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Optimisation of steamer jet-injection to extend the shelflife of fresh-cut lettuce

Daniel Rico [a], Ana B. Martín-Diana [a,*], Catherine Barry-Ryan [a], Jesus M. Frías [a], Gary T.M. Henehan [a], Jose M. Barat [b]

[a] *School of Food Science and Environmental Health, Postharvest Research Unit, Dublin Institute of Technology (DIT), Cathal Brugha, Dublin 1, Ireland*
[b] *Institute of Food Engineering for Development, Department of Food Technology, Universidad Politécnica, Camino de Vera s/n, 46022 Valencia, Spain*

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Abstract

Optimisation of short time blanching (steaming) was investigated using response surface methodology by analysing quality and microbial and nutritional markers over the shelflife of packaged fresh-cut lettuce. Steamer treatment time (5–10 s) and storage (1–10 days) were used as independent factors in order to optimise the process. Longer treatments (>10 s) were not feasible because of extensive damage caused to lettuce tissue. Significantly ($p < 0.05$) higher values of luminosity (L^*), greenness ($-a^*$) and sensory panel scores (fresh appearance, general acceptability and absence of browning) were obtained with samples treated for longer times with the steamer. Activity of browning-related enzymes present in photosynthetic tissue decreased in all the cases, however, longer treatment time (7.5–10 s) was needed with vascular tissues to reduce to similar activity values. These differences could be explained by the variation in tissue thickness. The longest steam treatment (10 s) reduced and maintained significantly ($p < 0.05$) lower mesophilic load than shorter treatments (5–8.5 s). However, significant ($p < 0.05$) decreases in vitamin C and carotenoids were observed in samples treated with longer treatments. Steamer treatment of 10 s could be considered the optimum time for maintaining the shelflife (mainly texture and browning) of fresh-cut lettuce for 7–10 days in optimum conditions.

Keywords: Steamer; Microbial; Quality; Nutritional; Lettuce; Response surface methodology

1. Introduction

Diets containing a high proportion of vegetables have been shown to reduce the incidence of chronic diseases (Block et al., 1992; Steinmetz and Potter, 1991). Lettuce is an important agricultural commodity available over the whole year worldwide. A set of potentially beneficial health effects are related to lettuce composition, particularly to micronutrients such as polyphenols or carotenoids. Epidemiological analyses indicate that carotenoids and vitamins E and C are among the molecules in the diet playing a role in preventing cancer (Hertog et al., 1992; Winter and Herrmann, 1986) and heart diseases (Hart and Scott, 1995).

Heat treatment is the most utilised method for stabilising foods because of its capacity to destroy micro-organisms and inactivate enzymes. However, since heat can impair many organoleptic properties of foods and reduce the content or bioavailability of some nutrients, there is growing interest in searching for new technologies that are able to reduce the intensity of the damage caused by these preservation methods (Lopez et al., 1994).

Lettuce, as a leafy vegetable, is very difficult to process mainly due to its fragility. Low temperature blanching (heat-shock) alone or combined with other agents (Martin-Diana et al., 2005a,b; Saltveit, 2000) has been used to preserve quality in fresh-cut lettuce. Textural firmness effects obtained with heat treatments in lettuce have been attributed to the heat-activation of pectin methylesterase (PME) and/or to increased diffusion of solutes into tissues at higher temperatures (Bartolome and Hoff, 1972; Garcia et al., 1996).

The use of heat-shock is reported to enhance the quality and bactericidal effect of sanitisers (Baur et al., 2005; Delaquis et al., 1999; Martin-Diana et al., 2005b, 2006a). Nevertheless, sanitising methods lacking the use of chemicals with bactericidal-properties have been questioned as unlikely to totally eliminate all pathogens from contaminated product

* Corresponding author. Tel.: +353 14024487; fax: +353 14024495.
E-mail address: anabelen.martindiana@dit.ie (A.B. Martín-Diana).

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(Parish et al., 2001). Blanching constitutes an important decontamination and stabilisation method in the vegetable processing industry (Arroqui et al., 2003). The use of blanching (water or steam) not involving any chemical treatment can reduce initial mesophilic counts on leafy salads by more than three orders of magnitude and *Enterobacteriaceae* counts to less than four log cfu per kg (Gartner et al., 1997). However, blanching itself introduces deleterious changes in the product by the loss of nutrients through thermal degradation, diffusion and leaching, while also increasing power consumption and generating effluents (Negi and Roy, 2000).

Previous analysis showed that short-time steam processing (10 s) can be used as an alternative to chlorine (100 mg kg^{-1}) in sanitising fresh-cut lettuce (Martín-Diana et al., 2006b). The use of steam produced a shocking effect on lettuce metabolism, reducing respiration of the product and causing partial inactivation of browning-related enzymes, thereby preventing browning apparition. Microbial results (mesophilic load) showed no differences between chlorine and steam, and an improvement over water washing alone (Martín-Diana et al., 2006b).

Time of exposure to steam is a critical factor in the case of the lettuce due to the high surface/volume ratio of the leaves. For this reason operating conditions should be designed to reduce microbial load and extend shelflife with minimal adverse effects. Over-blanching may result in undesirable loss of quality (loss of texture, colour, nutrients, etc.).

Optimising the time of exposure to steam is the main objective of this work. The response of the vegetable in quality, nutritional and microbial parameters is influenced by the variables studied (time of exposure to steam and storage). The final aim of this paper is to develop a reliable method to extend the shelflife of fresh-cut lettuce with optimum quality, nutritional and microbial characteristics for the industry.

2. Materials and Methods

2.1. Sampling and experimental design

The experiments consisted of three independent trials that were carried out between May and August, 2005. Each trial consisted of 50 kg of fresh-cut lettuce (∼140 heads of ∼300–350 g), which were prepared according to minimal process procedure (see section below) and divided into 10 groups according to the RSM design (Table 1). For each group, three bags were prepared per marker (respiration, potential browning, enzymes, pH, etc.). For sensory analysis, a parallel study was carried out, but with only three selected treatments (extreme steamer times (5 and 10 s) and intermediate time (7.5 s) at three different storage times (1, 5 and 10 d)), in order to compare with the previous RSM analyses and compare steamer treatment with chlorination samples. Use of the full RSM design could have produced fatigue in the judges due to the large number of samples and affect the results.

Shelflife is defined as the length of time in which the vegetable can maintain the appearance, and microbial and antioxidant values that appeal to the consumer (Delaquis et al., 1999). Consequently, the storage time studied was estimated to be within the self-life range of up to 10 days after processing. The blanching exposure time was limited by the minimum time possible for the manipulation (4 s) of the vegetables inside the steamer cabinet and the maximum time at which the lettuce samples lost the attributes of apparent freshness due to tissue damage caused by the heating (10 s).

Table 1
Response surface methodology design

Points	Steamer time (s)	Storage time (day)
1	7.5	0.86
2	3.96	5.5
3	10	1
4	7.5	5.5
5	5	10
6	5	1
7	7.5	11.86
8	7.5	5.5
9	11.03	5.5
10	10	10

Response surface methodology (RSM) is a powerful mathematical and statistical tool for process optimisation which analyses the response of a factor (dependent variable) to a group of factors (independent variables) (Sefa-Dedeh et al., 2003). RSM was used in this work to study the effect of treatment conditions (time of exposure to steam) and storage of the samples applying a composite central design (CCD). Central composite design (2^2 + star design) consisted of a total of 10 factorial points (Table 1) with centred points and "star" points to estimate curvature. The star points represented extreme values (low and high) for each of the independent factors. The order of the experiments was fully randomised to provide protection against the effects of lurking variables. RSM analyses the effects of the independent variables (steamer time (FA) and storage (FB)) on different dependent variables (markers) generating a mathematical equation for each marker (a total of 21 equations for all the analyses). The systems of equations were solved using the method of least squares (MLS) which assumes that random errors are identically distributed with a zero mean and a common unknown variance and they are independent of each other. The predictive capability of the model was quantified by the coefficient of determination (R^2).

