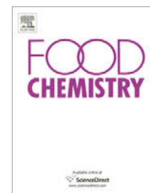
Food Chemistry 119 (2010) 1502–1506

Contents lists available at ScienceDirect

Food Chemistry

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



Salinity effects on enzymatic browning and antioxidant capacity of fresh-cut baby Romaine lettuce (*Lactuca sativa* L. cv. Duende)

Marco Chisari *, Aldo Todaro, Riccardo N. Barbagallo, Giovanni Spagna

DOFATA – Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari, University of Catania, Via Santa Sofia 98, 95123 Catania, Italy

A R T I C L E I N F O

Article history:
Received 4 June 2008
Received in revised form 25 August 2009
Accepted 8 September 2009

Keywords:
Browning
Polyphenol oxidase
Peroxidase
Baby lettuce
Hydric stress
ORAC

A B S T R A C T

Enzymatic browning is a main problem encountered in minimal processing and further storage of leafy vegetables, leading to shorter shelf-life of products. Changes in the two oxidative activities, polyphenol oxidase (PPO) and peroxidase (POD), as well as in total phenolic content, colour parameters and antioxidant capacity (assayed with ORAC method), were monitored during 10 days of storage at 4 °C of minimally processed baby Romaine lettuce (*Lactuca sativa* L. cv. Duende) cultivated under three different salinity conditions (2.8, 3.8 and 4.8 dS m^{-1}), in order to determine the most suitable condition for further processing. Increasing levels of salinity reduced both oxidases activities immediately after cutting and throughout 7 days of storage. Samples cultivated under high salinity had also the lowest change in colour, expressed as ΔE^* $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}]$, and showed the lowest reduction in total phenolic content and antioxidant capacity after 3 days of storage.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Minimally processed lettuce has become popular in recent years because of the increased consumption of fast food and prepared salads. Unfortunately, the damage inflicted to plant tissues by shredding or slicing accelerates reactions that lead to quality defects such as browning of cut edges, discolouration and reduced turgidity (King Jr., Magnuson, Torok, & Goodman, 2001). Enzymatic browning is a main problem which arises during minimal processing and further storage of lettuce (Loaiza-Velarde, Tomas-Barberan, & Saltveit, 1997; Tomas-Barberan, Gil, Castaner, Artes, & Saltveit, 1997), caused by polyphenol oxidase (EC 1.14.18.1; PPO) and peroxidase (EC 1.11.1.7; POD). PPO is a copper-enzyme that catalyses the *o*-hydroxylation of monophenols (monophenolase activity) and the oxidation of the *o*-diphenols to *o*-quinones (diphenolase activity) using molecular oxygen. The formed *o*-quinones undergo a complex series of non-enzymatic chemical changes which ultimately yield melanins and cause a loss of nutritional value due to involvement in reactions with amino acids. Peroxidase is mainly involved in lignification processes but it can also form melanins as previously described (Richard-Forget & Gauillard, 1997). These two enzymatic activities, widely taken as indexes of quality loss in products under optimum cultivation conditions, could also be utilised to evaluate the impact of unfavourable agronomic conditions, such as hydric stress, on product composition. In fact, the oxidation of phenolic compounds may also influence the antioxidant capacity since polyphenols are known to reduce oxidative stress and prevent chronic diseases (Muller, Bub, Watzl, & Rechkemmer, 1999). The antioxidant properties of these compounds are responsible for their anticancer, antiviral and anti-inflammatory properties (Cao, Sofic, & Prior, 1997).

The aim of the present work was to evaluate the changes of polyphenol oxidase and peroxidase activities, as well as of total phenolic content, phenolic profile, colour parameters and antioxidant capacity, during 10 days of storage at 4 °C of minimally processed baby Romaine lettuce (*Lactuca sativa* L. cv. Duende) cultivated under three different salinity conditions (2.8, 3.8 and 4.8 dS m^{-1}), in order to determine the most suitable condition for further processing.

