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Kinetics of Enzymatic Browning of Minimally Processed Iceberg Salad

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Iceberg lettuce (*Lactuca sativa L.*) is a highly perishable product and it is considered one of the most popular minimally processed vegetables. Commercially available iceberg lettuce has a short shelf-life of 7 days at 7°C. Enzymatic browning is one of the main factors affecting product shelf life when oxygen is present in the packaging system. For this purpose, the aim of this work was (i) to investigate the decay kinetics of minimally processed iceberg salad following polyphenols and browning index evolution at different temperature (4°C, 8°C, 12°C); (ii) to evaluate the effect of salicylic acid on enzymatic browning. Minimally processed iceberg samples were stored at 4°C, 8°C, 12°C in air for 14 days. Samples treated with salicylic acid were stored at 8°C for 14 days. Polyphenols and browning index were evaluated by spectrophotometric analysis. The kinetics constant and activation energy (Ea) has been calculated by non-linear regression. The enzymatic browning was well described by a pseudo first order kinetic. The kinetic constant at reference temperature (8°C) was 0.00948 h^{-1} for polyphenols decay and 0.0184 h^{-1} for browning index. Ea for polyphenols and browning index were 83.21 kJ/mol and 63.49 kJ/mol, respectively. Salicylic acid showed a significant effect on enzymatic browning kinetic by delaying the evolution of the reaction during the first days of storage.

Keywords: lettuce; oxidation kinetics; salicylic acid; storage temperature; polyphenols; quality index

1. Introduction

Shelf-life can be defined as the period during which the food product will remain safe and will retain its desired sensory, chemical, physical, microbiological, and functional characteristics (Robertson, 1993). The quality deterioration is a dynamic process in which different factor such as product composition, microbial population, temperature, relative humidity, light and package atmosphere interact (Koukounaras et al., 2006). Minimally processed vegetables produce deteriorates because of physiological ageing, biochemical changes and microbial spoilage, which may result in degradation of the colour, texture and flavour of the product. Fresh leafy vegetables have a short shelf-life since they are exposed to unfavorable postharvest storage conditions. In particular, iceberg lettuce (*Lactuca sativa L.*) ca be considered a highly perishable product and it is one of the most popular minimally processed vegetables present on the market. Commercially available iceberg lettuce has a short shelf-life of 7 days at 7°C.

During peeling and cutting operations, many cells are ruptured, and intracellular products such as oxidizing enzymes are liberated. Enzymatic browning is one of the main factors affecting product shelf life when oxygen is present in the packaging system. Polyphenol oxidase (PPO), found in most fruit and vegetables, is responsible for enzymatic browning of fresh horticultural products, following bruising, cutting or other damage to the cell.

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It can be divided into two phases: cresolasic activity, in which the monophenols are converted into o-diphenols, and catecholase activity, in which the o-diphenols are converted into o-quinones and melanoidins, responsible for the dark coloration of the food product.
Nowadays, extend the postharvest life by chemicals is limited because of their negative impact on the environment and human being health. Therefore, its need to develop more safe and effective strategies for shelf life extension of minimally processed product (Mandal et al., 2009). Recently more attention is being paid to plant-based metabolites as anti-ripening and anti-microbial agents for sustenance of maximum postharvest quality of products. Salicylic acid (SA) is a group of phenolic compounds (Awad, 2013). SA has been reported to play an important role in regulating of many physiological processes (Zavala et al., 2017) and in controlling quality losses of horticultural crops in post-harvest.
In addition it has been demonstrated that the use of SA in pre- and post-harvest period has been effective in maintenance of quality and extension of storage life for some horticultural crops such as banana (Srivastava and Dwivedi 2000), peach (Wang et al., 2006), pomegranate (Sayyari et al., 2017) and Chinese chestnut (Zhou et al., 2015). Moreover, in order to evaluate the effect of new post-harvest technology on product shelf life, it is important to quantify the kinetic of alteration and the effect of environmental factor on this kinetic. A mathematical model of the alteration kinetic is mandatory if simulation in different storage condition would be done to predict its effect on shelf life. For this purpose, the aim of this work was (i) to investigate the decay kinetics of minimally processed iceberg salad following polyphenols and browning index evolution at different temperature (4 °C, 8 °C, 12 °C); (ii) to evaluate the effect of salicylic acid on enzymatic browning.