The headspace of the bags (three bags per treatment) was monitored throughout the entire storage. One bag per treatment and storage day was used for each of the other markers. Potential browning, enzymatic activity, microbial load, pH, water, ascorbic acid and carotenoid content and image analysis were measured in triplicate. Texture and colour were measured in all the pieces of one bag per treatment and day (∼25 pieces).

2.2. Processing and experimental set up

Iceberg lettuce grown in Valencia (Spain) was purchased from a local grower. The product was brought into the laboratory within 12 h after harvesting and stored at 4 °C before processing. The two outer leaves of the lettuce head were discarded and the core excised with a stainless steel knife. The lettuce was cut

perpendicularly to the main axis, dividing it into top and bottom halves. Only the top part of the head was used and further cut into four pieces. The temperature in the processing plant was 25 °C.

Blanching was carried out using a metal chamber (60 cm × 50 cm × 20 cm) equipped with a steam spreader at the bottom designed with more than 100 steam fluxing holes to homogeneously distribute the steam. The blanching temperature was monitored and kept constant during the whole procedure (100 °C). The chamber was fed with steam at a constant rate of approximately 7.2×10^3 kg s^{-1}. A thin layer of lettuce was placed on a supporting aluminium basket, allowing the steam to reach both sides of the product. Immediately after blanching the product was immersed in water (room temperature ~25 °C) for 1 min with agitation and then the excess of water on the product removed using an automatic salad spinner for 5 min.

To minimise product heterogeneity the processed vegetable was pooled and mixed. Packaging bags (200 mm × 320 mm) were made of 35 μm oriented polypropylene (OPP) film (Amcor Flexibles Europe-Brighouse, United Kingdom). The permeability (transmission rates) to oxygen and carbon dioxide of the film were $\sim 1.37 \times 10^{-3}$ and 1.48×10^{-3} L m^{-2} s^{-1} Pa^{-1} at 5 °C, respectively. Each package was filled with ~100 g of product and quickly chilled in a blast freezer set at 0 °C for 2 min. Bags were heat-sealed under atmospheric conditions. The packaged product was stored for 10 days at 4 °C. Three different batches were repeated.

2.3. Shelflife analysis. Quality markers

2.3.1. Headspace composition

A Gaspace analyser (PBI Dansensor, model CheckMate 9900, Ringsted, Denmark) was used to measure CO_2 and O_2 concentration of the bag headspace during storage. Sampling was made piercing a hypodermic needle into the bag through an adhesive septum, previously stuck to the bag. Sample was extracted at a flow rate of 0.035 mL s^{-1} for 1 min. Sensitivity of the gas analyser was 0.001 (O_2) and 0.1 (CO_2) in percentage. The accuracy (in percentage of the reading) is ±1% for O_2 and ± 2% for CO_2.

2.3.2. pH

A 10 g sample of lettuce tissue was blended for 2 min in 20 mL of deionised water. The pH of the slurry was measured at room temperature using a pH-meter (Consort, model C830, Turnhout, Belgium).

2.3.3. Water content

Lettuce sample were heated at 100 °C for 2 h in a Universal Oven (Memmert, Schwabach, Germany). Weights before and after heating were recorded and water content calculated as the difference and expressed as percentage of the fresh weight (before heating).

2.3.4. Potential browning

Potential browning was measured according to Viña and Chaves (2006). Ten grams of frozen and crushed tissue was treated with ethanol for 60 min and then centrifuged at 100 s^{-1} at 10 °C for 10 min, retaining the supernatant. Ethanol was added to bring the final volume to 25 mL. Absorbance at 320 nm was recorded and the results expressed as Absorbance units (AU) kg^{-1}.

2.3.5. Browning-related enzymes

Peroxidase (POD, E.C.1.11.1.7) and Polyphenol oxidase (PPO, E.C.1.10.3.1) activities were measured in extracts prepared as follows: a 10 g sample of lettuce in 0.5 mol L^{-1} phosphate buffer pH 6.5 containing 50 g L^{-1} polyvinylpyrrolidone at a 1:2 (w:v) ratio was homogenised (Ika-Labortechnik, model UltraTurrax T25, Stanfen, Germany) at 92 s^{-1} for 2 min, with a break of 3 min in the middle to avoid excess of heat to the sample which was kept in ice during the processing. The homogenate was then centrifuged at 212 s^{-1} for 30 min at 4 °C and the supernatant filtered through crepe bandage. The resulting crude extract was used without further purification. All the extracts were kept at 4 °C under dark conditions as much as possible during the processing and the enzymatic activity was measured immediately after the extract was prepared.

PPO and POD activities were assayed spectrophotometrically (Thermo electron corporation, model CE 1020, Madrid, Spain) by modified methods based on Galeazzi et al. (1981) and Tan and Harris (1995). PPO reaction mixture consisted of 0.1 mL of the extract and 2.9 mL of substrate solution (0.02 mol L^{-1} catechol in 0.05 mol L^{-1} phosphate buffer, pH 6.5). The rate of catechol oxidation was monitored at 400 nm for 2 min at 25 °C. An enzyme activity unit (EAU) was defined as the increase of 1.67×10^{-3} AU s^{-1} kg^{-1}. POD reaction mixture consisted of 0.2 mL of extract and 2.7 mL of substrate solution (0.05 mol L^{-1} phosphate buffer pH 6.5, containing 100 μL of hydrogen peroxide (1% v/v) as oxidant and 200 μL of p-phenylendiamine as hydrogen donor). The oxidation rate of p-phenylendiamine was monitored at 485 nm for 1 min at 25 °C. An enzyme activity unit (EAU) was defined as the increment of 1.67×10^{-3} AU s^{-1} kg^{-1}.

2.3.6. Colour (colorimeter)

Colour was determined using a Minolta CM-3600d colorimeter (Minolta Ltd., Buckinghamshire, UK). Single pieces (20–30 measurements per treatment and day) of lettuce were placed one at a time directly on the colorimeter sensor (35 mm aperture). The instrument was previously calibrated using white ($L^* = 93.97$, $a^* = -0.88$ and $b^* = 1.21$) and green tile ($L^* = 56.23$, $a^* = -21.85$, $b^* = 8.31$) standards. CIE $L^*a^*b^*$ parameters were obtained: L^* (lightness index scale) ranges from 0 (black) to 100 (white), a^* measures the degree of redness (+a) or greenness (−a*), and b^* measures the degree of yellowness (+b) or blueness ($-b^*$). CIE $L^*a^*b^*$ parameters were used to calculate other colour attributes: Hue (arctan b^*/a^*), Chroma $(a^2 + b^2)^{1/2}$ and total colour difference ($\Delta E = [(L_f - L_i)^2 + (a_f - a_i)^2 + (b_f - b_i)^2]^{1/2}$) where: L_i: initial luminosity, L_f: final luminosity, a_f: a^* value at final time, a_i: a^* value at initial time, b_f: b^* at final time and b_i: the value b^* at initial time).

