



# Effect of mild heat-shock treatments on pink discoloration and physiological parameters in fresh-cut iceberg lettuce

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## ABSTRACT

Pink discoloration is one of the major quality issues occurring in fresh-cut lettuce. Low oxygen atmosphere in modified atmosphere packaging prevents pinking but may lead to other major quality losses such as fermentation, growth of lactic acid bacteria and associated production of off-odours. The application of mild heat-shock treatment on fresh-cut lettuce is known to reduce the activity of Phenylalanine Ammonia Lyase (PAL), an enzyme involved in pink discoloration. Heat-shock treatment, however, may also affect the vitality of the plant tissue, leading to early senescence symptoms. In the present study several heat-shock treatments were tested for their effect on pink discoloration and on lettuce tissue vitality. The optimal heat-shock treatment was found within a narrow range of temperatures (between 45 and 47.5 °C) and application times (between 30 and 180 s). Heat-shock treatment reduced significantly the PAL activity by a factor 3 during the first 5 d of storage but increased the respiration rate of the produce by a factor 2 on day 5. We recommend to optimize the packaging to suit the heat-shock associated higher respiration rate of the treated product in order to increase the shelf life of the fresh-cut lettuce.

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## 1. Introduction

Pink discoloration of fresh-cut lettuce is one of the major quality issues occurring when product is exposed to oxygen. Pink discoloration is judged by consumers as non-esthetical and the product is rejected. Pink discoloration is mainly the visual symptom of the oxidation of phenolic and quinone compounds. These compounds are the products of a complex biochemical pathway (phenylpropanoid pathway) involved in the response of wounding. Two key enzymes in the production of pink and brown compounds are Phenylalanine Ammonia Lyase (PAL) and Polyphenol Oxidase (PPO) (Degl'Innocenti, Pardossi, Tognoni, & Guidi, 2007).

Food producers try to avoid pink discoloration by application of modified atmosphere packaging (MAP) containing a low concentration of oxygen and an increased concentration of carbon dioxide. To avoid pink discoloration, the residual oxygen content in the package should be close to zero within three days after production. This solution evokes other quality issues later on during the storage

period. Due to the lack of oxygen, volatile compounds responsible for off-odour characteristics such as ethanol and acetaldehyde are produced and accumulated inside the packaging headspace (Deza-Durand & Petersen, 2014). In addition, the anaerobic conditions greatly stimulate the growth of lactic acid bacteria. These bacteria are responsible for the typical acetic acid smell perceptible at the end of the shelf life of fresh-cut vegetables (Paillart et al., 2016).

The application of heat-shock (HS) treatment on fresh-cut lettuce significantly reduced the PAL activity (Baur, Klaiber, Wei, Hammes, & Carle, 2005). HS, being a short heat exposure just before or after cutting, stresses the plant tissue. The tissue responds by producing heat shock proteins (HSPs). The production of HSPs competes with the production of PAL protein, resulting in decreasing PAL production (Kang & Saltveit, 2003b).

The specific interactions between the HS treatment and the vitality of the plant are poorly documented. In our study, we investigated the effect of HS on pink discoloration and vitality of fresh-cut iceberg lettuce. In addition, the effect of HS on PAL activity and respiration rate of fresh-cut lettuce stored in MAP was studied.

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## 2. Material and methods

### 2.1. Plant material and heat-shock treatment

Iceberg lettuce (*Lactuca sativa*) was obtained in two batches at a local vegetable supplier and stored at 4 °C till processing: the first batch for determination of the optimal HS treatment was purchased in December (Spain production season) and the second batch for the respiration rate and PAL activity measurements in June (Dutch production season). For establishing optimal HS parameters, a pinking assay was used. Disks of 9.7 mm diameter were excised from the lettuce leave using a cork borer. The HS was applied by plunging the disks into a water bath (GFL, Germany). The following temperatures and application times were applied: 45 °C for 30, 60, 120 and 180 s; 47.5 °C for 30 and 60 s; 50 °C for 30 and 60 s; 52.5 °C for 15, and 30 s and 55 °C for 30 s. Immediately after HS, disks were cooled down in 2 °C water and subsequently drained. Discs prepared as described above were used for determination of pinking and for measuring electrolyte leakage (see 2.2 and 2.3).

