



The effects of storage temperature on the quality and phenolic metabolism of whole and minimally processed kale leaves

Adriano do Nascimento Simões^{1*}, Silvino Intra Moreira², Paulo Roberto Mosquim², Nilda de Fátima Ferreira Soares³ and Rolf Puschmann²

¹Unidade Acadêmica de Serra Talhada, Universidade Federal Rural de Pernambuco, Fazenda Saco, s/n, 56903-970, Serra Talhada, Pernambuco, Brazil.

²Departamento de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. ³Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil. *Author for correspondence. E-mail: adriano@uast.ufpe.br

ABSTRACT. We studied the effects of storage temperature on the activities of phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) in minimally processed kale (*Brassica oleracea* var. *acephala*) that was stored for 15 and 9 days at $5 \pm 1^\circ\text{C}$ and $10 \pm 1^\circ\text{C}$, respectively. The main visual evidence for quality loss in whole leaves was yellowing and loss of turgescence. Minimally processed leaves presented significant browning, indicating increased POD and PPO activities. The PAL activity in minimally processed leaves stored at 5°C was fourfold higher than that of whole leaves after two days of storage. We showed that minimal processing influenced PAL, POD and PPO activities. The activity of all enzymes studied increased during storage, indicating that changes in phenolic metabolism play an important role in the decline of kale quality. PAL activity increased rapidly at the beginning of storage and exhibited a reduced rate of increase over time, while the PPO and POD activities increased continuously over time. The storage at 5°C was a great ally in delaying changes in phenolic metabolism; however, the absolute PAL activity was higher at 5°C than at 10°C .

Keywords: *Brassica oleracea* cv. *acephala*, enzymatic browning, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase.

Efeito da temperatura de conservação na qualidade e no metabolismo fenólico de folhas de couve inteiras e minimamente processadas

RESUMO. Estudou-se o efeito da temperatura de armazenamento e do processamento mínimo sobre a atividade da fenilalanina amônia-liase (PAL), peroxidase (POD) e polifenol oxidase (PPO), em folhas de couve (*Brassica oleracea* var. *acephala*) por 15 e 9 dias, nas condições de $5 \pm 1^\circ\text{C}$ e de $10 \pm 1^\circ\text{C}$, respectivamente. O amarelecimento e a perda de turgescência foram as principais causas para a redução da qualidade visual das folhas inteiras, enquanto nas folhas minimamente processadas apresentaram também, escurecimento, que coincidiu com o aumento na atividade da POD e PPO nas duas temperaturas estudadas. A atividade da PAL em folhas minimamente processadas mantidas a 5°C mais que dobrou até o segundo dia de conservação em relação às conservadas a 10°C . O processamento mínimo influenciou a atividade da PAL, POD e PPO. Todas as enzimas avaliadas, aumentaram a atividade durante a conservação, seja no início, no caso da PAL, ou no final, para a PPO e POD, indicando que as mudanças no metabolismo fenólico desempenha um papel importante no declínio da qualidade de couve. A conservação a 5°C retardou as alterações no metabolismo fenólico, embora nessa temperatura, a atividade da PAL foi maior, em grandeza numérica, em relação às folhas armazenadas a 10°C .

Palavras-chave: *Brassica oleracea* cv. *acephala*, escurecimento enzimático, peroxidase, polifenol oxidase, fenilalanina amônia-liase.

Introduction

Currently, there is a high level of interest in the production of minimally processed fruits and vegetables for consumption at home, in fast-food restaurants and in hotels; this demand has been attributed to the desire for reduced preparation time (BEAULIEU et al., 1997). In developed countries, approximately seventy percent of minimally

processed products are used in industrial cooking and fast-food restaurants (WILEY, 1994). Brazil has many commodities that are useful when they are minimally processed, such as cabbage, taioba and serralha (CARNELOSSI et al., 2005). The Brazilian population consumes a significant amount of kale (*Brassica oleracea* cv. *acephala*), and minimally processed kale is popular in Brazil. In the state of Brasília, for example, the production of minimally

processed kale exceeds 12 tons per month; the shelf life of kale, however, is less than four days (CARNELOSSI et al., 2005).