2.3.7. Digital image analysis

Images of lettuce samples were obtained using a digital camera (Canon Power Shot, CCD 3.1 MPixels, Japan). The camera was set to 1.0 digital zoom on automatic indoor focus and no flash. The camera was mounted on a stand with two fluorescent lamps providing the only constant illumination, in order to obtain repeatable conditions of brightness and contrast (Kaiser Fototechnik, RB 5000 DL. Copy Lighting Unit 5556, Buchen, Germany). These settings provided a close up view of the lettuce. The digital images were analysed for RGB colour parameters using image analysis software (Photoshop® image Adobe System, 2002). Pictures of three bags per treatment were analysed at day 1, 3, 7, and 10.

2.3.8. Texture analysis

Textural properties of the samples were assessed using an Aname TAXTPlus texture analyser (Stable Micro Systems, London) equipped with a 500 N load cell. Lettuce texture is difficult to measure mainly due to the high heterogeneity of the product surface. A three-point shear test accessory (Flexion-Fracture) with a single blade probe was used. Test speed was set to 1.67 mm s^{-1} and the maximum load needed to perform the test and minimum load immediately after breaking the sample were reported. More than 25 samples were analysed per treatment. Textural values were calculated according to the crispness coefficient (CC): max load–min load/max load (Martin-Diana et al., 2006a).

2.3.9. Sensory analysis

Analytical–descriptive test was used to evaluate sensory quality attributes of fresh-cut lettuce treated with steam. The panel consisted of 12 judges aged 22–35 years (eight females and four males, all members of the University Politecnica of Valencia) with sensory evaluation experience and trained in discriminate evaluation of fresh-cut lettuce. Panellists were asked to score fresh appearance, photosynthetic browning, vascular browning and general acceptability. Before the sensorial tests started, panel members were familiarised with the product and scoring methods. This consisted in demonstration exercises involving examination of packs at different levels of deterioration and agreeing appropriate scores. When the panel members had become familiar with the test facilities and scoring regime, they were invited to score samples. This procedure was repeated several times until a level of consistency in scoring was obtained.

Two sensory tests were carried out. The same packages were evaluated during the entire storage time (10 days) in order to avoid variability of the product. During the analyses, samples were presented in randomised order to minimise possible carry-over effects. In the first test each panellist was asked to evaluate 10 samples (according with RSM design, Table 1) at regular intervals during storage (1–10). The second experiment consisted in the scoring of four different samples by the panel to measure the reproducibility of the judges' scores and their capability in discriminating among samples at regular intervals during storage (1, 5 and 10).

Fresh-cut was evaluated for appearance using a five point numerical rating scale. (I)Photosynthetic Browning, where 1 = nothing, 3 = moderate and 5 = a lot (severe browning); (II)Vascular Browning, where 1 = nothing, 3 = moderate and 5 = a lot (severe browning); (III)General acceptability, where 1 = very good, 3 = moderate and 5 = very bad and (IV)Fresh appearance, where 1 = very good/fresh appearance, 3 = moderate, and 5 = poor/no fresh appearance.

Sensory evaluation was used to determine the shelflife of the product, and it was agreed with the panellists that scores equal to or below three would describe the sample as unacceptable, indicating the end of the shelflife. Fresh-cut lettuce washed with chlorine was used as internal control. The results of the sensory analysis were reported as means of three separate trials. Data were analysed using Compusense® Five software (Release 4.4, Ontario, Canada).

2.4. Shelflife analysis. Microbial markers

2.4.1. Mesophiles

Microbiology load of the samples was analysed before the treatment and afterwards at regular intervals throughout the storage. A 25 g lettuce sample was blended in 225 mL of peptone saline with a Stomacher circulator homogeniser. Total counts evaluation was carried out at 30 °C on plate count agar (PCA) over 72 h. The results were expressed as $\log_{10}$ colony forming units per kg (CFU kg^{-1}).

2.5. Shelflife analysis. Nutritional markers

2.5.1. Ascorbic acid

Ascorbic acid determination was carried out according to the 2,6,-dichlorolindophenol method recommended by AOAC (1995) for the analysis of Vitamin C in fruits and juices. Fresh-cut product (20 g) was homogenised with 50 mL of acetic-methaphosphoric acid at low speed (to avoid foaming) for 3 min at 4 °C avoiding light on the sample. The homogenate was filtered and acetic-methaphosphoric acid added to reach a final volume of 100 mL. Aliquots of 10 mL were titrated rapidly in triplicate, using acetic-mehtaphosphoric acid as a blank. The results were expressed as μg ascorbic acid per kg of sample, according to Eq. (1).

$$(X - B)\left(\frac{F}{E}\right)\left(\frac{V}{Y}\right) \quad (1)$$

where X mL is volume of 2,6,-dichlorolindophenol to permanently colour the titration flask (more than 5 s), B is the volume used in permanently colour the control, F^* is the 2,6,-dichlorolindophenol factor ($F^* = 2$ g L^{-1} 2,6,-dichlorolindophenol (for standard ascorbic acid g L^{-1})), E is the weight (g) of sample used in the assay, V the final volume used (100 mL) and Y the volume of the aliquot titrated (10 mL).

2.5.2. Carotenoids

Total carotenoids were extracted in dark conditions by homogenising lettuce tissue (5 g) in 30 mL of an acetone/ethanol (50:50 v:v) solution, containing 200 mg L^{-1} butylated hydroxytoluene (BHT). The homogenate was filtered and washed with acetone/ethanol until colourless and final volume adjusted to

100 mL with acetone/ethanol. An aliquot was placed in a 1 cm^3 quartz cuvette and its absorbance measured at 470 nm in a spectrophotometer (Thermo electron corporation, model CE 1020, Madrid, Spain). Total carotenoids ($\mu g\,kg^{-1}$) were calculated as described by Gross (1991).

2.6. Statistical analysis

Response surface methodology (RSM) was used to fit the experimental data to quadratic polynomial equations. The model and statistical analyses and contour plots were analysed using Statgraphics software 2.1 (Statistical Graphics Co., Rockville, USA).

For comparison of steamer with chlorine treatment for the sensory trials analysis of ANOVA (Multifactor and one-way) were performed to examine differences between treatments, storage and interaction of both factor for each one of the variables studied. Means were compared by significant difference (LSD) test, at a significance level of $p = 0.05$, using Statgraphics software (version 2.1; Statistical Graphics Co., Rockville, USA).

3. Results and discussion

3.1. Shelflife analysis. Quality markers

Browning appearance and texture degradation are the main causes of quality loss in fresh-cut lettuce. Quality markers related with these phenomena were evaluated in order to compare the effects of different steam treatment times over storage (headspace, pH, water content, potential browning, browning-related enzymes, texture and sensory analysis).

3.1.1. Headspace

Eqs. (2 and 3) describe the models obtained for oxygen and carbon dioxide headspace composition. The models explained 81% of variation of oxygen and 56% of carbon dioxide due to the effect of storage and treatment time. Significant linear effects ($p < 0.05$) of the storage and time of exposure to steam were observed for both gases during storage. The oxygen decreased and the carbon dioxide increased throughout storage, as expected. An interaction effect occurred between the two independent variables (storage and exposure time).