Determination of PAL activity and respiration rate was done on fresh-cut lettuce prepared according to commercial specifications. From lettuce heads, the first two outside leaves were removed, as well as its core. Heads were then cut in strips of 13 mm width with an Urschel cutting machine (Model G-A, Urschel Laboratories Inc, Chersterton, Indiana). The HS and the washing/cooling treatments were applied on batches of 1.5 kg. The fresh-cut lettuce was immersed at 47.5 °C for 180 s in a water bath. Directly after HS, the product was cooled down in two successive 4 °C water baths for 1 min. The reference treatment consisted on non-heat-treated fresh-cut lettuce, washed in three successive 4 °C water baths. The excess water was removed by centrifugation (60 tour min<sup>-1</sup> for 1 min) using a centrifuge (TRISTAR Europe BV, Tilburg, the Netherlands). After processing, the fresh-cut lettuce was packed in commercial modified atmosphere bag (BOPP) and flushed with 6 kPa O<sub>2</sub> and 11 kPa CO<sub>2</sub>. Storage of the samples was done at 7 °C and at regular intervals; samples for PAL activity measurement were taken. Samples for respiration rate measurement were treated and directly packed per 200 g in glass cuvette (2 L) and continuously flushed with air (see 2.5).

### 2.2. Pinking assay

Pinking in treated disks was evaluated using a pinking assay. Seven disks were placed between two Whatman® filters (filter 5981 S&S, Sigma, St Louis, Missouri) in a petri dish and 5 mL of 50 mmol L<sup>-1</sup> MES buffer (pH 3.0) was added to saturate the filter paper. After 3 d of incubation at 7 °C, filters were collected, dried and disk marks were scored on a zero to 5 scale (Table 1). The individual score of the 7 disks on both the upper and lower paper filters were summed and the results were expressed on a score scale from 0 to 70 points. Reference treatment was washed for 60 s in 21 °C tap water and subsequently cooled down in 7 °C tap water for 180 s.

### 2.3. Electrolyte leakage from disks

The disks used for the pinking assay were after 3 d incubation transferred to a test tube (7 disks per tube). The electrolyte leakage was measured using an electro-conductivity meter (CDM80) equipped with CDC-304 probe (Radiometer A/S Copenhagen, Denmark) directly after adding 20 mL of milli-Q water (EC<sub>0</sub>) and after 2 h (EC<sub>1</sub>) of incubation at room temperature (20 °C) with continuously shaking (30 rpm). After the second measurement, samples were placed at -21 °C to destroy the cells. Samples were then thawed and brought back to room temperature before measuring its maximal electro-conductivity (EC<sub>max</sub>). Electrolyte leakage was calculated and expressed in percentage of its maximal value as follow: EC(%)=(EC<sub>1</sub>-EC<sub>0</sub>)/(EC<sub>max</sub>-EC<sub>0</sub>).

### 2.4. PAL activity

On each evaluation day, samples of fresh-cut iceberg tissue were frozen in liquid nitrogen and stored at -80 °C till PAL activity measurement. PAL activity was measured as described by Ke and Saltveit (1986). Four gram of frozen samples were homogenised with Ultra-Turrax (maximum speed for 30 s) in 16 mL of 50 mmol L<sup>-1</sup> borate buffer (pH 8.5), 5 mmol L<sup>-1</sup> 2-mercaptoethanol and 0.4 g of PVPP. The homogenate was centrifuged for 20 min at 25,000 g and filtrated with 0.45 µm filter. The extract was kept on ice till PAL activity determination. Subsequently 0.55 mL of 100 mmol L<sup>-1</sup> L-phenylalanine was added to 5 mL of extract. Absorbance at 290 nm of the reagent was measured at time 0 and after 1 h incubation at 40 °C. One unit of PAL activity was defined as the amount of PAL that produced 1 µmol g<sup>-1</sup> of cinnamic acid in 1 h at 40 °C. PAL activity were analysed in duplicate. All the chemicals were supplied by Sigma-Aldrich (St Louis, Missouri).