The main problems with the quality of minimally processed kale leaves (*Brassica oleracea* cv. *acephala*) are yellowing, browning, dryness and odor (CARNELOSSI et al., 2005), similar to enzymatic browning in lettuce (SALTVEIT; CHOI, 2007).

Enzymatic browning in lettuce occurs when the metabolism of phenylpropanoids is activated, which is triggered by phenylalanine ammonia-lyase (PAL) activity (EC 4. 3. 1. 5). This enzyme transforms phenylalanine into cinnamic acid (KE; SALTVEIT, 1989). The accumulation of phenolic compounds may increase susceptibility to browning because these compounds are natural oxidation substrates for peroxidases (POD) (EC 1. 11. 1. 7) and polyphenol oxidase (PPO) (EC 1. 14. 18. 1).

During the storage of minimally processed kale, Carnelossi et al. (2005) observed and measured increasing darkness in leaves and attributed this change to PPO activity.

Increased POD and PPO activities can decrease food quality significantly by causing browning, loss of sensorial characteristics (such as color, smell, and texture) and loss of nutrients (WHITAKER, 1995); however, there is disagreement regarding the possible contributions of PAL, POD and PPO to the browning of minimally processed lettuce during storage (CASTANER et al., 1999).

The effect of different temperatures on the activity of the enzymes of phenolic metabolism in kale during postharvest storage was studied.

The assessment of other variations in the biochemistry properties (SIMÕES et al., 2010) and phenolic metabolism of collard greens that may occur immediately after their minimal processing and during medium- and long-term storage at both suitable and non-suitable temperatures may provide additional knowledge of quality in collard greens. It is expected that the phenolic metabolism changes that occur soon after the minimal processing of collard greens will not progress if the product is stored at 10°C.

Our objective was to measure the qualitative effects of PAL, POD and PPO on minimally processed kale by studying enzymatic activity in whole and minimally processed kale leaves stored at 5 and 10°C.

Material and methods

The cabbage plants (*Brassica oleracea* var. *acephala*) of accession BGH 1578 used in our study are known as Portuguese cabbages. These plants were grown at the experimental farm of the Plant Science Department. The leaves were detached from the

mother plant after the plant had grown to a height of approximately 0.35 m and were taken to the laboratory immediately. The stems were put in a tray with water for 24 hours to regain turgescence and then placed into a cold chamber at $5 \pm 1^\circ\text{C}$ (CARNELOSSI et al., 2005). After this 24-hour period, a portion of the leaves was minimally processed, and the remainder was kept whole.

We established the following processing steps (requiring approximately 1 hour): selection, standardization (leaves of approximately 35 cm in length) and washing (in tap water), cutting (slicing, 1 mm thick), sanitization (sodium dichloroisocyanurate dihydrate) in 100 mg L⁻¹ active chlorine for 10 min. at 5°C, rinsing (immersion in water at 5°C for 3 s) and centrifugation (10 min. at 800 g). After processing, the product was packed in polypropylene (PP) bags. Whole leaves were packed in sacks that were 0.39 m long, 0.26 m wide and 6 µm deep, with a ribbon of PP that was 4 µm wide and 5 cm long, and perforated with 648 punctures (810 µm in diameter) at the ends with the stems. For minimally processed leaves, we used sacks that were 0.22 m long, 0.26 m wide and 6 µm deep, with a ribbon of PP that was 4 µm wide and 5 cm long, and perforated with 648 punctures (810 µm in diameter) on the ends. The ribbons of PP were sealed at the front and back of the sacks. We put approximately 200 g of whole leaves (four leaves) and approximately 100 g of fresh-cut kale (approximately 4 leaves) in the packages.

The packed leaves were stored away from light in a refrigerated expositor with forced ventilation of air at $5 \pm 1^\circ\text{C}$ and $10 \pm 1^\circ\text{C}$, both with $90 \pm 5\%$ RH, for a period of 15 and 9 days, respectively. The activity of PAL, PPO and POD was quantified at intervals of two days until the sixth day; then, assessments were made at intervals of three days. Each package consisted of one repetition, with a total of three packages per treatment.