Oxygen decreased from atmospheric concentration (21%) to values in the range of 11–18% after the first day of storage (Fig. 1(1)). Fresh-cut lettuce treated with longer steam treatments maintained higher values of oxygen than samples treated with shorter times. This differences due to changes in the respiration rate can be due to a different degree of stress on the vegetable depending on the treatment time (Surjadinata and Cisneros-Zevallos, 2003) or/and differences in microbial load, which can affect the respiration of the vegetable. Lower oxygen levels were also found at the end of the storage in the samples treated with shorter exposure times. Toivonen and Stan (2004) reported that a decrease in respiration could be caused by the washing treatment, due to a microbial load reduction. The authors observed a decrease in respiration rate of treated samples compared to the untreated control.

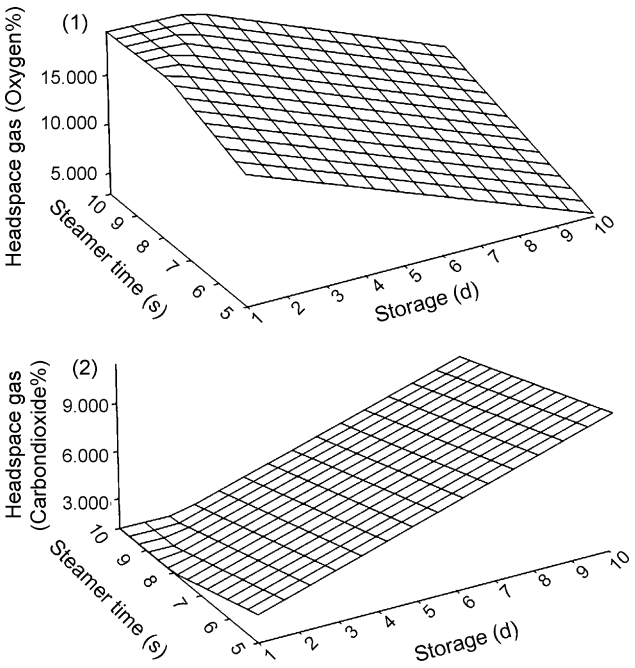

Fig. 1. Effect of steamer time (s) and storage (day) on the headspace oxygen (%) (1) and carbon dioxide (%) (2) in lettuce packaged and stored at 4 °C.

$$Z_{\text{Oxygen}} = 21.79 - 3.15FA - 0.27FB + 0.24FA \times FB; \quad R^2 = 81.63\% \tag{2}$$

Carbon dioxide reached levels from 3.5 to 9% at the end of the storage. Differences due to treatment time were found, mainly during the first days of storage. The use of longer treatments resulted in a significant ($p < 0.05$) reduction of the respiration, reflected in lower CO_2 concentrations (Fig. 1(2)).

$$Z_{\text{CarbonDioxide}} = 2.21 + 1.35FA + 0.46FB - 0.12FA \times FB; \quad R^2 = 55.6\% \tag{3}$$

3.1.2. pH

The model obtained for pH (Eq. (4)) shows a significant ($p < 0.05$) linear and quadratic effect of treatment time and an interaction effect of treatment time and storage. The model explained 98% of pH variation.

$$Z_{\text{pH}} = 8.23 + 6 \times 10^{-4}FA - 0.47FB - 9 \times 10^{-4}FA^2 + 8 \times 10^{-3}FA \times FB + 0.02FB^2; \quad R^2 = 98.52\% \tag{4}$$

As shown in Fig. 2, a general increase of pH was observed over storage, which could be due to an increase in the bacterial growth (Beuchat and Brackett, 1990; Delaquis et al., 1999). The use of longer steam treatment times resulted in lower pH values, perhaps related to the lower microbial load (Fig. 10). Similar statements were reported by Babic and Watada (1996) and Roura et al. (2000), which attributed the gradual increases in the pH values of spinach leaves and Swiss chard to the microbial growth.

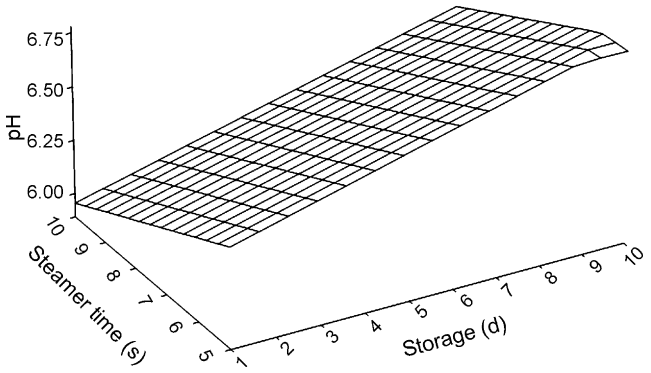

Fig. 2. Changes in pH as a function of steamer time (s) and storage time (day) in fresh-cut lettuce packaged and stored at 4 °C.

3.1.3. Water content

The model obtained, shown in Eq. (5), poorly explained the changes in water content ($R^2 = 38\%$) based on storage or treatment exposure time.

$$Z_{\text{W.Cont.}} = 78.87 + 0.31FA + 3.92FB + 0.06FA^2 + 0.10FA \times FB - 0.20FB^2; \quad R^2 = 38.13\% \tag{5}$$

The water loss increased during storage (Fig. 3), although not significantly. Water loss was not influenced by the time of treatment with steam.

3.1.4. Potential browning

Models on Eqs. (6 and 7) explained 91 and 51% of the potential browning for photosynthetic and vascular tissues, respectively. A significant ($p < 0.05$) linear effect of the storage time on the potential browning of both tissues was observed.

$$Z_{\text{P.Br.Ph}} = 0.07 + 0.04FA + 5 \times 10^{-3}FB - 10^{-3}FA^2 - 10^{-3}FA \times FB - 5 \times 10^{-4}FB^2; \quad R^2 = 90.84\% \tag{6}$$

$$Z_{\text{P.Br.V.}} = 0.09 - 3 \cdot 10^{-3}FA + 0.02FB + 2 \times 10^{-4}FA^2 + 9 \times 10^{-4}FA \times FB - 2 \times 10^{-3}FB; \quad R^2 = 51.20\% \tag{7}$$

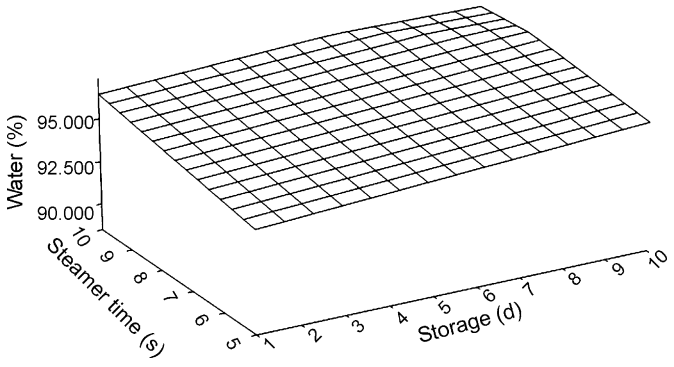

Fig. 3. Changes in water content (%) as a function of steamer time (s) and storage time (day) in fresh-cut lettuce packaged and stored at 4 °C.