2. Materials and methods

2.1. Materials

Iceberg lettuce (*Lactuca sativa L.)* was purchased from a local farm and brought to the laboratory within 1 h of the experiment. Sodium bicarbonate, Folin-Ciocalteu reagent, trichloroacetic acid (TCA) (≥99%), salicylic acid (≥99%), citric acid (99%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All chemicals used for analyses were of analytical grade.

2.2. Sample preparation, treatments and storage conditions

The external leaves of lettuces were removed and the remain leaves were washed roughly under a tap water (8 ± 2°C) to remove any visible soil. The leaves were cut into 30 x 30 mm pieces, using a stainless-steel knife. The pieces were dipped into SA solution (2 mM) or in distilled water (control) for 60 sec at 10°C and then rinsed for 60 sec with distilled water and allowed to drip with a manual centrifuge for 2 minutes.
All the samples were conditioned in air in refrigerated cell at temperatures of 4 °C, 8 °C, 12 °C for variable times. To avoid polyphenols oxidation due cutting and shredding, samples were rapidly frozen before each analysis, by plunging into liquid nitrogen. At different time interval the total phenol content and the browning index were determined.

2.2.1. Total phenol content determination

The total phenol content (TPC) of minimally procced salad was determined according to a Folin-Ciocalteu method as described by Singleton and Rossi (1965). Briefly, 1 g of frozen samples were homogenized with 6% sodium bicarbonate (10 ml) for 1 min. The extract was then filtered using filter paper (Whatman, No. 2, Tokyo, Japan). 0.5 g of the supernatant was mixed with 2.5 ml of Folin - Ciocalteu reagent and 2 ml of sodium bicarbonate. The samples were incubated for 1h at 35°C and then for 1h at 6°C. The total phenolic content was spectrophotometrically measured by reading the Absorbance at 750 nm (Jasco V-550 UV/VIS Spectrophotometer, Japan).

A standard curve of gallic acid was used to quantify the total phenolic content expressed as μg Gallic acid equivalents (GAE eq) / g of fresh weight (FW). The estimation of total phenolic compounds in the samples was calculates as a mean value of three different replicates.

2.2.2. Browning index

Browning index (BI) was determined with the procedure described by Kim et al. (2014). Briefly, 1 g of frozen sample was diluted with tricloroacetic acid 2% (TCA) (25 ml), and incubated for 2 h at 30°C. The solution was then filtered using filter paper (No. 2, Whatman, Tokyo, Japan). The absorbance of the filtrate was measured at 420 nm on a spectrophotometer (Jasco V-550 UV/VIS Spectrophotometer, Japan).

2.2.3. Colour properties

The colour of the samples were evaluated using a colorimeter (Minolta CHROMA METER CR-300). The colour values of L (black/white), a*(redness/greenness) and b* (yellowness/blueness) were measured.

A standard plate CR-200/CR-30 was used as standard. The total colour difference (ΔE^*) was calculated by equation 1:

$$\Delta E = \sqrt{[(\Delta \mathrm{L})2\ + (\Delta \mathrm{a})2 + (\Delta \mathrm{b})2]} \quad (1)$$

Where $\Delta L = L_t - L_{t0}$, $\Delta a = a^*_t - a^*_{t0}$, $\Delta b = b^*_t - b^*_{t0.}$ The subscript letters t and t_0 refer to the time of analysis and time zero respectively. The analysis was carried out on a single chromatic section, always measuring on the most extreme part of the leaf. The analyses were performed on three leaves stored inside a Petri dish. For each time, three replicas were made, and the results were reported as means ± deviation standard.