We evaluated the early senescence of leaves through visual observations by comparing the leaves evaluated during each time interval with freshly harvested leaves and newly processed leaves. The onset of senescence was characterized visually by yellowing, loss of turgor, dryness and browning.

For extraction and testing of PAL (EC 4.3.1.5), we used the methodology of Ke and Saltveit (1986) with certain modifications. The enzymatic extract was obtained by homogenization of 3.0 g of the vegetal material in 6.0 mL of sodium borate buffer (0.1 M), pH 8.8 with β-mercaptoethanol (5 mm), EDTA (2 mm) and 1% insoluble polyvinyl pyrrolidone (PVPP) (p/v). The extract was centrifuged at 25,000 g

for 20 min. with a 4°C desalinate in a Sephadex G25 column (Sigma-Aldrich, EUA).

For this assay, 2.0 mL of L-phenylalanine (Sigma-Aldrich, EUA) (60 mM) in sodium borate buffer (0.1 M), pH 8.8, was pre-incubated at 40°C for 15 min. After pre-incubation, 0.5 mL of enzymatic extract was added, followed by 2 hours of incubation at 40°C; the absorbance at 290 nm was measured before and after incubation. Sodium borate buffer (0.1 M) was used instead of L-phenylalanine as a blank. The activity of PAL was expressed as the increase in absorbance (ΔAbs) at 290 nm in 1 hour per milligram of protein in enzymatic extract, according to Bradford (1976).

For the extraction and assay of POD (EC 1.11.1.7) and PPO (EC 1.14.18.1), the enzymatic extract was obtained by homogenization of 1.0 g of vegetal material in 6.0 mL of sodium phosphate buffer (0.2 M), pH 6.0; the samples were then centrifuged at 10,000 g for 21 min. at 4°C.

The POD assay was performed added 10 μL of enzymatic extract to 1.0 mL of sodium phosphate buffer (0.2 M), pH 6.0, at 25°C until the temperature stabilized. We then added 100 μL of guaiacol (Sigma-Aldrich, EUA) (0.5 %) and 100 μL of hydrogen peroxide (0.08 %) as an electron donor; the absorbance at 470 nm was measured every 30 s for 2 min.

For the determination of PPO activity was added 1.3 mL of sodium phosphate buffer (0.2 M), pH 6.0, mixed with 1.5 mL of catechol (Sigma-Aldrich, EUA) (0.2 M) as a substrate; the reaction mixture was incubated at 30°C until the temperature stabilized. We then added 30 μL of enzymatic extract, and the absorbance at 425 nm was measured every 30 s for 2 min.

One enzymatic unit (EU) of POD or PPO was defined as the amount of extract that was capable of increasing the absorbance by 0.001 absorbance unit in 1 minute. As a blank, we used the enzymatic extract, which was boiled for 10 min., for both POD and PPO. For the three enzymes, the control corresponded to a previously boiled enzyme extract, as well as a replacement of the enzyme extract extraction buffer.

We used a randomized design, with three replications in the subplot for each temperature (5 and 10°C), different storage times for the main plot (0, 2, 4, 6, 9, 12, and 15 days for leaf samples stored at 5°C and 0, 2, 4, 6, and 9 days for leaves stored at 10°C) and two types of leaves (whole and minimally processed). We conducted an analysis of variance using the SISVAR software. For qualitative treatment (leaf types), we used a Tukey test at 5% probability, and the quantitative factors (conservation time) were analyzed for their ability to fit a regression equation.

Results and discussion

The main symptoms of visual whole leaf senescence observed were yellowing and loss of turgor. In leaves stored at 5°C, the onset of these symptoms began at nine days. For leaves stored at 10°C, senescence began at six days (Figure 1), a difference of 3 days, which is significant in commercial terms.