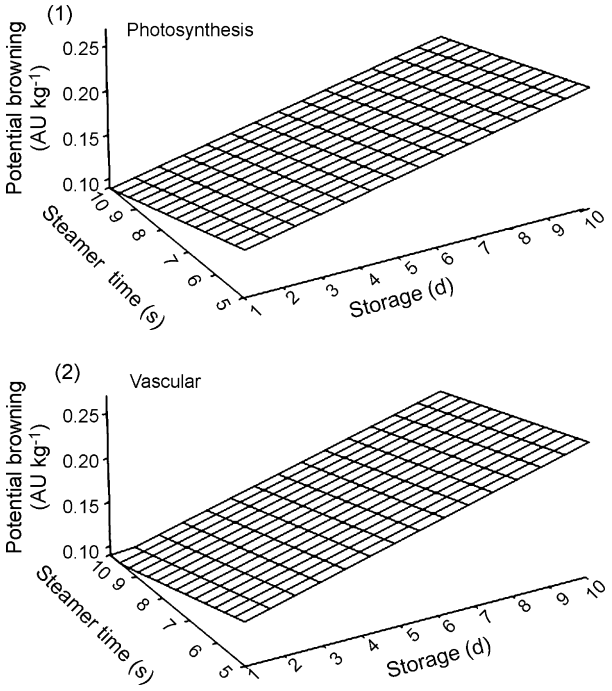

Fig. 4. Effect of steamer time (s) and storage (day) on the Potential browning (AU kg^{-1}) for photosynthetic tissue (1) and vascular tissue (2) in fresh-cut lettuce packaged and stored at 4 °C.

Potential browning (Fig. 4) of the samples significantly ($p < 0.05$) increased over storage. Initial values were lower in photosynthetic (0.12 ± 0.008) than in vascular (0.145 ± 0.006) tissues, according with results from other authors (Choi et al., 2005) that suggest the browning appears earlier in vascular than in photosynthetic tissue due to the stress caused by the minimal procesdure (cutting, washing, etc). After processing phenolic compounds accumulate in the cut area, effect that later propagates to non-wounded tissue. At the end of the storage higher values for potential browning were also found in vascular tissue (0.17 ± 0.006) compared with photosynthetic tissue (0.15 ± 0.005) (Fig. 4). When preparing fresh-cut products based on Iceberg lettuce a good practise could be the negative selection of the vascular tissue, increasing the amount of greener parts in the packages, which might extend the product shelflife.

The concentration and composition of phenolic compounds and/or activity of PPO are often the major factors determining tissue browning development and intensity (Mathew and Parpia, 1971; Vamos-Vigyazo, 1981). Higher potential browning was observed in vascular tissue compared with photosynthetic. Nevertheless, lower PPO and POD activity was found in vascular tissue (Fig. 5). Other causes of browning in vegetables than enzymatic have been explained by Namiki (1988), which described that the decomposition produced in the ascorbic acid due to the thermal treatment or the reaction of carbonyl groups (reducing sugars, aldehydes, ketones, lipid oxidation products and amino compounds) can be associated with browning appearance.

Treatment time significantly ($p < 0.05$) affected the potential browning (linear effect) of vascular tissue (Fig. 4(2)) but not the potential browning of the photosynthetic tissue (Fig. 4(1)).

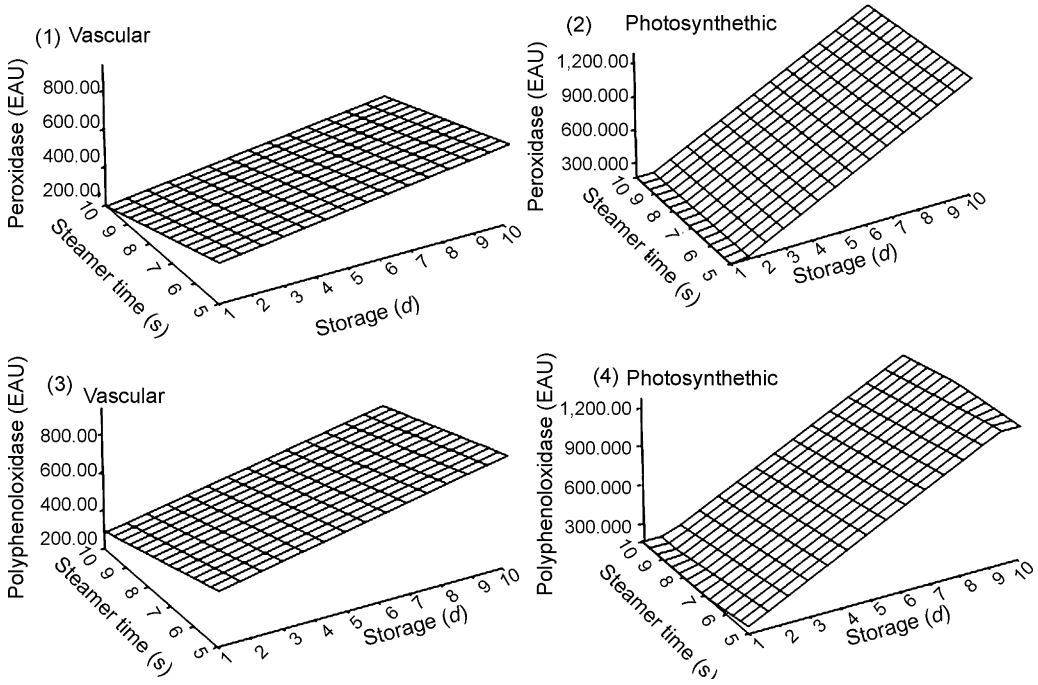

Fig. 5. Changes in Peroxidase (POD) a and Polyphenol oxidase (PPO) activity (EAU) as a function of steamer time (s) and storage time (day) for photosynthetic tissue (2, 4) and vascular tissue (1, 3) in fresh-cut lettuce packaged and stored at 4 °C.

3.1.5. Browning-related enzymes

One of the most relevant effects of blanching is on enzymatic activity. The effect of treatment time on two main enzymes involved in browning reactions, POD and PPO, was evaluated. The models of Eqs. (8 and 9) explained 92 and 53% of POD activity in photosynthetic and vascular tissues. The content of POD enzyme was higher in photosynthetic than in vascular tissue, as occurred in previous studies (Martin-Diana et al., 2005b). Storage time significantly ($p < 0.05$) affected (linear effect) (Fig. 5(1 and 2)) the activity of POD in both tissues. However, treatment time significantly ($p < 0.05$) affected POD activity present in vascular tissue and not in photosynthetic tissue. This could be caused by the different thickness of the tissues. POD activity increased over storage, reaching the highest values by the end of the storage (day 10).

$$Z_{\text{POD-Ph.}} = -456.9 + 62.5FA + 199.4FB + 3.5FA^2 + 2.7FA \times FB - 14.6FB^2; \quad R^2 = 92.46\% \tag{8}$$

$$Z_{\text{POD-V.}} = 329.8 + 61.0FA + 119.8FB + 3.1FA^2 - 0.4FA \times FB - 7.3FB^2; \quad R^2 = 66.90\% \tag{9}$$

Results for PPO were similar to those for POD (Fig. 5(3 and 4)). The model (Eq. (10)) better explained PPO activity in the photosynthetic tissue (92.6%) than that in the vascular tissue (Eq. (11)) (53.5%). A significant ($p < 0.05$) linear effect caused by the storage on PPO activity of both tissues was observed, meanwhile in vascular a significant ($p < 0.05$) linear effect of the exposure time to steam was observed, a similar result as the POD.

$$Z_{\text{PPO-Ph.}} = -1877 + 1846FA + 559FB - 42FA^2 - 31FA \times FB - 47FB^2; \quad R^2 = 92.60\% \tag{10}$$

$$Z_{\text{PPO-V.}} = 234 + 130FA + 33FB - 5FA^2 - 6FA \times FB - 3FB^2; \quad R^2 = 53.55\% \tag{11}$$

Although POD and PPO were not inactivated with the steam treatment, initial activities were reduced when compared with those of non-thermally treated chlorinated samples, which showed initial values 10 times higher (Martín-Diana et al., 2006b). In this study, the reduction in POD and PPO activities was correlated with lower visual browning and better colour parameter results.