2. Materials and methods

Plant materials: Baby Romaine lettuce (*Lactuca sativa* L. cv. Duende) was cultivated in three separated lots (500 m^2 each) of the experimental field of Dipartimento di OrtoFloroArboricoltura e Tecnologie Agroalimentari (DOFATA, Catania, Italy), adopting three different watering regimes and obtaining then three different salinity conditions (2.8, 3.8 and 4.8 dS m^{-1}). The salinity conditions were determined by measuring the electrical conductivity of soil paste extracts with a conductivity meter (CTS, Vicenza, Italy) according to the method proposed by Rhoades (1982). About 50 lettuces for each lot were harvested at commercial maturity stage, transported to the laboratory and stored at 4 °C until they were

* Corresponding author. Tel.: +39 0957580220; fax: +39 0957141960.
E-mail address: mchisari@unict.it (M. Chisari).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.09.033

processed the next day. Average fresh weight was 124.1, 117.5 and 111.3 g per head cultivated under 2.8, 3.8 and 4.8 dS m^{-1} salinity conditions, respectively. Wrapper leaves were removed and next seven lettuce leaves were carefully excised and cut into 2 × 2 cm pieces. Midribs were excised and discarded. Batches of 2 kg of excised tissues were dipped in 50 L of a 100 ppm chlorine bath for 1 min at 4 °C and then hand centrifuged to remove surface moisture. Aliquots of about 150 g tissue were placed in perforated polypropylene bags (250 g capacity) and stored at 4 °C during 10 days. Three samples for each condition were taken for analysis at days 0, 3, 7 and 10. One batch for each salinity condition was submitted to the same processing without the chlorine bath, in order to verify the impact of chlorine on enzymatic activities.

Chemicals: All reagents were of analytical grade and purchased from Sigma (Milan, Italy).

PPO and POD extraction and assay: Twenty grams of lettuce were added of 40 mL cold acetone (−20 °C) and homogenated for 10 min with an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany) homogeniser set at maximum speed. The homogenate was filtered through Whatman No. 42 paper under vacuum on a Buchner funnel; the acetone powder, after elimination of the acetone under vacuum, was collected and suspended in 30 mL 0.1 M citrate phosphate buffer pH 7.5 and kept overnight at 4 °C, before again filtering through Whatman No. 42 paper under vacuum on a Buchner funnel. Then, the solution was ultrafiltered in a Millipore stirred cell with 10 kDa membrane (Millipore 8050, Milan, Italy) and utilised as partially purified enzymatic extract.

Enzymatic assay was performed according to a reliable spectrophotometric method, using 3-methyl-2-benzothiazolinone hydrazone (MBTH) to trap the enzyme-generated orthoquinone (Espin, Morales, Varon, Tudela, & Garcìa-Canovas, 1996; Pifferi & Baldassari, 1973). PPO activity was assayed spectrophotometrically at 505 nm using 3,4-dihydroxyphenyl acetic acid (DOPAC) as phenolic substrate with MBTH. The standard reaction mixture contained 0.9 mL of 40 mM phenolic substrate, 0.1 mL of 2% (w/v) MBTH in methanol, 0.05 mL of dimethylformamide (DMF), 1.5 mL of 50 mM sodium acetate buffer pH 7.0 and 0.5 mL of enzymatic extract. Reaction was stopped at 1 min intervals with 0.5 mL of 5% H_2SO_4. Total reaction time was 10 min. Blank was prepared by inverting the order between the enzymatic extract and H_2SO_4. One unit of PPO activity is defined as the amount of enzyme which produces 1 μmol of adduct per min at 25 °C under the conditions above described.

POD activity was determined spectrophotometrically as the change in absorbance at 470 nm. The reaction mixture contained 2 mL of 0.01 M citrate phosphate buffer (pH 7.0) containing 1.0% (v/v) guaiacol, 0.25 mL of 32 mM H_2O_2 and 0.1 mL enzyme extract (Flurkey & Jen, 1978). One guaiacol unit (U) is defined as the amount of enzyme which oxidises 1 μmol of guaiacol per min at 25 °C and pH 7.0 under the conditions above described.