Figure 1. General appearance of whole cabbage leaves (left) and minimally processed leaves (right) maintained for 6 days at 10 and 5°C.

The signs indicating loss of quality in the minimally processed leaves consisted primarily of yellowing, turgidity loss that caused drying (particularly in the region near the micro holes in the packaging), and signs of hardening at the cut edges (data not shown). The onset of the signs of visual senescence occurred on the sixth day of storage in the minimally processed leaves stored at 10°C and on the 11th day of storage in the leaves stored at 5°C, indicating that although there was mechanical damage to the minimally processed leaves, storage at 5°C delayed the onset of the senescence symptoms.

Generally, the minimally processed leaves had a longer shelf life post-harvest when stored at 5°C compared with the intact leaves. Carnelossi et al. (2005) reported that for minimally processed kale green leaves of identical variety, quality was maintained over a period of up to 10 days at 10°C and 15 days at 5 and 1°C in PD 961, PD 941 and plastic boxes. Therefore, we did not observe the increase in useful life that was observed by the above-referenced authors. However, a storage period of up to 11 days at 5°C was determined to be commercially viable.

There was a significant interaction between the leaf types and the storage times for PAL and PPO in leaves stored at 5°C and for PPO in leaves stored at 10°C (Figure 2). Analyses of PAL in leaves stored at 10°C

and of POD in leaves stored at 5 and 10°C showed that the interaction was not significant when the enzymes were considered independently. The results of these analyses were plotted as a function of storage times (Figure 3). We conducted a Tukey test at 5% probability to compare the leaf types (Table 1).

Table 1. Mean values for all days for PAL and POD activity in whole and minimally processed leaves stored at 5 and 10°C.

Leaves	PAL, 10°C ($\Delta 290 \text{ nm h}^{-1} \text{ mg}^{-1}$ protein)	POD, 5°C ($\text{EU min}^{-1} \text{ mg}^{-1}$ protein)	POD, 10°C ($\text{EU min}^{-1} \text{ mg}^{-1}$ protein)
Intact	0.002 b	3871.07 b	4257.77 b
Minimally Processed	0.006 a	5643.03 a	6157.31 a

Averages followed by an identical small letter in the column do not differ significantly according to a Tukey test at 5% probability.

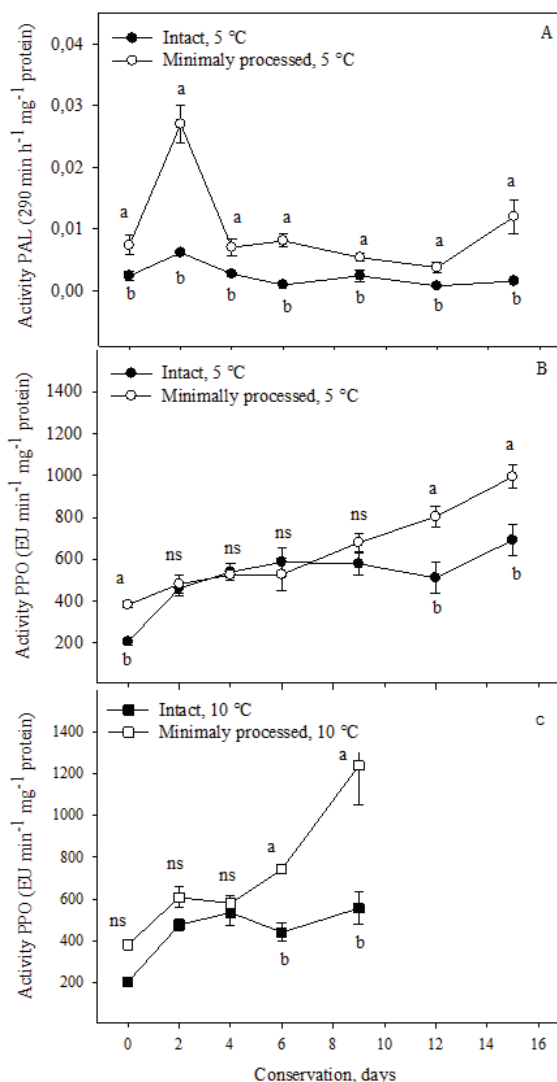


Figure 2. PAL and PPO activity in whole and minimally processed cabbage leaves stored at 5 and 10°C. The letters within the graph represent the results of the Tukey test at 5% probability. ns = not significant.

