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Green tea extract as a natural antioxidant to extend the shelf-life of fresh-cut lettuce

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Keywords: Green tea, Camellia sinensis L., Fresh-cut lettuce, Polyphenols, Browning

A B S T R A C T

Green tea extract (GT) was evaluated as a preservative treatment for fresh-cut lettuce. Different quality markers, e.g. respiration, browning, ascorbic acid and carotenoid content were evaluated. GT concentration (0.25, 0.5 and 1 g 100 mL− 1) and temperature (20 °C and 50 °C) were tested. Optimal GT treatment (0.25 g 100 mL− 1 at 20 °C) was compared with chlorine (120 ppm at 20 °C). High GT concentrations (0.5 g 100 mL− 1 and 1.0 g 100 mL− 1) maintained better prevent ascorbic acid and carotenoid loss than 0.25 g 100 mL− 1 GT and chlorine. GT increased browning of samples, probably due to the content of polyphenols of the treatment; the use of heat-shock reduced this negative effect. GT and heat-shock combined also showed negative effects, reducing the antioxidant content (ascorbic acid and carotenoids). No significant differences were observed between chlorine and optimal GT (0.25 g 100 mL− 1 at 20 °C) in browning appearance and sensory properties. GT better kept the antioxidant activity of the samples than chlorine.
Industrial relevance: An alternative treatment for minimally processed Iceberg lettuce is tested, based on its antioxidant capacity. Minimally processed industry is constantly looking for new treatments to avoid the use of chlorine which is a standard at the moment.

1. Introduction

Green tea (Camellia sinensis L.) is a popular beverage and it has been reported to exert beneficial effects on several life-style related diseases: chemopreventive, anticarcinogenic, antiatherogenic, antioxidant and antimicrobial activities (Si et al., 2006; McKay & Blumberg, 2002; Rietveld & Wiseman, 2003; Cooper, Morre & Morre, 2005). The ability to inhibit food-borne pathogens such as Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Staphylococcus aureus, and Campylobacter jejuni has been reported for different varieties of tea or tea extracts, including Oolong, Jasmine and Black (Si et al., 2006; Hamilton-Miller, 1995; Hamilton-Miller & Shah, 2005; Yeo, Ahn, Lee, Lee, Park & Kim, 1995).

However, results showing antimicrobial activity of tea extract components against bacterial pathogens have been inconsistent due to the variability of the methods used (e.g. different varieties of tea, different processing and extraction procedures) and the mechanisms of action described in the literature remain controversial (Si et al., 2006).

Green tea is an excellent source of polyphenols, which are natural antioxidants that can be used as alternatives to synthetic antioxidants as they are typically less harmful than synthetic ones and appears to have an equivalent effect upon the inhibition of oxidation (Cao, Sofic & Prior, 1996). These antioxidants, which inhibit the oxidation of organic molecules, are very important, not only for living systems and their defence against oxidative stress but also for food preservation (Masuda et al., 2003). Phenolic antioxidants interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, with the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes (Kaur & Kapoor, 2001).

Fresh-cut vegetable industry (~ 10% p.a.) has undergone an important growth due to the increasing demand for fresh, healthy and convenient foods. A substantial portion of vitamins and minerals in the diet comes from fruit and vegetable consumption (Klein, 1987). There is mounting evidence to support the alleviation of many degenerative diseases including cardiovascular disease, cancer and ageing by the consumption of fruit and vegetables (Kaur & Kapoor, 2001). These beneficial health effects of fruit and vegetables have been attributed to the presence of antioxidants that act as receptors of free radicals. Ascorbic acid and β-carotene are the antioxidants present in the greatest quantities in fruit and vegetables and usually are destroyed after processing or decontamination procedures.