Colour of lettuce samples: The colour was determined on photosynthetic tissue with a compact tristimulus chromameter (Minolta CR-300, Ramsey, NJ, USA) with an 8 mm diameter viewing aperture, white plate reference (Y = 94.3; x = 0.3142; y = 0.3211) and C illuminant (CIE, 2° observer). All measurements were carried out in triplicate on the surface area, initially after cutting and after different storage periods. Readings were expressed as L^*, a^* and b^* parameters. ΔE^* $[(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}]$ parameter was calculated.

Total phenolics determination: Aliquots of lettuce (25 g) were homogenised using a Ultra-Turrax T25 homogeniser set at maximum speed and extracted with 50 mL of methanol, under continuous stirring for 1 h at room temperature. Samples were then centrifuged at 4000×g for 20 min at 4 °C and filtered through Whatman No. 42 paper under vacuum on Buchner funnel.

Total phenolics content was determined according to the Folin–Ciocalteau method (Singleton, Orthofer, & Lamuela-Raventos, 1999), using catechin as a standard. Results were expressed as μg catechin equivalents per gram of fresh weight.

HPLC analysis: The methanolic extracts were evaporated to dryness under vacuum (Rotavapor 144 R, Buchi, Switzerland) at room temperature and rinsed with a $CH_3CN/CH_3OH/H_2O$ (pH 2 by HCOOH) 60:20:20 to a final volume of 5 ml. Samples of 25 μl were analysed by HPLC/DAD. Analyses were conducted using a Shimadzu LC10A liquid chromatograph equipped with a DAD detector and managed by a Shimadzu LCsolution workstation (all from Shimadzu, Milan, Italy). Polyphenol compounds were separated using a 150 × 3.0 mm (5 μm) Luna C18 column (Chemtek Analytica, Bologna), operating at 25 °C, equipped with a 4 mm l × 3.0 mm ID C18 ODS precolumn. The mobile phase consisted by H_2O (adjusted to pH 3.2 by H_3PO_4) (eluent A) and CH_3CN (eluent B). The gradient program was as follows: 7% B–14% B (12 min), 14% B–25% B (4 min), 25% B–75% B (4 min), 75% B–7% B (5 min). Chromatograms were recorded at 350 nm at a flow rate of 0.6 mL/min.

Antioxidant capacity: The total antioxidant capacity of lettuce methanolic extracts was determined by means of oxygen radical absorption capacity (ORAC) assay. The automated ORAC assay was carried out on a Wallac 1420 spectrofluorometric analyser (Perkin Elmer, Turku, Finland; excitation wavelength = 485 nm and emission filter = 515 nm), basing on a slightly modified procedure proposed by Ou, Hampsch-Woodill, and Prior (2001). Fluorescein (116 nM) was the target molecule for free radical attack from 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH, 153 mM) as the peroxyl radical generator. The reaction was conducted at 37 °C at pH 7.0 with (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 1 μM) as the control standard and phosphate buffer as the blank. All solutions were freshly prepared prior to analysis. All samples were diluted with buffer (1:25–100, v/v) prior to analysis and results were expressed as micromoles of Trolox equivalents (TE) per 100 g of fresh weight.

Statistical analysis: All determinations were done in triplicate, unless noted otherwise. Analysis of variance (ANOVA) of the data was evaluated by the Statistical Analysis System (SAS ver. 9.0). Duncan's Multiple Range Test was employed to determine the statistical significance of the differences between the means ($p \leqslant 0.05$).