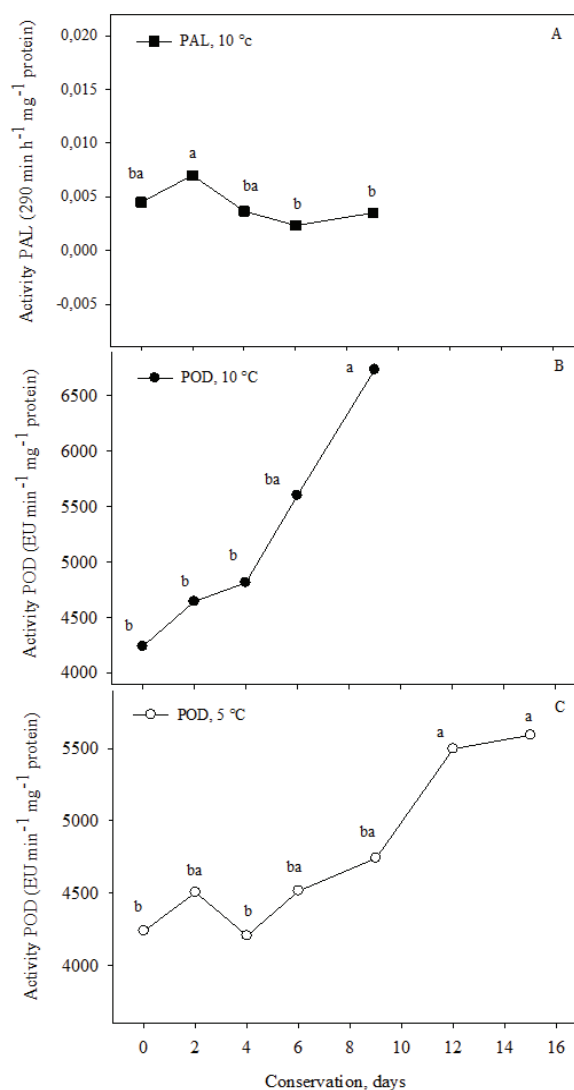


Figure 3. PAL and POD activity in whole and minimally processed cabbage leaves stored at 5 and 10°C. The letters within the graph represent a Tukey test at 5% probability.

No adjustment was needed for the regression equation to obtain insight into the interactions among storage times for the different types of leaves. Thus, we split the interaction of leaf types, depending on the shelf life of each type, using the Tukey test at 5% probability (Figure 2).

PAL activity increased approximately fourfold 48 hours after minimal processing (Figure 2A). When whole leaves and minimally processed leaves were stored at 10°C, the amplitude of the difference was reduced but still significant (Table 1). This response may be the result of the effects of cutting and ethylene, as suggested by Hyodo and Fujinami (1989), who reported increased ethylene production with increases in PAL activity, even when the mechanisms involved were proportionally different.

When minimally processed leaves were stored at 5°C, the PAL activity on the second day of storage was approximately 5.5-fold greater than that of whole leaves at an identical temperature (Figure 2A); at 10°C, however, this value was only 1.5-fold greater (data not shown). This difference may be the result of the stimulation of PAL by the stress of cold temperatures (VAMOS-VIGYAZÓ, 1981). Molisch (1896) reported that chilling injury can alter the metabolism of phenylpropanoids and, therefore, the activity of PAL, which may result in the accumulation of phytotoxic compounds. In this experiment, despite not having observed symptoms of chilling injury, the leaves stored at 5°C exhibited more stimulated PAL activity than those stored at 10°C.