Consumers have also become more critical to the use of artificial additives in order to preserve food or enhance characteristics such as colour, flavour and nutritional value (Bruhn, 2000). Ohlsson (1994) suggests that minimal processing techniques have emerged to meet the challenge of replacing traditional methods of preservation while retaining nutritional and sensory quality. RTU vegetables typically involve peeling, slicing, dicing or shredding prior to packaging and storage (Barry-Ryan & O'Beirne, 1999). The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as fruit or vegetables that have been trimmed and/or peeled and/or cut into

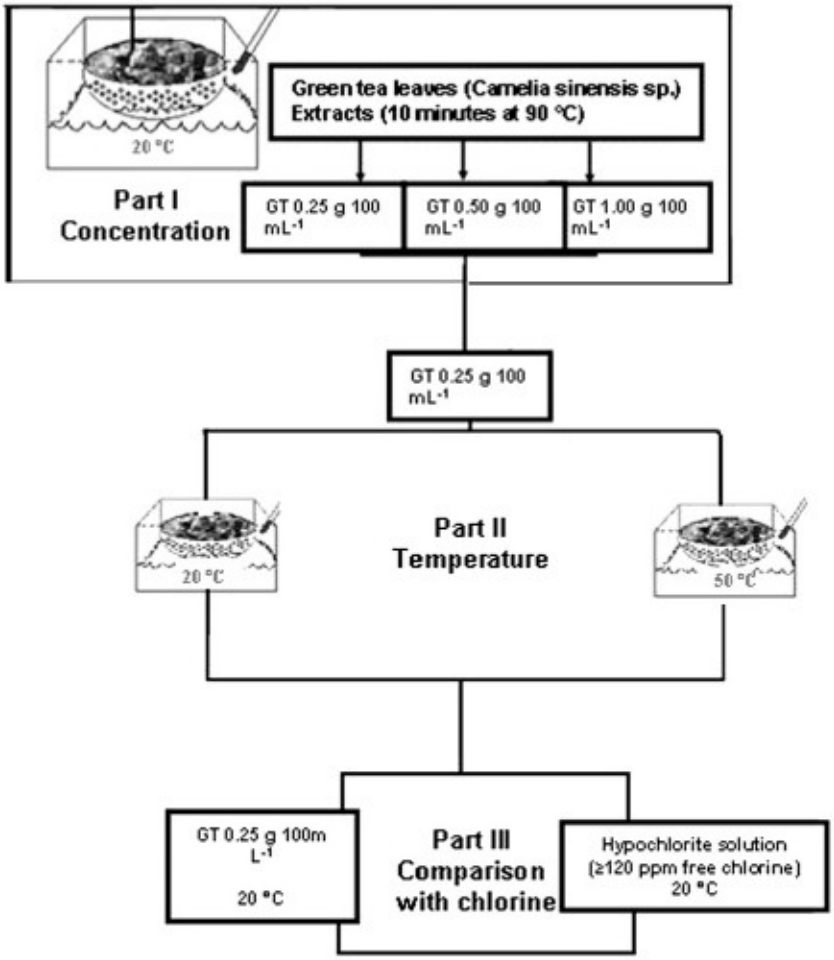

Fig. 1. Experimental design for the testing of Green tea extracts treatments on fresh-cut lettuce.

100% usable product that is bagged or pre-packaged to offer consumers high nutrition, convenience, and flavour while still maintaining its freshness (Lamikanra, 2002).

It is well-known that processing of vegetables promotes a faster physiological deterioration, biochemical changes and microbial degradation of the product even when only slight processing operations can be used (O'Beirne & Francis, 2003), which may result in degradation of the colour, texture and flavour (Kabir, 1994; Varoquaux & Wiley, 1994). While conventional food-processing methods extend the shelf-life of fruit and vegetables, the minimal processing to which fresh-cut fruit and vegetables are subjected renders products highly perishable, requiring chilled storage to ensure a reasonable shelf-life (Garcia & Barret, 2002).

The vast majority of fresh minimally processed produce manufacturers use chlorine in washing and decontamination procedures (Seymour, 1999). There is controversy about the formation of carcinogenic chlorinated compounds in water (chloramines and trihalomethanes), calling into question the use of chlorine (Wei et al., 1995).