3.1.6. Colour (colorimeter)

Colour is an important sensorial attribute and a critical factor affecting quality. The model obtained for luminosity (Eq. (12)) explained 58% of colour variation. A significant ($p < 0.05$) quadratic effect of the treatment time on colour was observed. Longer times of treatment increased sample luminosity. A significant ($p < 0.05$) interaction effect between storage and treatment time was also observed.

$$Z_{\text{Lum.}} = 81.03 - 2.42FA - 2.22FB + 0.33FA^2 - 0.23FA \times FB + 0.27FB^2; \quad R^2 = 58.12\% \tag{12}$$

Luminosity decreased, as expected (Fig. 6(1)), and this can be explained by the appearance of browning. The use of longer steam exposure time increased luminosity in the samples.

Redness/greenness parameter (a^*) significantly ($p < 0.05$) increased (linear effect) during the storage (Fig. 6(2)), indicating chlorophyll degradation and/or appearance of browning. The model (Eq. (13)) explained 90% of parameter variability. Treatment time also significantly ($p < 0.05$) affected a^* values. Samples exposed longer times to steam showed lower a^* values,

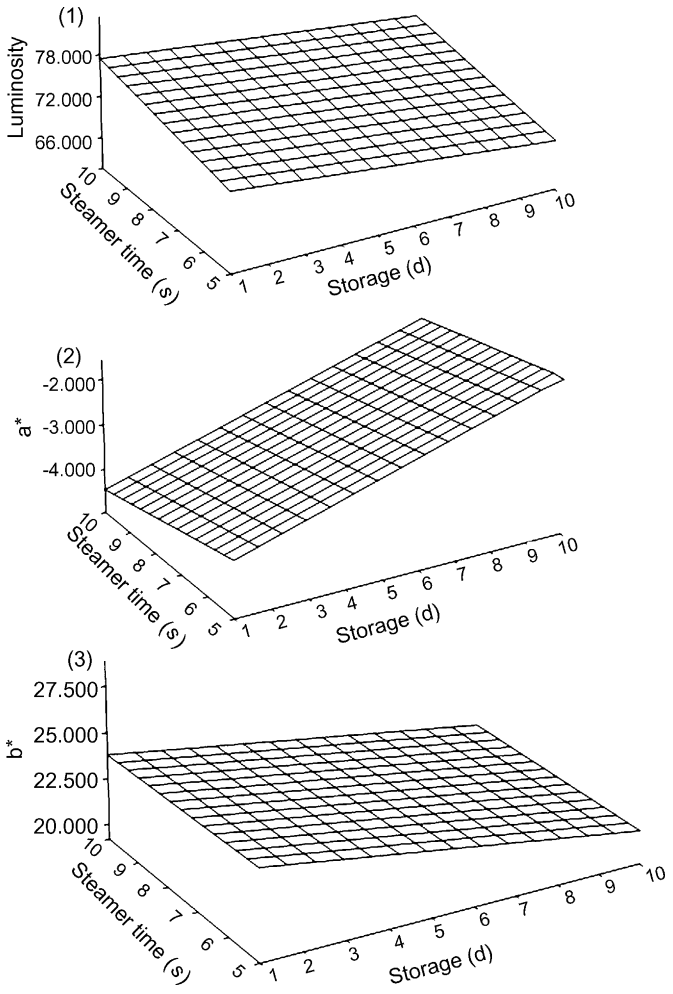

Fig. 6. Effect of steamer time (s) and storage (day) on the colour (CIE $L^*a^*b^*$) parameter in fresh-cut lettuce packaged and storage at 4 °C. Luminosity (1), a^* (2) and b^* (3).

effect better observed at the beginning of the storage.

$$Z_{a*} = -3.74 + 0.25FA + 0.03FB - 0.01FA^2 + 0.01FA \times FB - 0.02FB^2; \quad R^2 = 92.89\% \tag{13}$$

Although blanching has been linked to loss of greenness due mainly to the degradation of chlorophylls, some authors (Lau et al., 2000; MacKinney and Weast, 1940; Meyer, 1960; Woolfe, 1979) have reported short-time blanching to better maintain the greenness in vegetables. Longer treatments increased initial greenness values of the samples although the rate of change in a^* parameter over storage did not depend on treatment time.

The effect of treatment and storage time on b^* parameter was explained with Eq. (14) model. Yellowness/blueness parameter (b^*) decreased over storage with no significant ($p<0.05$) differences due to treatment time (Fig. 6(3)).

$$Z_{b*} = 16.74 - 3.40FA + 3.80FB + 0.26FA^2 + 0.03FA \times FB - 0.27FB^2; \quad R^2 = 76.03\%. \tag{14}$$

3.1.7. Digital image analysis

Fig. 7 shows packages at day 1 (Fig. 7(1, 3 and 5)) and at day 10 ((Fig. 7(2, 4 and 6)) of samples treated with steam for 5 ((Fig. 7(1 and 2)), 7.5 ((Fig. 7(3 and 4)) and 10 s ((Fig. 7(5 and 6)). At day 1 no significant ($p<0.05$) differences in RGB colour parameters were observed between treatment times (data not shown). At day 10, samples treated longer times with steam showed lower browning ((Fig. 7(3)) than samples treated with shorter times ((Fig. 7(1 and 2)).

3.1.8. Textural analyses

Texture was evaluated using the crispness coefficient (CC). The model (Eq. (15)) explained 77% of CC variation. Significant decrease of CC over storage (linear effect) was observed in all the samples (Fig. 8).

$$Z_{\text{Text.}} = 16.97 - 0.10FA + 1.37FB - 0.05FA \times FB; \quad R^2 = 76.84\% \tag{15}$$

The loss of turgor of the cells after treatment due to dehydration can explain the decrease in crispy characteristic textural properties of lettuce, although no significant differences in water content over storage were observed.

The time of treatment with steam significantly ($p<0.05$) affected CC results. Longer times of treatment resulted in higher values of CC in the samples. Firming effects of heat treatments could be linked to the action of heat-activated pectin methyl esterase (PME) (Bartolome and Hoff, 1972; Garcia et al., 1996). Previous work in this laboratory showed that heat-shock alone or combined with calcium lactate better maintained textural properties of fresh-cut lettuce than non-thermal treatments (Martin-Diana et al., 2005b, 2006a; Rico et al., 2006, 2007). Another possible reason of higher CC can be due to the temperature of the treatment, causing a higher retention of water in the vegetable. The heating of the air within the lettuce tissue and the subsequent cooling and contraction of this air would have absorbed the surrounding water into the lettuce, therefore increasing the moisture and turgor of the cells (Martin-Diana et al., 2006a).