2.3. Data analysis

Anova analysis was conducted to estimate the effect of the time and temperature on quality indices ($p < 0.05$). The software SPSS v17.0 for Windows (SPSS, Milan, Italy) were used.
The evolution of the quality indices versus time has been described by first order kinetic equation, such as:

$$\frac{dQ}{dt} = \pm K \cdot Q^n \quad (2)$$

Where Q is the quality index; t is the time; K is the kinetics constant (h^{-1}) of the qualitative parameter variation; n = order of the reaction (n=1).
Arrhenius equation has been used to describe the effect of temperature on kinetic constant:

$$ln\frac{k}{k_0} = -\frac{\mathrm{Ea}}{\mathrm{R}} \cdot \left(\frac{1}{T} - \frac{1}{T_0}\right) \quad (3)$$

Where K_o is the kinetic constant at reference temperature (T_0); K is the kinetic constant (h^{-1}); T is the absolute temperature (K), Ea is the activation energy (kJ mol^{-1}) and R is the universal gas constant (8,31 J/mol*K).
The activation energy (Ea) was estimated by linear regression of eq. 3

3. Results

Folin - Ciocalteu phenol content is used to obtain a crude estimate of the amount of phenolic groups present in minimally processed iceberg salad. The results showed that total phenolic content decreased during storage time from an initial value of 8 ± 3 µg GAE eq / g FW to an average value of 1.6 ± 0.2 µg GAE eq / g FW after a storage time of 200 h of storage. Data analysis showed a significant effect of time and temperature on phenolic content ($p<0.05$). Experimental data were described by a pseudo first order equation (Figure 1).

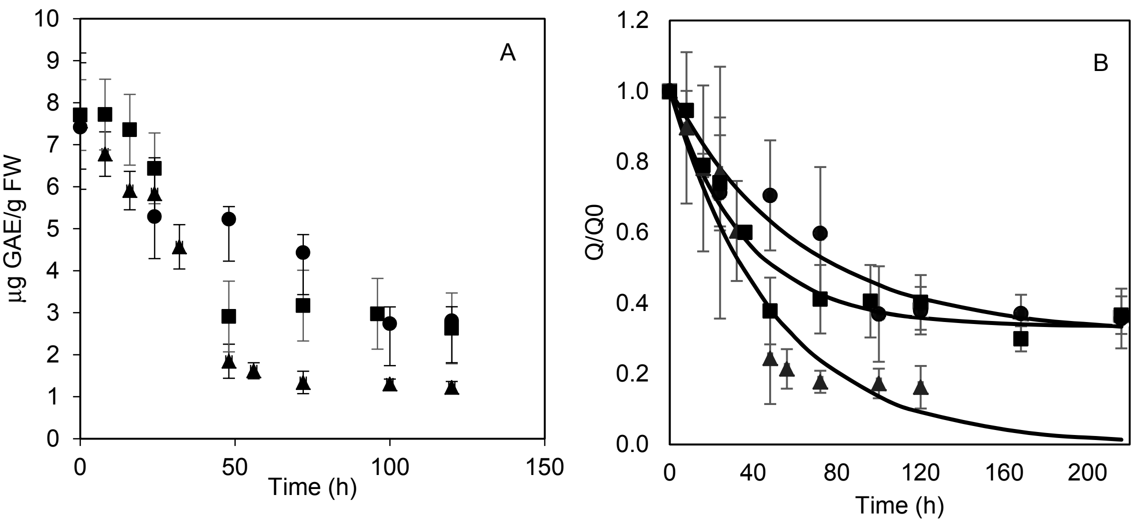

Figure 1: (A) total phenol values expressed as µg GAE eq/gFW; (B) total phenol values expressed as the ratio between the total phenol at time "t" (Q) and the total phenol at time zero (Q_o), of iceberg lettuce vs storage time at 4°C (●), 8°C (■), and 12°C (▲). The points represent experimental data, while the lines represent the prediction by using the eq. 2. Error bars indicate ± standard deviation

This results is in agreement with Altunkaya and Gokmen (2012) who describe with a first order kinetic the oxidation of two different variety of fresh cut lettuce. At 12 °C the model well describes the experimental data,

whereas at 4 °C and 8 °C there is a greater error. The kinetic constants at 4 °C, 8 °C and 12 °C, were 0,007 ± 0.001 h^{-1}, 0,009± 0.001 h^{-1} and 0,011± 0.006 h^{-1}, respectively.
The Arrhenius equation was applied for assessing the influence of temperature on the decay kinetic. The Ea allows to estimate the change of quality index of food samples as function of temperature; the estimated Ea was equal to 83.21 $kJmol^{-1}$ (Table 1).