These results suggest that PAL activity was stimulated at the start of storage, particularly when the leaves were minimally processed and stored at lower temperatures (5°C), and decreased in later stages, as was also observed by Cantos et al. (2001). The decrease in PAL activity may have resulted from inhibition due to the accumulation of chlorogenic acid in the lettuce, a by-product of PAL (SARMA et al., 1997). In addition, the decrease may also have resulted from the biosynthesis of a protein known as the PAL inactivation factor (PAL-IF), which occurs slowly (approximately 12 hours after PAL induction) and is sufficient to allow for enzyme activation and phenol compound accumulation, causing the leaves to darken (RITENIOUR; SALTVEIT, 1996).

In our study, throughout the time intervals tested, the greatest PAL activity was observed at 48 hours after leaf processing (Figure 2). In a previous study on minimally processed lettuce, PAL activity peaked after 24 hours of storage at 5°C, thus inducing enzymatic darkening (KE; SALTVEIT, 1989). One likely explanation for the peaks in PAL

activity concerns the sequential induction of mRNAs of this enzyme, as previously reported by Ishizuka et al. (1991) in pieces of potato. Furthermore, Ke and Saltveit (1989) reported that cutting induces a repeated signal for PAL synthesis in lettuce.

Inducing PAL activity also leads to the synthesis of lignin precursor compounds, as observed in lettuce by Ke and Saltveit (1989) and in fresh-cut cassava in stick by Junqueira et al. (2014). In our study, lignin synthesis most likely occurred and was then mediated both by PAL and by POD (Figures 2 and 3, Table 1), which may contribute to sensorial quality loss in the product.

Cantos et al. (2001) and Castaner et al. (1999) did not observe a correlation between susceptibility to darkening and PAL activity in lettuce cultivars. However, our results showed that increases in PAL activity at the start of storage may have contributed to phenol compound accumulation; these phenols may have been oxidized by PPO and POD, inducing darkening in the minimally processed leaves beginning at six days at 10°C and at 11 days at 5°C.

On average, minimally processed leaves presented approximately twice the POD activity of whole leaves (Table 1). Furthermore, POD activity increased steadily throughout the storage time (Figure 3B and C). A similar observation was reported by Ke and Saltveit (1989) in cut lettuce stored at 5°C.

Between 4 and 9 days of storage at 10 and 5°C, we observed an increase in POD activity for minimally processed leaves that coincided with the start of darkening (Figure 3B and C, Table 1); this increase in activity is likely significant in the darkening process (RICHARD-FORGET; GAUILLARD, 1997) and in tissue senescence (VAMOS-VIGYAZÓ, 1981).

The increase in POD activity after minimal processing (Table 1) may have been the result of certain chemical signals that induce the re-synthesis of this enzyme, as observed by Ke and Saltveit (1989) and Cantos et al. (2001) in lettuce. The increased POD activity may reflect a greater rate of lignin biosynthesis and a greater healing rate adopted by the leaves in an attempt to compensate for the damage suffered after cutting and to protect the tissue from pathogen attack, as suggested by Ke and Saltveit (1989) and Howard and Griffin (1993).

Although no significant interaction was observed, these results highlight the important role of POD in leaf quality and, thus, in the senescence of the collard green leaves studied by the above-mentioned authors, by either acting on tissue

lignification or darkening. However, these mechanisms still must be studied in collard greens.

Minimal processing induced increases in PPO activity, resulting in a doubling of enzymatic activity compared with activity of the control on day zero (Figure 2B).

The minimally processed leaves presented significantly greater PPO activity compared with whole leaves immediately before the start of visual darkening at both storage temperatures (Figure 2B and C). Castaner et al. (1999) and Carnelessi et al. (2005) reported the involvement of PPO in the darkening of minimally processed lettuce and minimally processed collard greens, respectively. However, Cantos et al. (2002) were not able to establish a positive relationship between susceptibility to darkening and PPO activity in minimally processed potato cultivars.

In our study, we observed that a storage temperature of 5°C delayed the increase in PPO activity (Figure 2B and C) and, consequently, the onset of darkening. The minimal processing likely activated the latent PPO in lettuce, as observed by Espín et al. (1999) and Cantos et al. (2001), or may also have been involved in its re-synthesis (CANTOS et al., 2002; THIPYAPONG et al., 1995).