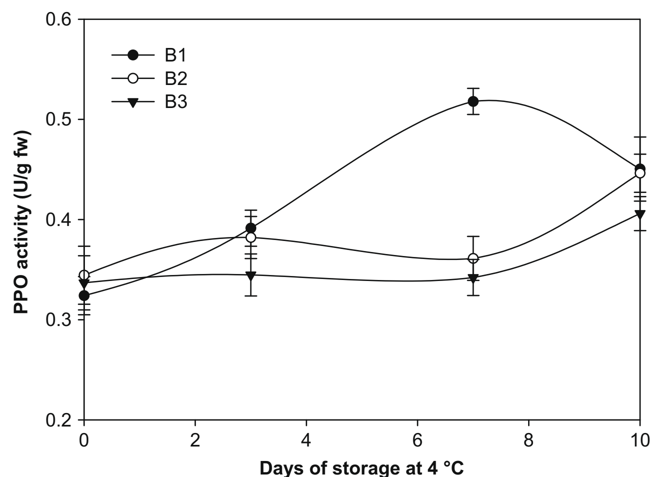


Fig. 1. Polyphenol oxidase activity in minimally processed baby Romaine lettuce during 10 days of storage at 4 °C. Salinity conditions: B1 = 2.8 dS m^{-1}; B2 = 3.8 dS m^{-1}; and B3 = 4.8 dS m^{-1}.

3. Results and discussion

Oxidase activities: Polyphenol oxidase and peroxidase activities were monitored during 10 days of storage at 4 °C. Results are shown in Figs. 1 and 2. Immediately after processing (day 0), PPO activity was very similar amongst samples cultivated at different salinity levels, whilst wounding of tissues due to minimal processing caused an activation of both enzymatic activities, much more evident after 7 days of storage. A similar PPO increasing trend was also noticed by Castaner, Gil, Ruiz, and Artes (1999) in photosynthetic tissues of minimally processed baby and Romaine lettuces throughout 7 days of storage at 5 °C. Moreover, the increase of PPO activity during storage was evident only in samples cultivated under low salinity, whereas no marked increase was observed at higher salt concentrations. In fact, the increase of polyphenol oxidase activity ranged from 2.3% to 21.0% at day 3 and reached 59.8% at day 7 in samples cultivated under low salinity condition. Under the same condition, PPO activity at day 7 (0.52 U/g fw) was significantly higher than in other samples (values ranging from 0.34 to 0.36 U/g fw). An increasing trend upon storage was also noticed relatively to peroxidase activity, with more similar activity amongst samples than PPO and a drastic increase up to day 7 (about four times higher than the initial value).

Moreover, at day 0 and 7 POD extracts from samples cultivated under low salinity showed the highest activity (0.16 and 0.43 U/g fw at day 0 and 7, respectively), significantly higher than the other two conditions (0.09 U/g fw at day 0 for both conditions, 0.36 and 0.40 U/g fw at day 7 for medium and high salinity conditions, respectively). The increase of PPO activity after wounding is mainly due to activation process from latent to fully active form. In fact, as previously reported by Cantos, Espin, and Tomas-Barberan (2001), tissue wounding involves the decompartmentalisation of cellular components with the subsequent release of proteases involving a cascade of reactions leading to the activation of latent PPO. On the other hand, Ke and Saltveit (1989) associated increased POD activity with cell wall lignification in response to wounding, because POD is involved in the lignin-specific pathway. The highest activity of oxidases extracts from samples cultivated under low salinity could be explained by the high availability of free water for the enzymatic reactions. Moreover, Cl^- ions may be formed in sodium chloride solutions used for lettuce cultivation and influence the expression of both oxidases.

