and in the content of its substrates have been observed between different cultivars of fruits

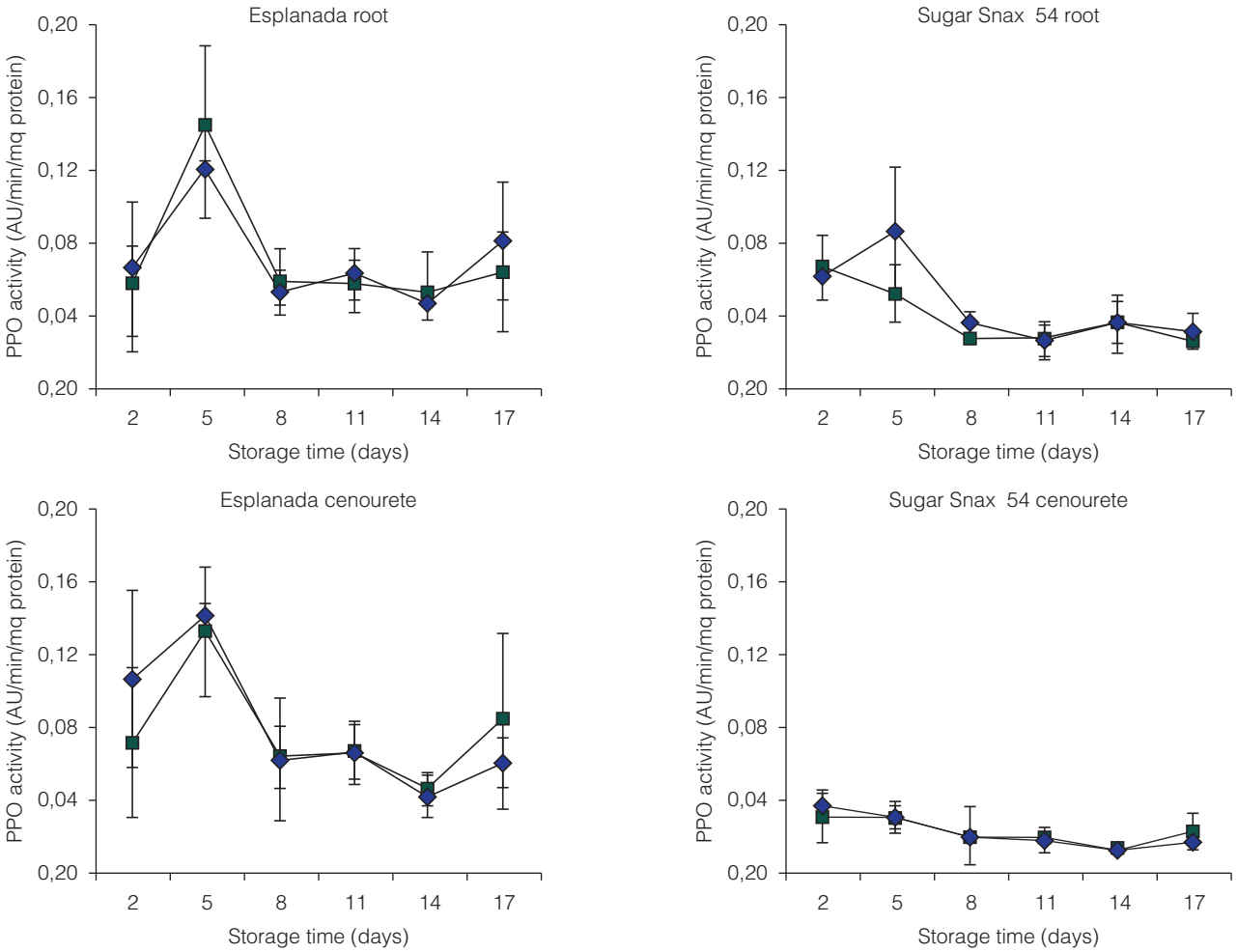

Figure 4. Poliphenol oxidase activity (AU/min/mg protein) of carrot cultivars Esplanada and Sugar Snax 54, stored at 5 + 1,5 °C in the dark (blue symbols) or under light (green symbols), as intact root or minimally processed in the form of mini carrot (Cenourete®). Values are the average of 5 replicates + standard deviation.

and vegetables, including carrot (MAYER and HAREL, 1979; ZHANG et al., 2005). Further investigations should explore the effect of developmental stage on PPO activity. It has been demonstrated that the activity of PPO changes markedly during the development of the plant (MAYER and HAREL, 1979). In the present work, both cultivars were harvested 90 days after sowing in order to obtain thinner roots for processing. However Esplanada has a shorter cycle than Sugar Snax 54, which was therefore in an earlier stage of development, which in turn could affect its PPO level of activity.

Contrary to expected, processing the roots had a marginal effect ($P > F = 0.0170$, $R^2 = 0.0077$) on PPO activity. An analysis of the distribution of the deteriorative enzymes in 4 Australian carrot varieties showed that PPO activity was only detected in the superficial tissues (VORA et al., 1999). An effect of processing similar to that observed for POD activity would then be expected, due to the removal of the tissues where the enzymes would be concentrated. The reason for the lack of effect of processing on both cultivars is not clear at the moment, but it should be stressed that during storage none of the treatments showed extensive change in surface colour (data not shown) that would result from the oxidation of phenolic compounds.

The presence or absence of light had no influence on PPO activity ($P > F = 0.4093$).

4 Conclusions

The processing operations to produce Cenourete®, namely cutting and abrading, induced a decrease in carotenoid content as compared to the intact carrot root when both were stored at 5 °C for 17 days. The total phenolic content decreased during storage for both intact and processed roots and differences among cultivars were not relevant.

The changes in phenolic content were not correlated with the activity of either POD or PPO. POD activity was inhibited in the processed carrot as compared to the intact roots for both cultivars. The PPO activity was much lower than POD activity and differences between the cultivars were more important, with Esplanada showing higher activity than Sugar Snax 54. These differences were not expressed as differences in shelf life neither in appearance. A rather small, but significant correlation between carotenoid content and peroxidase activity was found. None of the factors studied was influenced by the presence of light.

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