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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.

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 PolyPenol 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 immerged 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^{-1} for 1 min) using a centrifuge (TRISTAR Europe BV, Tilburg, the Netherland). After processing, the fresh-cut lettuce was packed in commercial modified atmosphere bag (BOPP) and flushed with 6 kPa O_2 and 11 kPa CO_2. 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^{-1} 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_0) and after 2 h (EC_1) of incubation at room temperature (20 °C) with continuously shacking (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_{max}). Electrolyte leakage was calculated and expressed in percentage of its maximal value as follow: $EC(\%)=(EC_1-EC_0)/(EC_{max}-EC_0)$.

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^{-1} borate buffer (pH 8.5), 5 mmol L^{-1} 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^{-1} 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^{-1} 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^{-1}) 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 Chromatographer - 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_2/CO_2 kg^{-1} h^{-1}.

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