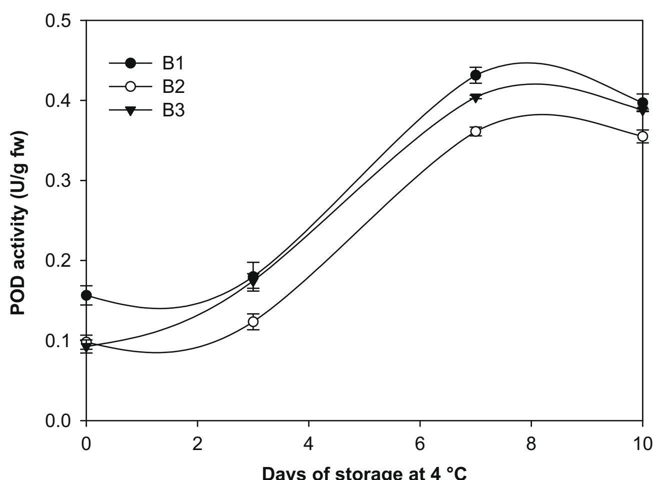


Fig. 2. Peroxidase activity in minimally processed baby Romaine lettuce during 10 days of storage at 4 °C. Salinity conditions: B1 = 2.8 dS m^{-1}; B2 = 3.8 dS m^{-1}; and B3 = 4.8 dS m^{-1}.

Many works in literature report the inhibitor effects of different antioxidant compounds and washing procedures on lettuce PPO (Altunkaya & Gokmen, 2008; Baur, Kleiber, Koblo, & Carle, 2004), whilst little attention was paid to the potential inhibitor effect of NaCl in irrigation water. In earlier work (Spagna, Barbagallo, Chisari, & Branca, 2005), we demonstrated the inhibition of PPO in presence of high concentrations of NaCl in the assay medium, due to the interaction of NaCl with the copper at the active centre of the enzyme. However, further studies are needed to confirm the relationship found between oxidase activities and salinity conditions, focusing on salt content of samples. In order to verify the effect of chlorine bath on oxidase activities of samples, a sample set of lettuces washed with unchlorinated water was tested. Results (data not shown) revealed that chlorine bath caused a reduction of both PPO and POD activity above all at day 0, with a range of reduction from 10% to 18%. No significant difference was found amongst samples cultivated at different salinity conditions relatively the percent reduction of oxidase activities during cold storage.

Browning of minimally processed lettuce: The colour of baby Romaine lettuce was measured during storage, taking ΔE^* ($\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$), a widely used parameter for determining colour differences perceptible by human eye, as an index for colour changes during cold storage. Results are reported in Table 1. It was possible to notice a sharp colour variation in all samples after 3 days of storage, more evident in samples cultivated under low salinity (ΔE^* = 9.92 and 5.41 for samples cultivated under low and high salinity, respectively). Degradation of colour evidenced by ΔE^* value was mostly due to variation in lightness (L^*) and green–red (a^*) values, whereas blue–yellow (b^*) chromatism did not influence significantly such parameter. Colour degradation was linear up to day 10 in samples cultivated under low salinity, whilst there was no significant difference between day 7 and 10 in samples cultivated under medium and high salinity.

Antioxidant capacity: Since polyphenolic compounds are the main antioxidants in leafy vegetables, as widely reported in literature (Caldwell, 2003; Llorach, Tomas-Barberan, & Ferreres, 2004), the variation of both total phenolics and antioxidant capacity was monitored throughout the cold storage of minimally processed lettuce. A general decrease of phenolic content was noticed in all samples (Fig. 3), reaching minimum values at day 7, in correspondence of maximum values of PPO and POD activity. At day 10, a slight rise of phenolics was noted only in low salinity samples. Extracts from samples cultivated under low salinity showed also the highest phenolics degradation after 7 days of storage (−65.3% of the initial value). Such decrease could be explained by the oxidation associated with the browning reaction, considering the increasing trend of oxidases activity upon storage. HPLC analyses were performed on methanolic extract from baby Romaine lettuce at medium salinity condition (3.8 dS m^{-1}) in order to verify which phenolic compound was mostly affected by enzymatic degradation. The amounts of the main polyphenol compounds in lettuce, namely *O*-caffeoyltartaric acid (caftaric acid), 5-*O*-caffeoylquinic acid (chlorogenic acid), 3,5-di-*O*-caffeoylquinic acid (isochlorogenic acid) and dicaffeoyltartaric acid (chicoric acid), are reported in

Table 1
ΔE^* values of minimally processed baby Romaine lettuce during 10 days of storage at 4 °C. Salinity conditions: B1 = 2.8 dS m^{-1}; B2 = 3.8 dS m^{-1}; and B3 = 4.8 dS m^{-1}.

