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Wound-induced phenylalanine ammonia lyase activity: factors affecting its induction and correlation with the quality of minimally processed lettuces

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Abstract

One of the major causes of quality loss in minimally processed lettuce (*Lactuca sativa* L.) is the browning of the cut pieces. Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) is a key enzyme in phenolic synthesis. PAL activity increased in lettuce midrib tissue with wounding and storage in the presence or absence of ethylene. PAL activity increased 2.5- and 3-fold at 5 and 15°C, respectively, by reducing midrib size from 2.5 × 15 to 0.5 × 1 cm. Wounding usually induced maximum levels of enzyme activity within 3 days at 5°C and 1 day at 15°C. Pre- and postharvest factors affected the kinetics of wound-induced PAL (WI-PAL) activity and of subsequent changes in the quality of minimally processed lettuce. The rate at which WI-PAL activity increased and the maximum level attained were influenced by the duration of storage before processing. These parameters were also affected by the cultivar and type of lettuce used. Butterhead and iceberg types had the highest and lowest levels, respectively, of WI-PAL activity; romaine, green leaf and red leaf had intermediate levels. The activity of PAL 1–2 days after processing, and the slope of the induction curve between days 0 and 2, were the enzyme measurements that gave the highest correlations with processed lettuce quality attributes, including overall visual quality, leaf edge browning, and leaf surface browning.

Keywords: Phenylalanine ammonia lyase (PAL); Wounding; Temperature; Preharvest factor; Postharvest factor; Visual quality; Browning

1. Introduction

A major defect of minimally processed lettuce is the browning of cut pieces. Techniques that retard surface and edge browning include low storage temperatures (Bolin and Huxsoll, 1991), modified or controlled atmospheres (MA or CA) using low

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oxygen and/or high carbon dioxide (Ke and Saltveit, 1989a; Mateos et al., 1993a,b; Heimdal et al., 1995), and antioxidants (McEvily et al., 1992).

Phenylalanine ammonia lyase (PAL, EC 4.3.1.5) catalyzes the first reaction in the biosynthesis of plant phenylpropanoid products. The phenolic compounds can then be oxidized by polyphenoloxidase (PPO) producing brown polymers that contribute to tissue browning in lettuce (Ke and Saltveit, 1986, 1989b). PAL activity increases in response to several kinds of stress including wounding (Ke and Saltveit, 1989c), exposure to ethylene (Hyodo et al., 1978), and fungal infection (Jones, 1984).

Hyodo et al. (1978) reported a correlation between PAL activity and the development of russet spotting (RS) in ethylene-treated lettuce midribs. They found a significant increase in some phenolic compounds (e.g., chlorogenic and isochlorogenic acid) in the RS-affected tissue. Ke and Saltveit (1989b) observed an increase in phenolic compounds and brown stain (BS) in excised midrib lettuce tissue after exposure to 11% CO_2 (in air or 1.5% O_2) for 8 days followed by transfer to air at 25°C. Mateos et al. (1993a) also demonstrated that the development of BS after a period of storage in air + CO_2 followed by transfer to air at 20°C was due to an increase in phenolic content. When the tissue was transferred from a high CO_2 atmosphere to air, the pH of the acidified cytoplasm returned to its normal value with a restoration of PAL activity (Siripanich and Kader, 1985).

Couture et al. (1993) reported that ethylene-induced PAL activity was negatively correlated with the storage life of minimally processed lettuce. In their study, different iceberg cultivars, stages of maturity and ethylene exposure were evaluated in relation to PAL activity and quality parameters. Although iceberg is the predominant lettuce used for prepared salads, other types of lettuce are now used in salad mixes. Information on the postharvest behavior of these other lettuces is limited (López-Gálvez et al., 1996), especially with regard to their physiology and quality as minimally processed products.

The objective of this research was to further characterize the kinetics of wound-induced PAL (WI-PAL) activity and to test the correlation of enzyme activity with various quality attributes in fresh-cut lettuces.