The current concern associated with chlorine outlines the need to find new alternatives for preservation of minimally processed vegetables. Traditional and novel technologies for maintaining quality while inhibiting undesired microbial growth are being investigated, driven by an increasing demand from the industry and consumers. These include use of temperature blanching/steamer (Abreu, Beirão-da-Costa, Gonçalves, Beirão-da-Costa & Moldão-Martins, 2003; Martín-Diana et al., 2007a; Rico et al., 2008a,b); short thermal shocks (Loaiza-Velarde, Mangrich, Campos-Vargas, & Saltveit, 2003; Martín-Diana et al., 2005a; Koukounaras, Diamantidis & Sfakiotakis, 2008); organic acids (Rosen & Kader, 1989; Agar, Massantini, Hess-Pierce, & Kader, 1999; Luna-Guzmán & Barrett, 2000; Martín-Diana et al., 2005b; Martín-Diana et al., 2007b), irradiation (Allende & Artes, 2003a; Han et al., 2004), modified atmosphere packaging (Kato-Noguchi & Watada, 1997, Allende & Artes, 2003b), ozone (García, Mount & Davidson, 2003; Zhang, Zhaoxin, Zhifang & Xiang, 2005; Rico, Martín-Diana, Frías, Henehan & Barry-Ryan, 2006), electrolysed water (Izumi, 1999; Koseki, Yoshida, Kamitani, Isobe & Itoh, 2004; Park, Hung, Doyle, Ezeike & Kim, 2001; Rico et al., 2008a,b), electric pulses (Giner et al., 2000), edible coating (Molnar-Perl & Friedman, 1990; Guilbert, Gontard, & Gorris, 1996; Son, Moon, & Lee, 2001) among others.

During the last years a special renewed interest in the use of natural products as decontaminants has been noticed. Research and commercial applications have shown that natural antimicrobials could replace traditional sanitizing agents (Graham,1997; Cherry,1999; Martín-Diana, Ricoet al., 2006). Green tea has been used to extend the shelf-life of dry-fermented sausage (Bozkurt, 2006), enrich candy jellies (Gramza-Michalowska & Regula, 2007) and cooked pork patties (Nissen, Byrne, Bertelsen & Skibsted, 2004). However, only a few studies can be found on the use of Green tea in fresh-cut fruits or vegetables.

Due to the lack of information regarding the use of Green tea in minimally processed vegetables, the objective of this research was to evaluate its ability to extend the shelf-life of fresh-cut lettuce. Quality markers, mainly indicators of respiration, browning, sensory and nutritional parameters were used to optimise the Green tea extract treatment conditions (concentration and temperature) and to compare its effect on fresh-cut lettuce quality with chlorine (standard treatment).

2. Materials and methods

2.1. Sampling and experimental design

The experiments were carried out between December 2004 and June 2005. All procedures were performed in a food-processing room at 20 °C. Each batch consisted of ~ 18–25 kg (~ 55–65 heads of lettuce of ~ 450 g each). For colour and texture analyses all pieces in the assigned bag (20 to 25 approx.) were analysed individually to minimise error due to product variability. For sensory analysis, samples from the whole storage bag were analysed. For destructive analysis (enzymes, ascorbic acid, carotenoids and pH) samples were taken from a pool of all the pieces in a storage bag.

The experimental design was divided in to three parts (Fig. 1): two-step optimisation of Green tea extract (GT) treatment conditions to extend the quality and nutritional value of fresh-cut lettuce (Parts I and II) and comparison of GT and chlorine treatments (Part III).

Preliminary sensory studies using GT concentrations from 0 to 5 g 100 mL^{-1} vs. 120 ppm chlorine as control were carried out in fresh-cut lettuce. Sensory results showed that it was necessary to use at least concentrations of 0.25 g 100 mL^{-1} and a maximum of 1 g 100 mL^{-1} to obtain samples considered as acceptable during the shelf-life (10 days).