Days of storage at 4 °C	B1	B2	B3
Day 3	9.92 ± 1.11 a	7.75 ± 0.67 a	5.41 ± 0.32 a
Day 7	15.01 ± 1.43 b	14.82 ± 1.34 b	12.25 ± 1.32 b
Day 10	20.18 ± 1.65 c	14.06 ± 1.11 b	12.77 ± 1.10 b

Means in a same column followed by the same letter are not significantly different at the $p \leqslant 0.05$ level according to Duncan's Multiple range test.

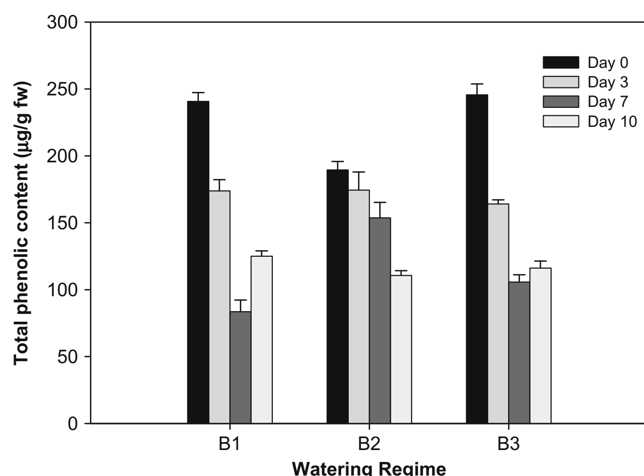


Fig. 3. Total phenolic content of minimally processed baby Romaine lettuce during 10 days of storage at 4 °C. Salinity conditions: B1 = 2.8 dS m^{-1}; B2 = 3.8 dS m^{-1}; and B3 = 4.8 dS m^{-1}.

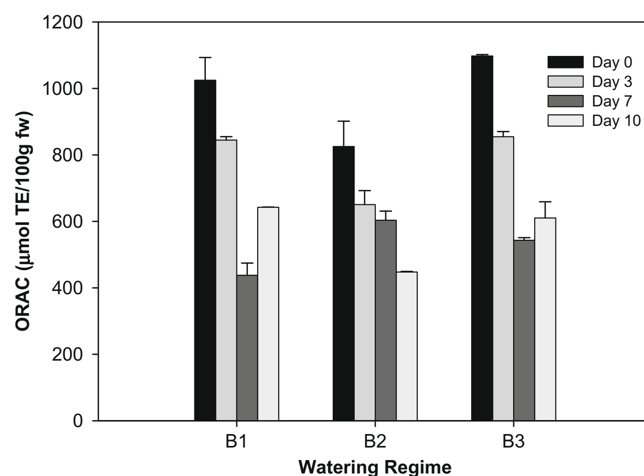


Fig. 4. Antioxidant capacity of minimally processed baby Romaine lettuce during 10 days of storage at 4 °C, expressed as ORAC units (µmol Trolox equivalent/100 g fw). Salinity conditions: B1 = 2.8 dS m^{-1}; B2 = 3.8 dS m^{-1}; and B3 = 4.8 dS m^{-1}.

Table 2. A sharp decrease of caftaric acid and chlorogenic acid was noticed above all between day 3 and 10 (25.0% and 36.7% decrease for caftaric and chlorogenic acids, respectively), whereas chicoric acid and isochlorogenic did not show any significant variation. These results could be related to the increasing course of oxidase activities (above all peroxidase) at latter days of storage.

As for total phenolic content, a decreasing course was noticed relatively to antioxidant capacity of lettuce methanolic extracts (Fig. 4). The highest variation was found after 7 days of storage at 4 °C in samples cultivated under low salinity (−57.3% of the initial value), although a general diminution of ORAC units was observed in all samples. As far as we know, no work in literature reports the changes of antioxidant capacity (measured with the ORAC method) during storage of fresh-cut lettuce, although a recent work (Altunkaya & Gokmen, 2008) reported a rapid decrease of antioxidant capacity, by means of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay, in fresh lettuce after homogenisation. Furthermore, the same work confirmed a decreasing trend of total phenolic content during storage at 4 °C.