### 2.5. Respiration

Glass cuvettes of 2 L volume were filled with 200 ± 2 g of fresh-cut lettuce, flushed from the bottom to the top with humidified air atmosphere (125 mL min<sup>-1</sup>) and stored at 7 °C. HS treated lettuce was compared to non-heat-treated product (3 times washed in cold water). The oxygen consumption and the carbon dioxide production were determined by measuring the oxygen and carbon dioxide contents (Gas Chromatograph - Thermo Fischer Scientific, Waltham, Massachusetts) over an accumulation period of 4 h. The measurements occurred on day 1, 2, 5, 7 and 9. The results are expressed in mL O<sub>2</sub>/CO<sub>2</sub> kg<sup>-1</sup> h<sup>-1</sup>.

## 3. Results and discussion

### 3.1. Testing HS parameters

Each HS treatment was judged on basis of its efficiency to block pink discoloration and its ability to preserve plant tissue integrity. For this purpose the non-heat-treated lettuce sample was used as a reference. HS significantly reduced the pink discoloration of leaf

**Table 1**

Visual judgement of pinking on a zero to 5 scale used for quantification of pink coloration on the disk print.

Points	Amount of discoloration	
0	None:	no pink coloration
1	Minor:	<20% of disk perimeter showed pink coloration
2	Slight:	20 to 40% of disk perimeter showed pink coloration
3	Medium:	40 to 80% of disk perimeter showed pink coloration
4	Intense:	80 to 100% of disk perimeter showed pink coloration
5	Full pink disk:	100% of disk perimeter including inside the disk print showed pink coloration

disks (Fig. 1A). Clear reduction of pink discoloration was observed for the lower temperature treatment (45 °C) with long application times (at least 120 s). The higher the temperature, the shorter application time was needed to reach similar pink discoloration reduction (e.g. 50 °C for 30 s). These results are in accordance with previously published data (Saltveit, 2000). A more severe HS treatment (high temperature and/or long application time), although effective in alleviating pinking, caused an increased electrolyte leakage (Fig. 1B) indicating loss of tissue integrity (Kim, Nimitkeatkai, Choi, & Cheong, 2011). On the basis of our results (Fig. 1) and for potential industrial application, we concluded that the most promising HS treatment should combine a mild temperature (45 °C) with a relatively long application time (180 s) for Spanish lettuce harvested in December.

### 3.2. Effect of HS on PAL activity

For PAL activity measurement, the fresh-cut samples were heat-treated and subsequently stored under MAP condition (commercial packaging). Although we defined the optimal HS treatment for lettuce harvested in December month, we adjusted the HS temperature to 47.5 °C (for 180 s) in order to reach a temperature about 10 °C above the maximum growing temperature of the heads as recommended by Saltveit (2000). The PAL activity was reduced by a factor 3 in HS samples compared to non-heat-treated samples immediately after the treatment (Fig. 2). Similar PAL activity reduction of samples treated with for 150 s at 45 °C was reported by Kang and Saltveit (2003a).

The PAL activity of non-heat-treated samples increased to reach a peak level of  $0.12 \mu\text{mol g}^{-1} \text{h}^{-1}$  on day 3. According to López-Gálvez, Saltveit, and Cantwell (1996), the PAL activity reached its production peaks within 24–72 h at 5 °C as response to wounding occurring during processing of fresh-cut lettuce. In the present study, the PAL activity of HS treated samples was maintained at a considerably lower level than that of the non-heat-treated samples for a prolonged period of time (Fig. 2).

### 3.3. Effect of HS on respiration rate

The respiration rate of non-heat-treated and heat-treated lettuce was followed during the complete storage period. With the exception of day 1, HS increased the respiration rate (oxygen consumption and carbon dioxide production) of the produce during its storage period (Table 2). After 5 d of storage, heat-treated lettuce still showed significantly higher respiration rate than non-heat-treated lettuce. Similar behaviour was observed by Baur et al. (2005).

Saltveit (2000) assumed that the short HS application times (shorter than 120 s) do not affect the respiration rate of the

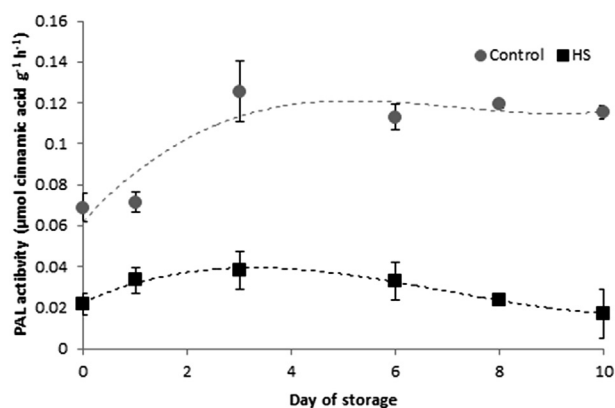


Fig. 2. PAL activity measured of non-heat-treated (● Control) and heat-treated (■ HS) fresh-cut iceberg lettuce during storage in modified atmosphere packaging at 7 °C (n = 2; ±SD).