Tudela et al. (2002) reported that higher vitamin C content in minimally processed potato tissues reduced the susceptibility of the leaves to darkening as a result of PPO activity. High vitamin C content has been observed in kale green leaves (SIMÕES et al., 2010) and may be one reason why these leaves did not present darkening symptoms shortly after the beginning of storage. Instead, darkening began after the vitamin C levels had decreased by almost half (SIMÕES et al., 2010) at both temperatures studied. Furthermore, recent study associated ascorbic acid with protection against enzymatic browning in rocket leaves (DEL'INNOCENTI et al., 2007).

Minimal processing influenced PAL, POD and PPO activities. All of the evaluated enzymes showed increased activity during storage, indicating changes in phenolic metabolism and a possible loss of quality. PAL activity increased rapidly at the beginning of storage and then increased at a slower rate over time, while the activity of PPO and POD increased continuously over time.

Furthermore, storage temperature helped delay certain changes in phenolic metabolism, particularly when the leaves were stored at 5°C; however, the absolute values of PAL activity were higher at 5 than at 10°C.

Conclusion

We found that the changes in the PAL, PPO and POD activities preceded the browning of minimally processed cabbage. This finding indicates that the phenolic metabolism was directly related to sensory quality of minimally processed kale. The conservation of the leaves at 5°C delayed changes in PPO and POD activity and in browning. Although PAL activity was shown to have been extremely altered, more studies on this subject are necessary.

These results show the importance of low temperature (5°C) for the maintaining the quality of whole and minimally processed kale.

Acknowledgements

The authors are grateful to CNPq, Prodetab and Fapemig for financial support.

References

- BEAULIEU, J. C.; OLIVEIRA, F. A. R.; FERNANDES, T. D.; FONSECA, S. C.; BRECHT, J. K. Fresh-cut kale: quality assessment of portuguese storage supplied product for development of a MAP system. **CA'97 Proceedings**, v. 5, p. 145-51, 1997.
- BRADFORD, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analysis in Biochemistry**, n. 72, p. 248-254, 1976.
- CANTOS, E.; ESPÍN, J. C.; TOMÁS-BARBERÁN, F. A. Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. **Journal of Agricultural and Food Chemistry**, v. 49, n. 1, p. 322-330, 2001.
- CANTOS, E.; TUDELA, J. A.; GIL, M. I.; ESPÍN, J. C. Phenolic Compounds and Related Enzymes are Not Rate-limiting in Browning Development of Fresh-cut potatoes. **Journal of Agricultural and Food Chemistry**, v. 50, n. 10, p. 3015-3023, 2002.
- CARNELOSSI, M. A. G.; SILVA, E. O.; CAMPOS, R. S.; PUSCHMANN, R. Respostas fisiológicas de folhas de couve minimamente processadas. **Horticultura Brasileira**, v. 23, n. 2, p. 215-220, 2005.
- CASTANER, M.; GIL, M. I.; RUIZ, M. V.; ARTES, F. Browning susceptibility of minimally processed Baby and Romaine lettuces. **European Food Research and Technology**, v. 209, n. 5, p. 52-56, 1999.
- DEL'INNOCENTI, E.; PARDOSSI, A.; TOGNONI, F.; GUIDI, L. Physiological basis of sensitivity to enzymatic browning in 'lettuce', 'escarole' and 'rocket' salad when stored as fresh-cut products. **Food Chemistry**, v. 104, n. 1, p. 209-215, 2007.
- ESPÍN, J. C.; VAN LEEUWEN, J.; WICHERS, H. J. Kinetic Study of the activation Process of a latent Mushroom (*Agaricus bisporus*) Tyrosinase by Serine Proteases. **Journal of Agricultural and Food Chemistry**, v. 47, n. 9, p. 3509-3517, 1999.