In order to verify the role of polyphenols in determining the antioxidant capacity of the product, the correlation between ORAC values and total phenolics was calculated (Fig. 5). The different phenolic contents of methanol extracts were positively correlated with the antioxidant capacity, with r^2 values ranging from 0.91 to 0.98. These results agree with those reported in literature relatively to lettuce (Llorach et al., 2004). In samples cultivated under high salinity (4.8 dS m^{-1}), it was noticed the highest ratio between ORAC values and total phenolics throughout the storage period.

Table 2
Polyphenolic compounds in baby Romaine lettuce (salinity condition: B2 = 3.8 dS m^{-1}) during 10 days of storage at 4 °C, evaluated by HPLC–DAD and expressed as µg g^{-1} fw.

	Day 0	Day 3	Day 7	Day 10
CTA	42.14 ± 1.65	40.45 ± 1.34	32.66 ± 1.11	30.32 ± 1.44
5-CQA	40.06 ± 3.43	39.67 ± 1.45	27.76 ± 0.99	25.11 ± 1.78
diCQA	7.16 ± 0.46	6.28 ± 0.33	7.01 ± 0.29	7.22 ± 0.42
diCTA	1.94 ± 0.11	1.76 ± 0.10	1.64 ± 0.32	1.89 ± 0.13

Abbreviations used: CTA, *O*-caffeoyltartaric acid (caftaric acid); 5-CQA, 5-*O*-caffeoylquinic acid (chlorogenic acid); diCQA, 3,5-di-*O*-caffeoylquinic acid (isochlorogenic acid); diCTA, dicaffeoyltartaric acid (chicoric acid).

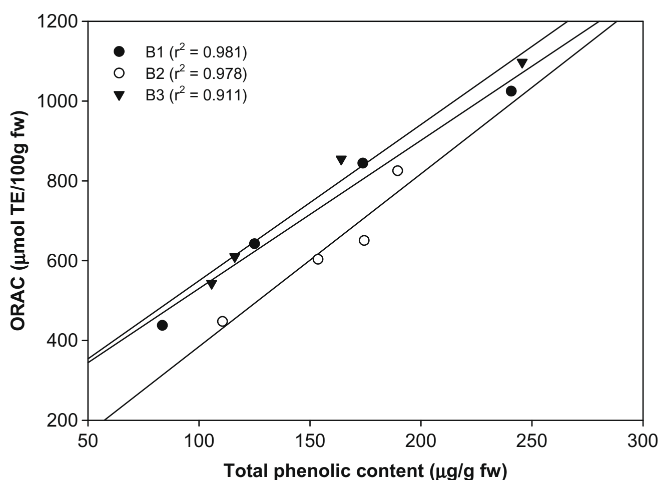


Fig. 5. Dependence of antioxidant activity (ORAC) on phenolic content in minimally processed baby Romaine lettuce during 10 days of storage at 4 °C. Salinity conditions: B1 = 2.8 dS m^{-1}; B2 = 3.8 dS m^{-1}; and B3 = 4.8 dS m^{-1}.

4. Conclusions

Cultivating baby Romaine lettuce under conditions of low salinity showed to be inappropriate for further minimal processing, due to exposure of the product to oxidase activities and browning phenomena throughout the cold storage. In fact, samples cultivated under low salinity showed significantly higher values of both PPO and POD activities throughout storage than the other samples. Moreover, the oxidation of phenolic compounds inevitably leads to a drastic decrease of the antioxidant capacity, since the two parameters are positively correlated. Increasing levels of salinity (up to 4.8 dS m^{-1}) were effective in reducing PPO and POD activities, colour changes and phenolics degradation, preserving then the antioxidant capacity of the product. These results could be of importance from an economic point of view, due to increased consumption of ready-to-eat products and considering the serious problems related to enzymatic browning in such products. However, relatively to salutary properties of fresh-cut lettuce, increasing only the salinity is not a suitable way to make the product healthier and further studies should focus on combined effects

for preservation and also protection of nutritional quality of lettuce.