2. Materials and methods

Lettuces were grown under standard commercial conditions and obtained freshly harvested, packed and cooled from a shipper in the Salinas Valley of California or were purchased from a local wholesale market. Various cultivars of iceberg lettuce were used for most of the experiments. In one experiment, lettuce was obtained from a fertilization trial (0, 22.5 or 45 t/ha of compost). In another experiment, lettuce was harvested and handled at ambient temperature, vacuum-cooled, or hydrocooled before processing. Different types of lettuce (iceberg, romaine, butterhead, green leaf and red leaf) were used in other experiments. Lettuce was prepared on the day of arrival or was stored at 2.5 or 5°C until processed.

Minimal processing of lettuce was carried out as described by López-Gálvez et al. (1996). Lettuce pieces (5 × 3 cm) including leaf and midrib were cut, rinsed with 5°C water containing 50 μl/l NaOCl, and centrifuged in a manual salad spinner. Tissue (~75 g) was placed in a glass jar covered with two layers of cheesecloth fixed with a rubber

band. Groups of 3 jars were placed into polyethylene bags at 5 ± 0.25°C and connected to flow-through systems providing humidified atmospheres (90–95% RH) of either air or 3% O_2 + 10% CO_2 balanced with nitrogen. Gas concentrations were monitored periodically and maintained within 10% of the indicated conditions.

Lettuce quality was evaluated by a trained judge (G.L.-G.) using the following parameters. Overall visual quality (OVQ) was rated on a scale from 9 to 1, where 9 = excellent and 1 = unuseable (Kader et al., 1973). An OVQ rating of 6 was considered the limit of salability. The parameter 'days to OVQ score of 6' was calculated using a linear regression. Leaf surface browning (LSB) and leaf edge browning (LEB) were scored on a scale from 1 to 5, where 1 = no browning and 5 = severe browning. The disorder brown stain (BS), if present, was included in the LSB score. Russet spotting (RS) was scored on a scale from 1 to 9, where 1 = none and 9 = severe (Ke and Saltveit, 1986). For each of these defects, an index was calculated by multiplying the scores by the percentage of pieces affected. The samples were inspected for visual quality attributes every 4 days over a period of 16 days. After evaluation, samples were transferred to air at 15°C for 12 h (to simulate retail conditions) and then re-evaluated.

The subjective color scale for LSB and LEB was correlated with L, a, and b values obtained by a color difference meter (Minolta Chroma Meter CR-200) using a white plate for calibration ($L = 97.63$; $a = -0.53$; $b = 2.38$). The 'a' color value of midrib pieces was highly correlated with the surface ($r = 0.97$) and edge ($r = 0.98$) browning scores.

PAL activity was routinely assayed from 0.5 × 1 cm midrib segments of minimally processed lettuce; for some experiments 2.5 × 4 or 2.5 × 15 cm midrib segments were used. Segments were stored at 5 or 15°C and flushed with humidified air, with or without the addition of 5 ppm ethylene, and periodically assayed for PAL activity as described by Ke and Saltveit (1986). Four grams of tissue were used for sample extraction and PAL activity was expressed as μmol cinnamic acid g^{-1} fresh weight h^{-1}.

PAL analyses were conducted with 3 replicates. The area beneath the curve of PAL activity (i.e., the cumulative estimated activity) was calculated using the trapezoidal rule for equally spaced 'x' values. The slope of the initial curve for PAL activity was calculated by linear regression analysis. Results of PAL activity were subjected to an analysis of variance, and when statistically significant differences were detected, LSD values were calculated at the $P \leq 0.05$ level. Quality evaluations were conducted with 3 replicates. Correlation analyses were performed to establish the association between PAL activity and the quality parameters evaluated (OVQ, LSB, LEB, RS) and calculated (days to OVQ score of 6).