Table 2

Oxygen consumption and carbon dioxide production of fresh-cut iceberg lettuce. Respiration of non-heat-treated (Control) and heat-treated (47.5 °C for 180 s) (HS) product was measured after 1, 2, 5, 7 and 9 d of storage in air at 7 °C.

Time (day)	O <sub>2</sub> consumption (mL O <sub>2</sub> kg <sup>-1</sup> h <sup>-1</sup> )		CO <sub>2</sub> production (mL CO <sub>2</sub> kg <sup>-1</sup> h <sup>-1</sup> )	
	Control	HS	Control	HS
1	12.9	9.2	11.0	7.4
2	9.4	9.6	8.2	8.3
5	7.9	14.9	5.6	10.1
7	8.9	25.8	6.1	19.4
9	9.2	34.4	6.5	26.8

produce. Bai et al. (2004) reported that applying HS on fresh-cut apple reduced the respiration rate of the product. Application of severe HS induced cell membrane damage, increased the microorganism growth and discoloration of the tissue (Moreira, Ponce, Del Valle, & Roura, 2006). These deterioration processes could lead to an increase of the respiration rate that occurred in our study. The higher respiration rate of heat-treated samples (2 times higher than non-heat treated samples) may be explained by a temperature stress imposed by a too high temperature and/or long application time. The HS treatment temperature applied in the experiments where the PAL activity and respiration rate were measured was 2.5 °C higher than the optimal temperature found in the earlier experiment (see 3.1). Saltveit (2000) and Artés and Allende (2005) recommended to adjust the HS treatment temperature in function of the maximum temperature applied during the growing period of the crop (25 and 28 °C for Spanish and Dutch

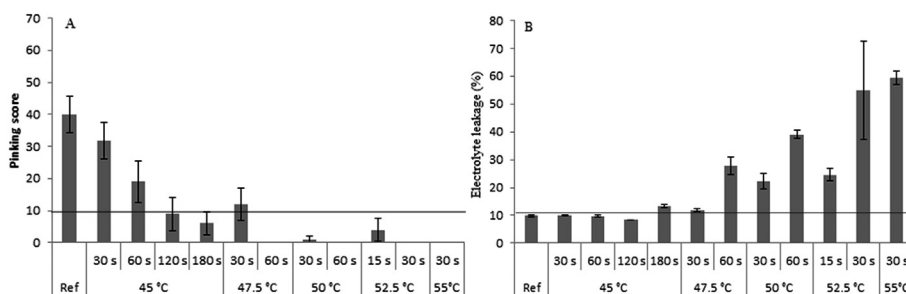


Fig. 1. Quantification of pink coloration (A) of leaf disks performed after application of HS treatments of different temperatures and durations. Quantification of pinking was done by visual judgment. Electrolyte leakage measurement (B) was performed on same leaf disks following incubation for 3 d in the pinking assay. The grey horizontal lines in both figures target the maximum pink coloration and maximum electrolyte leakage of the optimal HS treatment. Each bar represents the mean ± SD (n = 6).

heads respectively). In our results, the HS temperature may likely have been too high and engendered tissue damages resulting to higher respiration rate.

#### 4. Conclusion

The pinking assay showed that HS significantly reduced pink discoloration of iceberg lettuce. However, temperature and application time that greatly reduced pinking may also induce tissue damage as reflected in an increased electrolyte leakage. The optimal HS treatment (45 °C for 180 s) greatly reduced pinking without affecting tissue integrity.

The HS treatment (47.5 °C for 180 s) significantly reduced the PAL activity of the fresh-cut product stored in MAP but at the same time slightly stimulated the respiration activity of the product. To avoid anaerobic conditions in the packaging headspace of heat-treated product, we recommend to increase the gas permeability properties of the new bags. .

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