References

Altunkaya, A., & Gokmen, V. (2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chemistry, 107*, 1173–1179.

Baur, S., Kleiber, R. G., Koblo, A., & Carle, R. (2004). Effect of different washing procedures on phenolic metabolism of shredded, packaged Iceberg lettuce during storage. *Journal of Agricultural and Food Chemistry, 52*, 7017–7025.

Caldwell, C. R. (2003). Alkylperoxyl radical scavenging activity of red leaf lettuce (*Lactuca sativa* L.) phenolics. *Journal of Agricultural and Food Chemistry, 51*, 4589–4595.

Cantos, E., Espin, J. C., & Tomas-Barberan, F. A. (2001). Effect of wounding on phenolic enzymes in six minimally processed lettuce upon storage. *Journal of Agricultural and Food Chemistry, 49*, 322–330.

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: Structure–activity relationship. *Free Radical Biology and Medicine, 22*, 749–760.

Castaner, M., Gil, M. I., Ruiz, M. V., & Artes, F. (1999). Browning susceptibility of minimally processed Baby and Romaine lettuces. *European Food Research and Technology, 209*, 52–56.

Espin, J. C., Morales, M., Varon, R., Tudela, J., & Garcìa-Canovas, F. (1996). Continuous spectrophotometric method for determining monophenolase and diphenolase activities of pear polyphenoloxidase. *Journal of Food Science, 61*, 1177–1181.

Flurkey, W. H., & Jen, J. J. (1978). Peroxidase and polyphenoloxidase activities in developing peaches. *Journal of Food Science, 43*, 1826–1828.

Ke, D., & Saltveit, M. E. (1989). Wound-induced ethylene production, phenolic metabolism and susceptibility to russet spotting in iceberg lettuce. *Plant Physiology, 76*, 412–418.

King, A. D., Jr., Magnuson, J. A., Torok, T., & Goodman, N. (2001). Microbial flora and storage quality of partially processed lettuce. *Journal of Food Science, 56*, 459–461.

Llorach, R., Tomas-Barberan, F. A., & Ferreres, F. (2004). Lettuce and chicory byproducts as a source of antioxidant phenolic extracts. *Journal of Agricultural and Food Chemistry, 52*, 5109–5116.

Loaiza-Velarde, J. G., Tomas-Barberan, F. A., & Saltveit, M. E. (1997). Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in iceberg lettuce. *Journal of the American Society for Horticultural Science, 122*, 873–877.

Muller, G., Bub, A., Watzl, B., & Rechkemmer, G. (1999). Plasma concentration of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. *European Journal of Nutrition, 38*, 35–44.

Ou, B., Hampsch-Woodill, M., & Prior, R. L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–4626.

Pifferi, P. G., & Baldassari, L. (1973). A spectrophotometric method for determination of the catecholase activity of tyrosinase by Besthorn's hydrazone. *Analytical Biochemistry, 52*, 325–335.

Rhoades, J. D. (1982). Soluble salts. In A. L. Page, R. H. Miller, & D. R. Keeney (Eds.), *Methods of soil analysis, Part 2: Chemical and microbiological properties* (2nd ed., pp. 149–157). Madison, WI, USA: American Society of Agronomy.

Richard-Forget, F. C., & Gauillard, F. A. (1997). 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, 45*, 2472–2476.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau Reagent. *Methods in Enzymology, 299*, 152–178.

Spagna, G., Barbagallo, R. N., Chisari, M., & Branca, F. (2005). Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. *Journal of Agricultural and Food Chemistry, 53*, 2033–2038.

Tomas-Barberan, F. A., Gil, M. I., Castaner, M., Artes, F., & Saltveit, M. E. (1997). Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. *Journal of Agricultural and Food Chemistry, 45*, 583–589.