3. Results and discussion

3.1. Wound-induced and ethylene-induced PAL activity

Different conditions of storage and preparation of lettuce tissue were tested for their effect on the induction of PAL activity. A prolonged induction of WI-PAL activity was observed at 5°C, while a more rapid climax and decrease, generally 24 h, were observed at 15°C (Fig. 1). Depending on the size of the lettuce pieces, PAL activity reached maximum levels within 6–16 h at 15°C, but required longer periods at

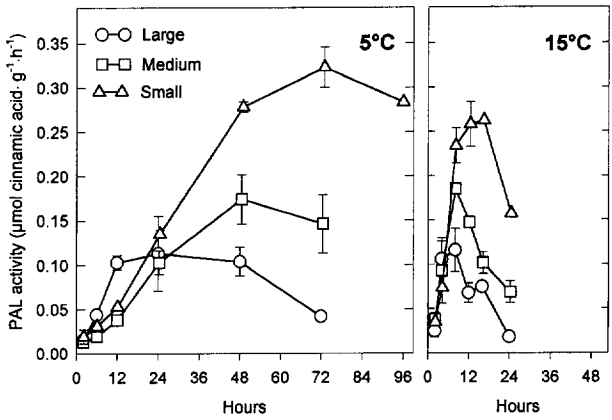

Fig. 1. PAL activity in midrib tissue of iceberg lettuce ('Salinas') during storage at 5°C (left) or 15°C (right). Midrib was prepared as large (2.5 × 15 cm), medium (2.5 × 4 cm) or small (0.5 × 1 cm) pieces. Each data point is the average of 3 replications ± standard deviation. Lettuce heads were stored for 4 days at 2.5°C before processing.

5°C. At both temperatures, the level of induced PAL activity was proportional to the level of wounding (i.e., piece size). Ritenour et al. (1995) showed that PAL activity increased more rapidly at elevated temperatures. Hyodo et al. (1978) hypothesized the development of an inactivation system following the PAL induction process which could be responsible for the more rapid decay of enzyme activity at higher temperatures.

Maximum WI-PAL activity for 'Salinas' iceberg lettuce was higher at 5°C than at 15°C in the finely chopped tissue, but similar in medium and large pieces. For other iceberg lettuce cultivars (e.g., 'El Toro' and 'RC-74') the maximum induced PAL activity at both temperatures was similar for a given sized piece (data not shown).

Maximum WI-PAL activity increased as the severity of wounding increased. There was a 2.5-fold increase in PAL activity at 5°C in the small (0.5 × 1 cm) midrib pieces compared to the large pieces (2.5 × 15 cm). At 15°C, the difference in piece size resulted in a 3-fold difference in maximum PAL activity. These results are in agreement with those obtained by Ke and Saltveit (1989c) who showed that the level of WI-PAL activity in lettuce was a function of the degree of injury. Similar kinetics of PAL induction were observed in sweet potato roots cut into disks (Tanaka and Uritani, 1977) and in wheat inoculated with *Erysiphe graminis* f. sp. *tritici* (Green et al., 1975).

According to Ke and Saltveit (1989c) WI-PAL activity occurred more rapidly than ethylene-induced PAL activity, but the combined effects of physical wounding plus ethylene on PAL activity of lettuce midribs were not systematically studied. Higher levels of PAL were induced by wounding plus ethylene as compared to wounding alone (Fig. 2). However, initial induction kinetics and time to reach maximum PAL levels were similar. Fig. 2 also illustrates the range of induced PAL activity that was observed among iceberg cultivars. Differences in ethylene-induced PAL (Couture et al., 1993) and WI-PAL activity (Ke and Saltveit, 1989d) for various iceberg cultivars were reported previously. The inducer of PAL activity in lettuce may modify the browning defect observed. In the visual quality evaluation of lettuce, russet spotting (RS) was the