The optimisation of GT concentration (Part I) was done using concentrations (0.25, 0.5 and 1.0 g 100 mL^{-1}). Part II consisted of a comparative analysis using GT treatments at a concentration obtained from the optimisation step (Part I) at two temperatures: 20 and 50 °C. The temperatures were selected to analyse the combined effect of heat-shock and GT. The two temperatures were selected based on previous research (Martin-Diana et al., 2005a,b,c). The last part of the experiment (Part III) was carried out in order to compare the optimal GT conditions obtained in the two previous steps (Part I and II) with an industrial standard treatment.

2.2. Raw material

Iceberg lettuce (Lactuca sativa sp.) was grown in Ireland and purchased from a local supplier. The lettuce was brought to the laboratory within 24 h after harvest and stored in cardboard boxes, in bulk and unwrapped at 4 °C until processing.

2.3. Processing (washing and packaging procedure)

Lettuce was prepared according to safety statements and recommendations (Beuchat & Ryu, 1997; Beuchat, 1998). The two outer leaves were removed and the heads cored with a stainless steel knife. The lettuce was cut in halves and each half was further cut into four pieces.

Washing treatments consisted of immersion of the fresh-cut lettuce in the treatment solution. Dried and ground Green tea (C. sinensis L.) leaves

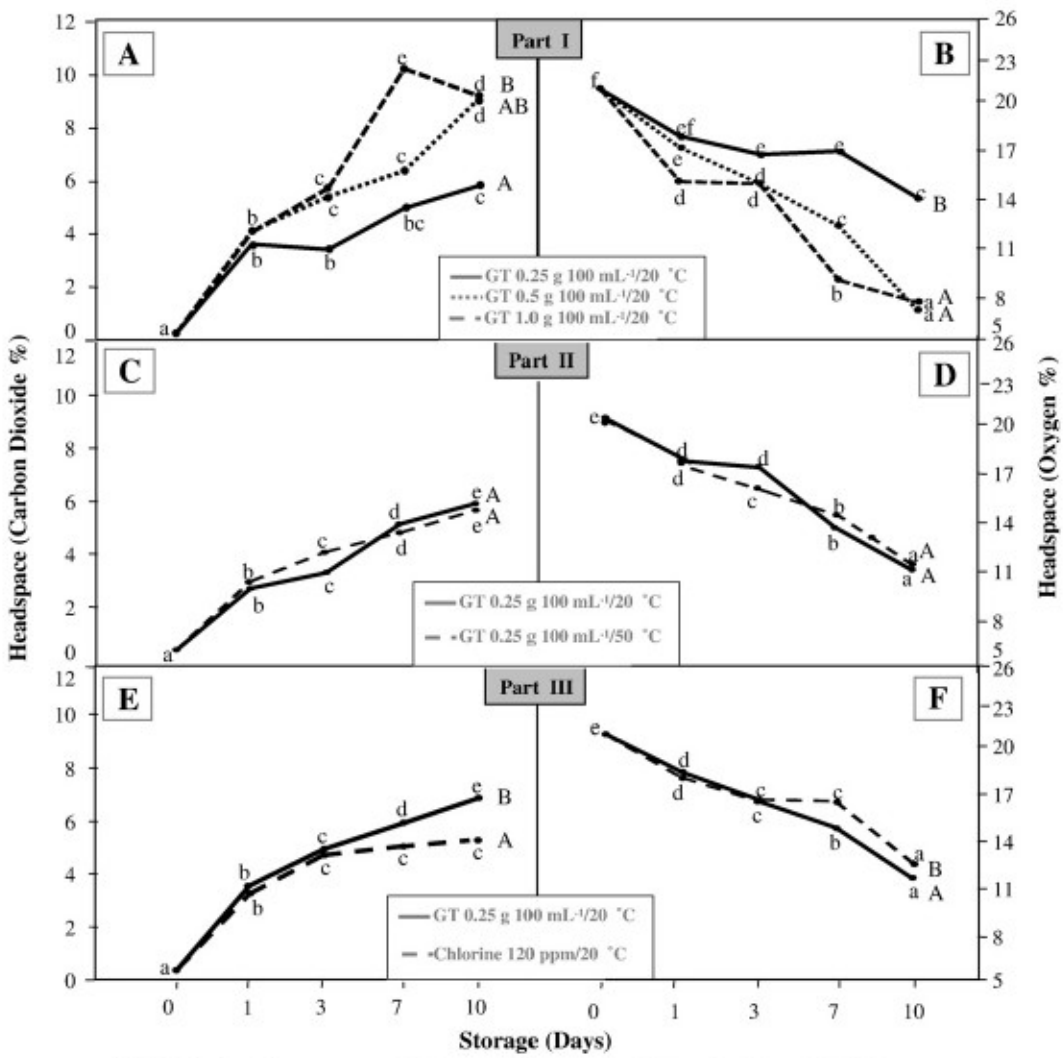

Fig. 2. Changes in gas composition (carbon dioxide (A,C,E) and oxygen (B,D,F)) of package headspace of fresh-cut lettuce treated with GT and stored at 4 °C over 10 days. Part I: GT at different concentrations (0.25, 0.5 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

were brewed for 5 min in distilled water at 90 °C at different concentrations (0.25 g 100 mL^{-1}, 0.5 g 100 mL^{-1} and 1 g 100 mL^{-1} with a content in total phenolics respectively of 350, 700 and 1260 mg of gallic acid per litre of infusion). The obtained extracts were filtrated two times trough a Whatman No. 1 filter paper. Chlorinated water was prepared by adding sodium hypochlorite (≥120 ppm available chlorine) to distilled water to obtain a 120 ppm free chlorine solution (pH ~8). Free chlorine concentration in the washing solution was determined with the DPD method, using a DREl/2000 spectrometer with appropriate AccuVac® pillows (Hach Company, Loveland, Colorado, USA).