3.1.9. Sensory analysis

Models in Eqs. (16–19) explained more than 96% of the sensorial test results variability (photosynthetic browning, vascular browning, fresh appearance and general acceptability). Significant linear ($p<0.05$) effects of the storage and the treatment time were observed for all the attributes scored by the panellist.

$$Z_{\text{Br.V.}} = -2.90 + 0.58FA + 1.22FB - 9 \times 10^{-3}FA^2 - 0.02FA \times FB - 0.09FB^2; \quad R^2 = 99.22\% \tag{16}$$

$$Z_{\text{Br.Ph.}} = -1.34 + 0.70FA + 0.83FB - 0.02FA^2 - 0.03FA \times FB - 0.07FB^2; \quad R^2 = 96.92\% \tag{17}$$

$$Z_{\text{Fr.App.}} = -3.19 + 0.64FA + 1.19FB - 0.01FA^2 - 0.03FA \times FB - 0.08FB^2; \quad R^2 = 97.38\% \tag{18}$$

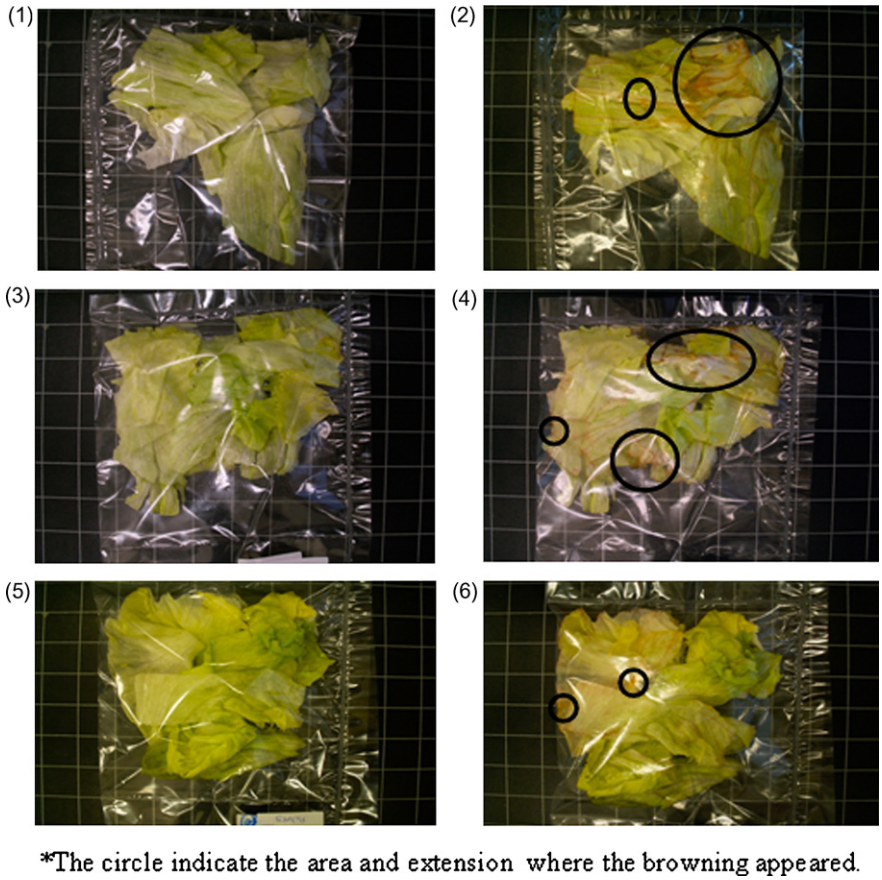

Fig. 7. Image of samples treated with 5 (1, 2), 7.5 (3, 4) and 10 (5, 6) s at day 1 (1, 3, 5) and day 10 (2, 4, 6) for fresh-cut lettuce packaged and stored at 4 °C.

$$Z_{\text{G.Acc.}} = -2.42 + 0.60FA + 1.03FB - 0.02FA^2 - 0.02FA \times FB - 0.07FB^2; \quad R^2 = 96.96\% \tag{19}$$

Samples treated with steam during three different times (5, 7.5 and 10 s) were compared with a chlorinated sample. Samples treated with steam for 10 s showed no significant differences with chlorinated samples regarding photosynthetic and vascular browning ((Fig. 9(1 and 2)), fresh appearance (Fig. 9(3)) and general acceptability (Fig. 9(4)). However, samples treated with shorter times showed a higher appearance of browning, lower acceptability and fresh appearance, and were considered not acceptable (score higher than three) at the end of the storage.

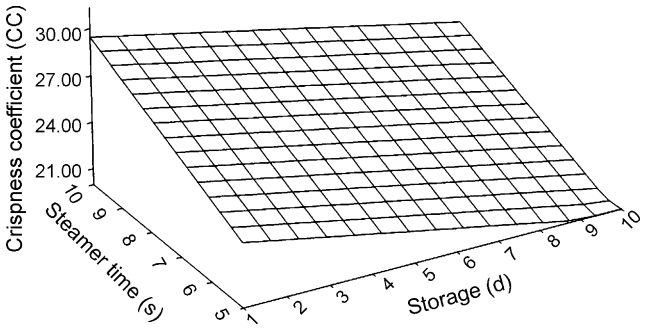

Fig. 8. Effect of steamer time (s) and storage (day) on the crispness coefficient (CC) in lettuce packaged and stored at 4 °C.

3.2. Shelflife analysis. Microbial markers

Ready-to-eat vegetables can be used without further washing if kept refrigerated and within the "use-by" date. Despite quality is affected by the microbial growth and although it is possible that certain types of treatments preserve the texture and browning appearance, they might not control the microbial load.

3.2.1. Mesophiles

The model described in Eq. (20) explained 98% of mesophilic load variation. A significant ($p < 0.05$) linear increase of mesophilic load over storage was observed. Also, a significant ($p < 0.05$) reduction with increasing treatment times occurred (Fig. 10).

$$Z_{\text{M.}} = -2.5 \times 10^7 + 1.9 \times 10^6 FA + 6.1 \times 10^6 FB + 2.1 \times 10^5 FA^2 - 3.5 \times 10^5 FA \times FB - 3.7 \times 10^5 FB^2; \quad R^2 = 97.99\% \tag{20}$$

Initial values in non-treated samples were higher ($\sim 10^{10}$ CFU kg^{-1}) than previous studies by other authors ($\sim 10^9$ CFU kg^{-1}) (Ponce et al., 2002; Rico et al., 2006, 2007). Particular conditions of the study, as the Spanish summer temperatures, might have favoured a higher microbial load. Longer steamer treatment time (10 s) produced a significant ($p < 0.05$) reduction in the initial counts ($\sim$1.8 log CFU reduction) with