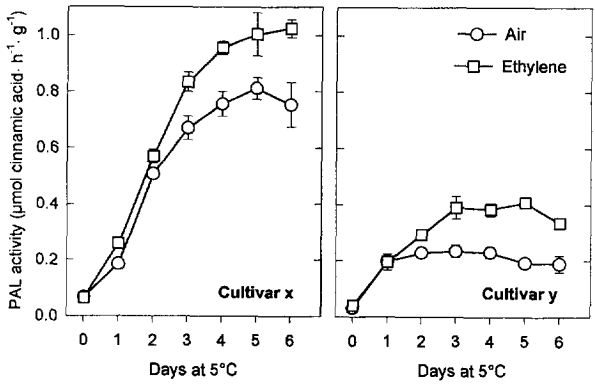

Fig. 2. PAL activity in midrib tissue (0.5 × 1 cm pieces) of two iceberg lettuce cultivars stored at 5°C in air or air +5 ppm ethylene. Each data point is the average of 3 replications ± standard deviation. Lettuce heads were stored at 2.5°C and processed within 24 h.

most evident defect in wounded ethylene-stored samples, while browning of the surface (LSB) and cut edges (LEB) was the major defect in wounded air-stored pieces.

For subsequent experiments, PAL assays were carried out on samples prepared as small sized pieces and stored at 5°C in air. These preparation and storage conditions provided tissue with the slowest increase in PAL activity, the longest duration of continued induction, the highest maximum level of PAL activity, and avoided the use of ethylene.

3.2. Effect of preharvest and postharvest treatments on wound-induced PAL activity

WI-PAL activity in lettuce was studied in a series of experiments simulating commercial pre- and postharvest handling practices. WI-PAL activity followed a similar pattern in 5 different types of lettuce (Fig. 3). However, PAL activity was most rapidly induced and reached highest levels in butterhead lettuce, showing 3-fold differences in rates of induction and 2-fold differences in maximum PAL activity compared with levels in iceberg. Romaine, green leaf and red leaf lettuces generally showed intermediate levels. The cumulative PAL activity over 7 days (i.e., the area beneath the curve of PAL activity) was 1.6, 2.4, 2.7, 3.2 and 4.3 for iceberg, green leaf, red leaf, romaine and butterhead, respectively.

Higher cumulative levels of WI-PAL were observed when higher amounts of organic fertilizer were applied to iceberg lettuce (data not shown). The availability of nitrogen may be a limiting factor, although Ke and Saltveit (1989b) did not observe an increase in PAL activity in lettuce tissue incubated in a solution of phenylalanine.

Different cooling methods of intact iceberg lettuce had no effect on maximum levels of WI-PAL (data not shown). However, the rate of induction in vacuum cooled samples was lower than in non-cooled and hydrocooled samples.

Storage of whole heads before processing affected WI-PAL activity (Fig. 4). Tissue from freshly harvested iceberg and romaine lettuces had a longer period of PAL induction than that of stored heads. For both lettuce types maximum PAL values

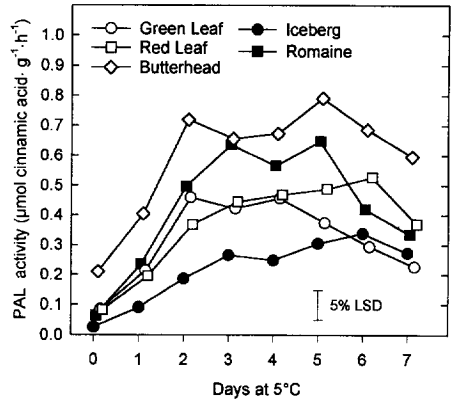

Fig. 3. PAL activity in midrib tissue (0.5 × 1 cm pieces) of 5 types of lettuce stored at 5°C in air. Each data point is the average of 3 replications. The vertical bar represents the 5% LSD value. Lettuce heads were stored at 2.5°C and processed within 24 h.

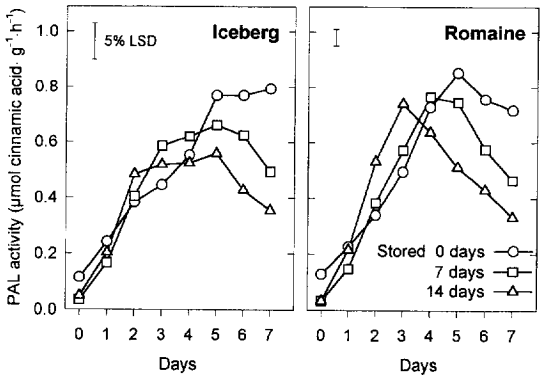

Fig. 4. PAL activity in midrib tissue (0.5 × 1 cm pieces) of iceberg and romaine lettuces stored at 5°C in air. Intact lettuce heads were stored at 5°C in air for 0, 7 and 14 days before processing. Each data point is the average of 3 replications. The vertical bars represent the 5% LSD values.

decreased with increasing storage time of the heads. The initial rate of induction, however, increased with increasing storage time.