For all treatments the solutions were prepared using distilled water stored at selected temperatures according to the experimental design. The cut produce was placed in a basket and immersed with agitation in the solution (~100 g vegetable product per litre) for 1 min and subsequently dried for 5 min using an automatic salad spinner.

To minimise product heterogeneity, processed vegetables were pooled, mixed and subsequently packaged in bags (200 × 320 mm) of oriented polypropylene (Amcor Flexibles Europe-Brighouse, United Kingdom). Film permeabilities to oxygen and carbon dioxide were ~ 12,000 and ~ 13,000 (mL m^{-2} day^{-1} atm^{-1} at 5 °C) respectively. Each package contained ~100 g of the product. The packages were chilled in a blast freezer set at 0 °C for 2 min before being sealed to avoid water condensation during storage (10 days at 4 °C).

2.4. Shelf-life analysis

The objective was to study the effect of GT on the quality of fresh-cut lettuce. Respiration, pH, browning appearance (browning-related enzymes, colour and potential browning) and nutritional changes (ascorbic acid and carotenoids) were evaluated as indicators of shelf-life. Shelf-life is defined as the length of time at which the vegetable can maintain the appearance, safety and antioxidant content that appeals to the consumer (Delaquis, Stewart, Toivonen & Moyls, 1999), therefore markers were monitored throughout the storage (10 days).

2.4.1. Headspace analysis

A Gaspace analyser (Systech Instruments, UK) was used to measure levels of CO_2 and O_2 during storage. Gas extractions were performed with a hypodermic needle, inserted through an adhesive septum previously fixed to the bags, at a flow rate of 30 mL/min for 10 s. The accuracy is in percentage of readings: ±0.5% below 10% and ±0.1% above 10% for O_2; ±0.1% below 1%, ±0.2% between 1% and 10% and ±2% above 10% for CO_2. Three bags per treatment and batch were monitored throughout the storage.

2.4.2. pH. Ten grams 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 an Orion research pH-meter.