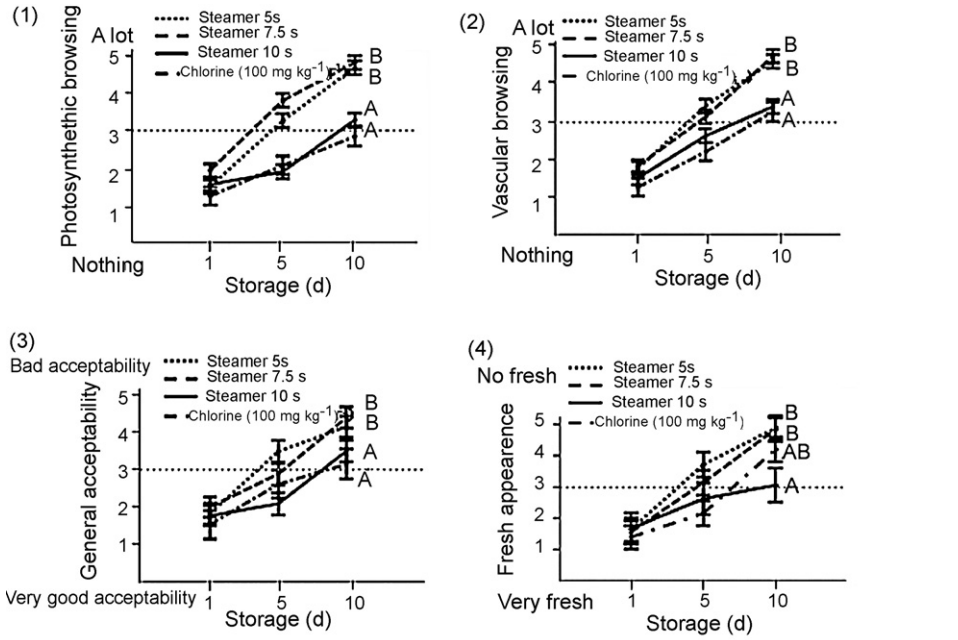

Fig. 9. Sensory evaluation of fresh-cut lettuce treated with 5, 7.5 and 10 s during 10 days of storage. Chlorine (~100 mg kg^{-1}, room temperature) was used as control. Parameters: Photosynthetic browning (1), Vascular browning (2), Fresh appearance (3) and General acceptability (4).

respect to shorter times (5 s), that showed similar reductions as with water (1 log CFU).

Microbial load increased in all the samples over storage (Fig. 9). The values at the end of storage were within the limit recommended (10^{12} CFU kg^{-1}) for consumption (Debevere, 1996) in all the cases. Only 10 s treatment kept similar microbial load values to chlorine over storage (data not shown).

3.3. Shelflife analysis. Nutritional markers

One of the valuable properties associated to fresh-cut vegetables is the antioxidant characteristic of some of their components, e.g. vitamin C and carotenoids, which present a high reducing power and free radical scavenging activity. Ascorbic acid and carotenoids were evaluated as indicators of the nutritional value of the fresh-cut lettuce.

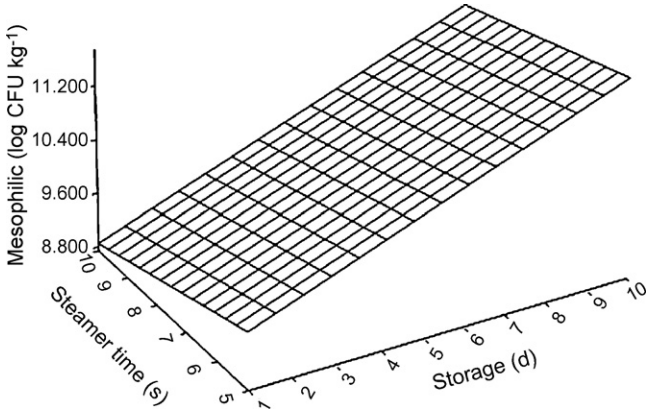

Fig. 10. Effect of steamer time (s) and storage (day) on the mesophilic counts (log CFU kg^{-1}) in fresh-cut lettuce packaged and storage at 4 °C.

3.3.1. Ascorbic acid

Previous studies showed blanching procedure using steam for short times caused a significant decrease in vitamin C content in fresh-cut lettuce, when compared with non-thermal treatments (Martín-Diana et al., 2006b), mainly due to the low thermal stability of Vitamin C (Liu et al., 2002; Nagy and Smooth, 1976). In this study the model shown in Eq. (21) explained 86% of ascorbic acid variability due to the effect of storage and treatment time. The ascorbic acid decreased significantly ($p < 0.05$) over storage (linear effect) with significant differences between treatment times (Fig. 11(1)). Increasing treatment time reduced the Vitamin C content of the samples.

3.3.2. Carotenoids

The model for carotenoid content with the two independent variables, storage and time of exposure to steam, is described in Eq. (22). A linear decrease of carotenoid content was observed during the storage. Carotenoid content decreased when the treatment time was increased (Fig. 11(2)).

$$Z_{\text{As.Ac.}} = 0.03 + 4 \times 10^{-4} FA - 0.003 FB - 10^{-4} FA^2 + 10^{-5} FA \times FB + 5 \times 10^{-4} FB^2; \quad R^2 = 86.09\% \tag{21}$$

$$Z_{\text{Car.}} = 0.72 - 0.02 FA - 0.06 FB + 0.003 FA^2 - 0.003 FA \times FB + 0.004 FB^2; \quad R^2 = 53.01\% \tag{22}$$

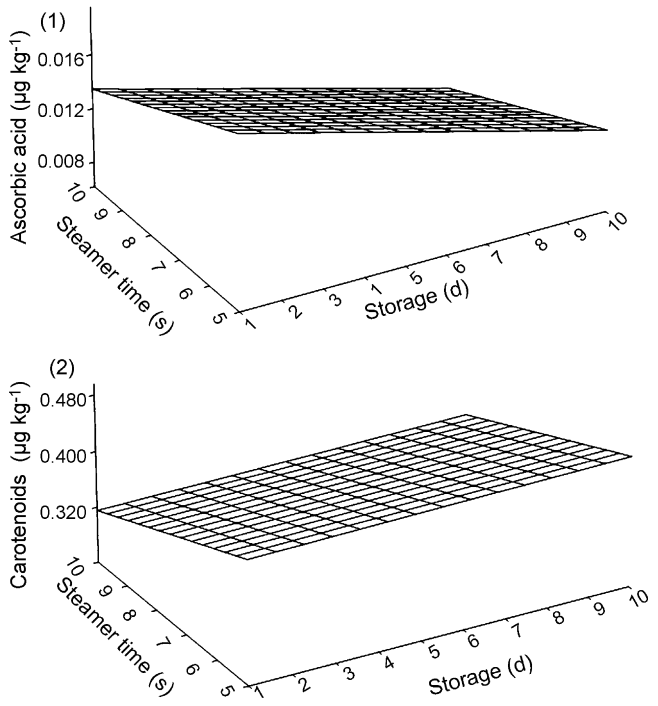

Fig. 11. Effect of steamer time (s) and storage (day) on the antioxidants markers (ascorbic acid (1) and carotenoids (2)) in fresh-cut lettuce packaged and storage at 4 °C.

4. Conclusion

The results showed that the variable time of exposure to steam affected most of the quality, microbial and nutritional markers. Based on the quality markers tested 10 s can be consider the best time for the application for keeping fresh-cut lettuce quality and microbial acceptable during 7–10 days as maximum storage. Increasing the time of the treatment resulted in better textural properties and later appearance of the browning over storage. However, the use of the steamer even for very short time (∼5 s), significantly reduced the ascorbic acid and carotenoid content of the samples. A compromise solution between quality, microbial and nutrition in the final product must be found in order to choose a suitable treatment time. The loss in nutritional value could be balance with the incorporation of an external source of vitamin C or others antioxidant compounds using atomising techniques after the steam treatment.

A significant reduction of mesophilic load was observed with long steam treatments (10 s). This type of ready to-use vegetables retain much of their indigenous microflora after minimal processing, and pathogens may form part of this microflora, therefore posing a potential microbial problem. Further research on the ability of steam to control the microbial spoilage of fresh-cut lettuce and protect consumers against microbiological hazard is recommended.

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