The dynamics of PAL activity were monitored in relation to head maturity. Two iceberg cultivars, 'Alpha' and 'Legacy' (known to be more and less tolerant to RS, respectively), were harvested at different maturity stages (head firmness) and stored 2 weeks at 2.5°C before processing. Levels of WI-PAL activity were similar for both cultivars at the immature stage (Fig. 5). However, at the mature or overmature stages, 'Legacy' showed higher levels of PAL activity than 'Alpha'. For a given cultivar, there were negligible differences in the level of PAL activity among the different stages of maturity. These results are consistent with the lack of differences in phenolic content among samples of minimally processed iceberg lettuce harvested at different maturity stages (Couture et al., 1993).

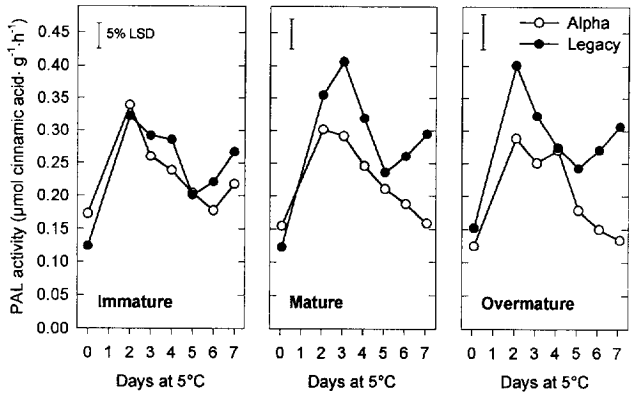

Fig. 5. PAL activity in midrib tissue (0.5 × 1 cm pieces) of iceberg lettuce 'Alpha' and 'Legacy' harvested at 3 maturity stages. Samples were stored at 5°C in air. Each data point is the average of 3 replications. The vertical bars represent the 5% LSD values. Lettuce heads were stored for 14 days at 2.5°C before processing.

3.3. Correlation of PAL activity with quality of minimally processed lettuce

Induced PAL activity has been correlated with the visual quality of minimally processed iceberg lettuce (Couture et al., 1993). In our study, we evaluated this relationship across a broad range of lettuce types and treatments. Correlation coefficients of WI-PAL activity with quality measurements were calculated in 5 types of minimally processed lettuce (Table 1). As expected, different parameters of PAL activity correlated negatively with overall visual quality (OVQ) and positively with leaf surface browning (LSB) and leaf edge browning (LEB). A larger number of significant correlations was found with the quality parameters measured at day 12 than at day 17 (data not shown). By day 17, the visual quality of some of the lettuces was limited by factors other than browning (e.g., decay and severe CO_2 injury) (López-Gálvez et al., 1996). More than 80% of the significant correlations were obtained for CA-stored samples; PAL parameters were most highly correlated with LSB. However, in air-stored samples PAL activity was most highly correlated with LEB.

In the cases where maximum PAL activity correlated significantly with quality measurements, other PAL parameters also correlated significantly (Table 1). Since maximum PAL levels are not reached until days 2–5, earlier enzyme measurements could be more useful to predict shelf-life. In addition, when lettuce was stored, maximum PAL levels decreased with storage time (Fig. 4) and the initial slope of PAL activity was more highly correlated with the visual quality of processed lettuce.

Correlation coefficients of WI-PAL activity with quality measurements were calculated for a pool of iceberg lettuce experiments (Table 2). The coefficients were estimated for a population of up to 30 treatments obtained from 8 experiments (different cultivars, stages of maturity, fertilization practices, cooling methods, and storage periods before processing). About 25% of the calculated correlation coefficients were statistically significant. The lack of significant correlations for PAL activity with russet spotting (RS) was due to the lack of development of this disorder under our experimental conditions.