2.4.3. Browning

2.4.3.1. Colour measurement. Colour was quantified using a colorimeter (Minolta, Model CM-3600d, UK). A lettuce piece was place directly on the colorimeter sensor (3.5 cm of diameter) and measured; 20–30 measurements were taken per treatment and day. The instrument was calibrated with a white tile standard (L* =93.97, a* =−0.88 and b*= 1.21) and a green tile standard (L8 =56.23, a* =−21.85, b* =8.31) under D_{65} luminosity conditions. The a* parameter measures the degree of red (+a) or green (−a*) colour and the b* parameter measures the degree of yellow (+b) or blue (−b*) colour.

2.4.3.2. Potential browning. Potential browning was measured according to the methodology of Viña and Chaves (2006). Ten grams of tissue frozen and crushed was treated with ethanol for 60 min and then centrifuged at 6000 rpm at 10 °C for 10 min, retaining the supernatants. After a further amount of ethanol was added to bring the final volume to 25 mL. Absorbance at 320 nm was measured on aliquots of these extracts. The results were expressed as absorbance units (AU) g^{-1} fresh tissue.

2.4.3.3. Browning-related enzymes (peroxidase (POD) E.C.1.11.1.7 and polyphenol oxidase (PPO) E.C.1.10.3.1.). Both enzymes were assayed in homogenates that were prepared as follows: 10 g of lettuce was placed in a polytron homogeniser (Polytron model PT 3000) in a 1:2 (w:v) ratio with 0.5 M phosphate buffer pH 6.5 containing 50 g L^{-1} polyvinylpyrrolidone. Homogenisation was carried out two times at 4 °C, and 5500 rpm, for 1 min each time with a break of 3 min between homogenisations in order to avoid excessive heating of the sample. The homogenate was then centrifuged at 12,720 ×g for 30 min at 4 °C. It was filtered through one layer of crepe bandage. The resulting crude extract was used without further purification. All the extracts were stored at 4 4 °C in the dark and used immediately.

POD activity was assayed spectrophotometrically. The reaction mixture contained 0.2 mL of extract and 2.7 mL of 0.05 M phosphate buffer pH 6.5 containing 100 µL of hydrogen peroxide (1 mL/L v/v) as an oxidant and 200 µL of p-phenylendiamine as hydrogen donor. The oxidation of p-phenylendiamine was monitored at 485 nm and 25 25 °C. An enzyme activity unit was defined as an increment of 0.1 in absorbance per minute. PPO activity was assayed spectrophotometrically by a modified method based on Galeazzi, Sgarbieri and Constantinides (1981) and Tan and Harris (1995). The reaction mixture contained 0.1 mL crude extract and 2.9 mL substrate solution (0.020 mol/L catechol in 0.05 mol/L phosphate buffer, pH 6.5). The rate of catechol oxidation was followed at 400 nm for 2 min at 25 °C. An enzyme activity unit was defined as an increase of 0.1 in absorbance per minute. Three independent trials were carried out. All the enzymatic measurements were carried out in duplicate.

2.4.4. Sensory analysis. Analytical-descriptive tests were used to evaluate the sensory quality attributes of fresh-cut lettuce prepared by different washing treatments. The panel, which consisted of 10 judges aged 20–30 years with sensory evaluation experience, were trained in discriminate evaluation of fresh-cut tissue. Panellists were required to score changes in total browning, photosynthetic browning, vascular browning and fresh appearance. Before the start of the sensory experiments, panel members were familiarised with the product and scoring methods. This consisted of 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. The same packages were scored during the entire test for sensory analysis (10 days), due to the high variability of the product. During this stage, the samples were presented to the panel to evaluate and measure the reproducibility of the judge's answers and their capability in discriminating among samples. During the analyses, samples were presented in randomized order to minimise possible carry-over effects.

Fresh-cut lettuce was evaluated for appearance using a ten point numerical rating scale, where: (I) General Browning, where 1 = nothing, 5 = moderate and 10 = a lot (severe browning); (II) Photosynthetic Browning, where 1 = nothing, 5 = moderate and 10 = a lot (severe browning); (III) Vascular Browning, where 1 = nothing, 5 = moderate and 10 = a lot (severe browning); (IV) Fresh appearance, where 1 = poor/no fresh appearance, 5 = moderate and 10 = very good/fresh appearance.