Table 1: Kinetic constant (k) at different temperatures and activation energy (Ea) of total phenol oxidation

T (°C)	k (h^{-1})	Q_{inf}/Q_o	Ea ($kJmol^{-1}$)
4	0.0072± 0.001	0.075± 0.001	83.21
8	0.0095 ± 0.001	0.075± 0.001	
12	0.012 ± 0.006	0	

The BI was affected by different temperature condition. As general trend, the BI increased by increasing temperature. At 4°C and 8°C, BI reached a value of 0.64 ± 0.02 and 0.70 ± 0.02 after 216 h of storage, respectively. At 12°C BI was equal to 0.800 ± 0.004 after 100 h of storage. The increase of browning index is described by a pseudo first order equation, until the index value reached an equilibrium. No statistically differences were found between the kinetics of browning at 4 °C and 8 °C.
For this reason, we made an average of the data and the kinetic constant at 6° has been estimated (Figure 2, Table 2).

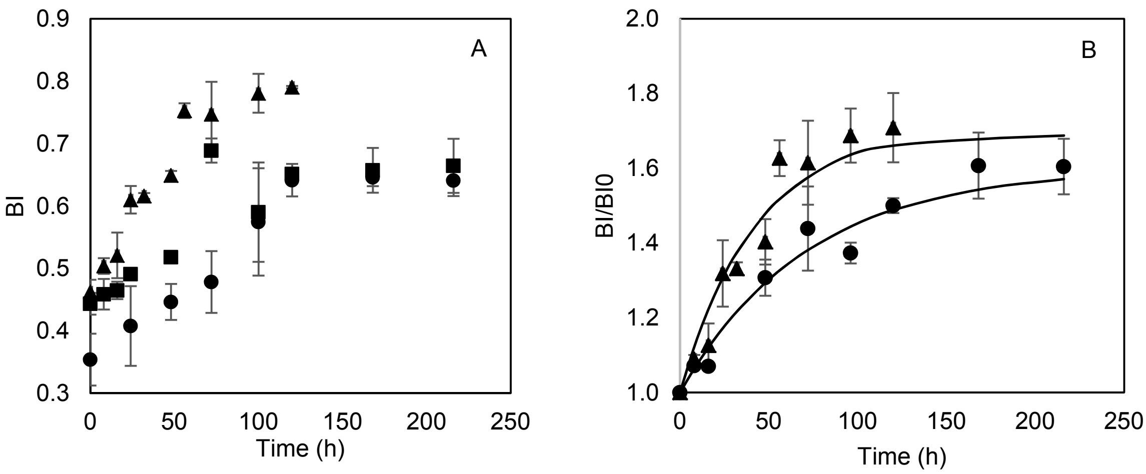

Figure 2: (A) browning index (BI) values at 4 °C (●),8°C (■), and 12 °C (▲); (B) normalized BI respect to time zero (BI/BI_0), of iceberg lettuce vs time during storage at 6°C (●), and 12°C (▲). The points represent experimental data, while the lines represent the prediction by using the eq. 2. Error bars indicate ± standard deviation

Table 2: Kinetic constant (k) at different temperatures and activation energy (Ea) of browning index

T (°C)	k (h^{-1})	Q_{inf}/Q_o	Ea ($kJmol^{-1}$)
6	0.014 ± 0.001	2.346	63.49
12	0.024 ± 0.006	1.700	

Salicylic acid showed an effect for the first 96 h of storage. As general trend, total phenol content was higher when iceberg salad is washed with salicylic acid. It was almost constant up to 96 h then dramatically decreased assuming the same value of the samples washed in water. BI of the acid-washed iceberg is constant up to 96 h while at 216 h of storage its value was the same of the water-washed sample (Figure.3).