Table 1
Correlation coefficients between visual quality parameters and PAL activity (measured at day 1, day 2, slope of the curve at day 1, slope of the curve at day 2 and maximum PAL levels) in 5 types (iceberg, romaine, butterhead, green leaf and red leaf) of minimally processed lettuces. For the evaluation of quality parameters samples were stored for 12 days in air or CA (3% O_2 + 10% CO_2) at 5°C

Atmosphere	Hour[a]	Parameter[b]	PAL activity				
			Day 1	Day 2	Slope day 1	Slope day 2	Maximum levels
Air	0	OVQ	−0.77[c]	−0.68	−0.78	−0.45	−0.70
		LSB	0.69	0.57	−0.41	0.24	0.58
		LEB	0.81	0.84	0.88[d]	0.88[d]	0.85[d]
	12	OVQ	−0.59	−0.48	−0.36	−0.23	−0.52
		LSB	0.80	0.71	0.51	0.34	0.63
		LEB	0.62	0.61	0.63	0.62	0.62
CA	0	OVQ	−0.88	−0.80	−0.68	−0.55	−0.78
		LSB	0.92[d]	0.89[d]	0.89[d]	0.81	0.95[e]
		LEB	0.82	0.74	0.62	0.49	0.74
	12	OVQ	−0.93[d]	−0.86	−0.76	−0.63	−0.86
		LSB	0.96[e]	0.93[d]	0.89[d]	0.80	0.95[e]
		LEB	0.86	0.81	0.64	0.51	0.69

[a] Hour 0, after storage; hour 12, after storage plus 12 h at 15°C in air.
[b] Parameters of evaluation for quality: OVQ, overall visual quality; LSB, leaf surface browning; and LEB, leaf edge browning.
[c] Each coefficient is based on 15 values.
[d,e] Significant at $P \leq 0.05$ or 0.01, respectively.

OVQ is a function of quality defects (i.e., LSB, LEB and RS) (López-Gálvez et al., 1996). The days to reach an OVQ score of 6 (limit of salability) were estimated by regression analysis in air- and CA-stored samples both after storage and after storage plus a transfer period of 12 h at 15°C in air. The parameter 'days to OVQ score of 6' was analyzed for correlation with WI-PAL activity (Table 3). As expected, all correlations were negative; approximately 50% of the correlation coefficients were significant.

Generally WI-PAL activity at day 1, day 2 and the slope of the curve at day 2 were the enzyme parameters most highly correlated with the quality of minimally processed iceberg lettuce (Table 2 and Table 3). There were more significant correlations of PAL activity with quality after the transfer period than with quality after storage. The warmer poststorage temperature led to an increase in both LSB and LEB. This is consistent with the increase in phenolic compounds during the transfer period after air (Ritenour et al., 1995) or CA storage (Mateos et al., 1993a).

Couture et al. (1993) found a negative correlation between ethylene-induced PAL activity at day 3 and the visual quality of lettuce after 6 or 10 days of storage in air, including 3 days in ethylene. These conditions are much more stressful than those typically experienced during commercial handling. We have determined that wounding by itself can induce PAL levels which correlate with the visual quality of lettuce after air or CA storage for 12 or 16 days. The storage duration and atmosphere used in this study more closely approximate those of commercial salad products, providing a more valuable test of the predictive potential of PAL. PAL was investigated because it has

Table 2
Correlation coefficients between visual quality parameters and PAL activity (measured at day 1, day 2, slope of the curve at day 1 and slope of the curve at day 2) in samples of minimally processed iceberg lettuce. For the evaluation of quality parameters samples were stored for 16 days in air or CA (3% O_2 + 10% CO_2 at 5°C)