- HOWARD, L. R.; GRINFFIN, L. E. Lignin formation and surface discoloration of minimally processed carrot sticks. **Journal of Food Science**, v. 58, n. 5, p. 1065-1068, 1993.
- HYODO, H.; FUJINAMI, H. The effects of 2, 5-norbornadiene on the induction of activity of phenylalanine ammonia-lyase in wound mesocarp tissue of *Cucurbita maxima*. **Plant Cell Physiology**, v. 30, n. 6, p. 857-860, 1989.
- ISHIZUKA, M.; YAMADA, F.; TANARA, Y.; TAKEUCHI, Y.; IMASEKI, H. Sequential induction of mRNAs for phenylalanine ammonia-lyase in slice of potato tuber. **Plant Cell Physiology**, v. 32, n. 1, p. 57-64, 1991.
- JUNQUEIRA, M. J.; SIMÕES, A. N.; SEDIYAMA, T.; CORREA, P. C.; PUSCHMANN, R. Biochemical and bioactive phytonutrients changes in tissues of two cultivars of fresh-cut cassava in stick form under refrigerated storage. **Ciência Rural**, v. 44, n. 7, p. 1284-1290, 2014.
- KE, D.; SALTVEIT, M. E. J. R. Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. **HortScience**, v. 21, n. 5, p. 1169-1171, 1986.
- KE, D.; SALTVEIT, M. E. Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. **Physiologia Plantarum**, v. 76, n. 3, p. 412-418, 1989.
- MOLISCH, H. **Das Erfriern von Pflanzen bei Temperaturen über dem Eispunkt**. Sitzungsber. D. Kaiserlichen Akademie der Wissenschaften zu Wien, 1996.
- RITENIOUR, M. A.; SALTVEIT, M. E. Identification of a phenylalanine ammonia-lyase inactivating factor in harvested iceberg lettuce (*Lactuca sativa* L.). **Physiologia Plantarum**, v. 97, n. 2, p. 327-331, 1996.
- RICHARD-FORGET, F.; GAUILLARD, F. A. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. **Journal of Agricultural and Food Chemistry**, v. 45, n. 7, p. 2472-2476, 1997.
- SALTVEIT, M. E.; CHOI, Y. Aromatic- and dicarboxylates inhibit wound-induced phenolic accumulation in excised lettuce (*Lactuca sativa* L.) leaf tissue. **Postharvest Biology and Technology**, v. 46, n. 3, p. 222-229, 2007.
- SARMA, A. D.; SREELAKSHMI, Y.; SHARMA, R. Differential expression and properties of phenylalanine ammonia-lyase isoforms in tomato leaves. **Phytochemistry**, v. 49, n. 8, p. 2233-2243, 1997.
- SIMÕES, A. N.; PUIATTI, M.; SALOMÃO, L. C. C.; MOSQUIM, P. R.; PUSCHMANN, R. Effect in the quality of intact and minimally processed leaves of collard greens stored at different temperatures. **Horticultura Brasileira**, v. 28, n. 1, p. 81-86, 2010.
- THIPYAPONG, P.; HUNT, M. D.; STEFFENS, J. C. Systemic wound induction of potato (*Solanum tuberosum*) polyphenol oxidase. **Phytochemistry**, v. 40, n. 3, p. 673-676, 1995.
- TUDELA, J. A.; ESPÍN, J. C.; GIL, M. I. Vitamin C in fresh-cut potatoes. **Postharvest Biology and Technology**, v. 26, n. 1, p. 75-84, 2002.
- VAMOS-VIGYAZO, L. 'Polyphenol oxidase and peroxidase in fruits and vegetables'. **Critical Reviews in Food Science and Nutrition**, v. 15, n. 1, p. 49-127, 1981.
- WHITAKER, J. R. Polyphenol oxidase. In: WONG, D. W. S. (Ed.). **Food enzymes structure and mechanism**. New York: Chapman Hall, 1995. p. 271-307.
- WILLEY, R. C. **Minimally processed refrigerated fruits and vegetables**. London: Chapman and Hall, 1994.

Received on August 2, 2012.

Accepted on December 4, 2012.

License information: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.