Colour properties

The colour analysis showed an increase in the colorimetric index a* and a decrease in the index b* (Table 3). However, the variability of the data was very high, so that these indices have not been considered as critical quality index parameters to be used to quantify the kinetic of alteration.

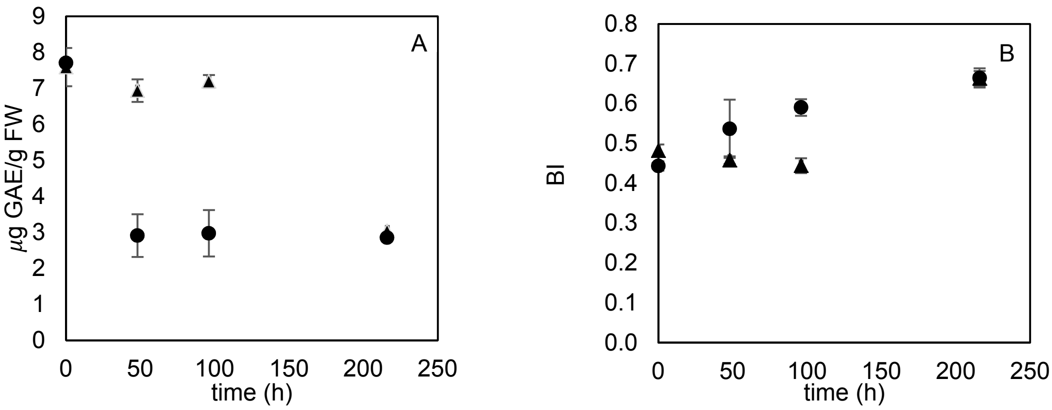

Figure 3: (A) effect of salicylic acid on polyphenols and (B) browning index during storage at 8 °C. ● water (control), ▲ salicylic acid. Value are given as mean ± standard deviation.

Table 3: a, b* and L* values of minimally processed iceberg salad, with and without salicylic acid treatment over time. Value are given as mean ± standard deviation.*

Time (h)	Sample treatments	a*	b*	L*
0	S.A	-4.62 ± 4.17	13.39 ± 5.66	70.16 ± 3.47
48		-6.95 ± -6.95	9.21 ± 9.21	71.25 ± 2.25
96		-7.02 ± 4.67	16.05 ± 6.26	68.29 ± 3.87
120		-9.23 ± 2.14	8.97 ± 8.97	70.64 ± 2.47
168		-3.88 ± 3.62	16.02 ± 5.08	68.29 ± 4.28
216		-8.24 ± 4.00	15.21 ± 6.64	70.48 ± 1.75
336		-9.01 ± 2.26	12.31 ± 4.62	69.17 ± 3.97
0	Control	-6.16 ± 3.16	16.22 ± 6.53	66.46 ± 2.07
8		-4.48 ± 5.24	17.67 ± 7.00	69.07 ± 3.16
16		-0.91 ± 0.98	26.56 ± 2.85	73.51 ± 3.06
24		-6.20 ± 5.41	16.50 ± 8.47	67.04 ± 3.17
48		-9.90 ± 6.43	18.91 ± 7.98	71.85 ± 4.40
72		-6.10 ± 5.58	14.20 ± 6.11	70.90 ± 2.81
96		-8.73 ± 6.43	19.84 ± 7.43	65.58 ± 3.70
120		-1.86 ± 1.48	5.74 ± 2.58	76.62 ± 3.18

4. Conclusion

Polyphenols and browning index can be used as a potential quality index for study enzymatic browning of iceberg lettuce. A pseudo first order kinetic model well described the evolution of the quality indices versus time. The effect of temperature was well explained by an Arrhenius type equation. The kinetic constant at reference temperature of 8°C were 0.018 ± 0.007 h^{-1} for browning index and 0.009 ± 0.001 h^{-1} for polyphenols. Ea for browning index was 63.49 KJ/mol, and for polyphenols was 83.21 KJ/mol. Moreover, preliminary results showed that salicylic acid were able to reduce the quality decrement of the product during the first 96 h of storage at 8 °C.

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