Atmosphere	Hour[a]	Parameter[b]	No. of treatments[c]	PAL activity			
				Day 1	Day 2	Slope day 1	Slope day 2
Air	0	OVQ	12	0.05	0.09	0.28	0.26
		LSB	7	0.42	0.33	0.11	−0.01
		LEB	7	0.36	0.52	0.68	0.64
		RS	7	0.05	−0.30	−0.31	−0.58
	12	OVQ	26	−0.36	−0.66[f]	0.08	−0.62[f]
		LSB	26	0.39[d]	0.60[f]	−0.04	0.45[d]
		LEB	26	0.44[d]	0.75[f]	0.06	0.71[f]
		RS	15	0.18	0.31	−0.33	0.12
CA	0	OVQ	11	−0.63[d]	−0.48	−0.25	−0.17
		LSB	11	0.72[e]	0.59	0.41	0.33
		LEB	11	0.11	0.43	0.51	0.50
		RS	11	−0.04	−0.15	−0.14	−0.22
	12	OVQ	30	−0.46[d]	−0.71[f]	−0.14	−0.62[f]
		LSB	30	0.34	0.59	0.03	0.50[e]
		LEB	30	0.19	0.58[f]	0.12	0.59[f]
		RS	19	0.06	0.25	−0.11	0.19

[a] Hour 0, after storage; hour 12, after storage plus 12 h at 15°C in air.
[b] Parameters of quality evaluation: OVQ, overall visual quality; LSB, leaf surface browning; LEB, leaf edge browning; and RS, russet spotting.
[c] Three replicates per treatment. Treatment data were pooled from 8 experiments, including different cultivars, stages of maturity, fertilization practices, cooling methods and storage periods before processing.
[d,e,f] Significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table 3
Correlation coefficients between days to reach an overall visual quality score of 6 and PAL activity (measured at day 1, day 2, slope of the curve at day 1 and slope of the curve at day 2) in samples of minimally processed iceberg lettuce. For the evaluation of quality parameters samples were stored in air or CA (3% O_2 + 10% CO_2 at 5°C)

Atmosphere	Hour[a]	No. of treatments[b]	PAL activity			
			Day 1	Day 2	Slope day 1	Slope day 2
Air	0	15	−0.62[c]	−0.50	−0.69[d]	−0.69[d]
	12	31	−0.22	−0.55[c]	−0.18	−0.42[c]
CA	0	11	−0.82[d]	−0.32	−0.07	−0.01
	12	35	−0.29	−0.49[d]	−0.03	−0.40[c]

[a] Hour 0, after storage; hour 12, after storage plus 12 h at 15°C in air.
[b] Three replicates per treatment. Treatment data were pooled from 8 experiments, including different cultivars, stages of maturity, fertilization practices, cooling methods and storage periods before processing.
[c,d,e] Significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

a key role in phenolic metabolism and because it increases in response to numerous biotic and abiotic stresses. Although the correlation between PAL activity and visual quality is therefore reasonable, there may be other factors contributing to this apparent relationship.

4. Conclusions

Processors and handlers of minimally processed lettuces currently experience large variations in the quality of the stored salad products. PAL activity could be useful to predict the shelf-life of minimally processed lettuce. For iceberg lettuce, wound-induced PAL activity at day 1, day 2 and the slope of the curve at day 2 were the parameters most highly correlated with the visual quality. These parameters of WI-PAL activity also significantly correlated with the days to reach a minimum acceptable quality. For a wide range of lettuce types, WI-PAL activity at day 1, day 2 and the slope of the curve at day 1 were highly correlated with the visual quality of the processed product. Since several days are required to reach maximum PAL levels, the correlations of earlier PAL parameters with quality could be more useful. More work is needed to determine which of the PAL measurements is a better overall predictor of the shelf-life of minimally processed lettuces. PAL activity may also be a useful predictor of the shelf-life of other fresh-cut products.

Assuming that WI-PAL activity is a useful parameter to predict the shelf-life and quality of fresh-cut lettuce, it would be necessary to further reduce the time of induction and analysis for practical application. We have demonstrated that substantial levels of PAL activity can be induced within 1 day. Other techniques, such as immunological assays, should be explored to obtain faster results.

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