The results of the sensory analysis were reported as means of three separate trials. Data analysis was collected using Compusense® Five software (Release 4.4, Ontario, Canada).

2.4.5. Nutritional markers

2.4.5.1. 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. grams of fresh-cut was homogenised with 50 mL of acetic–methaphosphoric acid at low speed –(in order to avoid foaming) for

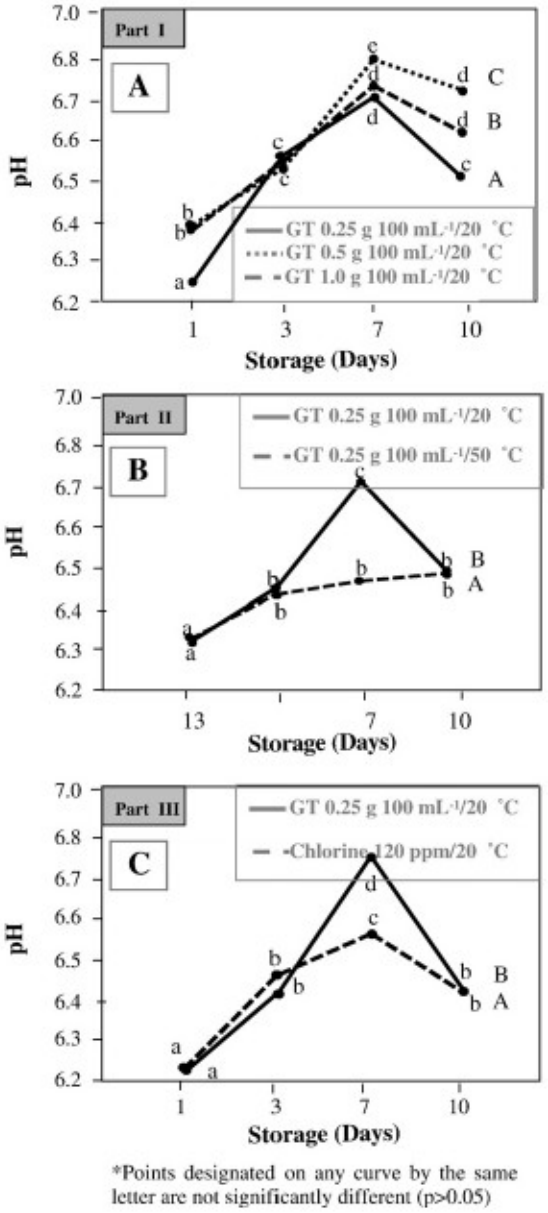

Fig. 3. Changes in pH of fresh-cut lettuce stored at 4 °C for 10 days. Part I: GT at different concentrations (0.25, 05 and 1 m 100 mL-1 at 20 °C). Part II: 0.25 g 100 L-1 GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL-1 GTE at 20 °C and 120 ppm chlorine at 20 °C.

3 min at 4 °C in darkness. –The homogenate was filtered and aceticmethaphosphoric added up to 100 mL. Aliquots of 10 mL were titrated rapidly in triplicate, using aceticmethaphosphoric acid as a blank. The results were expressed as mg ascorbic acid in g of sample.

$$\text{mg ascorbic acid/g sample} = (X - B)^*(F/E)^*(V/Y)$$

where X is mL 2,6,-dichlorolindophenol (DCPIP) used to obtain a permanent pink colour which persists more than 5 s, B is the DCPIP volume used for the control (blank), F* is the DCPIP factor (F□ = 2 mg ascorbic acid (standard) mL-1 DCPIP), E is g of sample (20 g), V is the final volume prepared (100 mL) and Y is mL titrated (10 mL).

2.4.5.2. Total carotenoids. Total carotenoids were extracted in the dark by homogenising lettuce tissue (5 g) with 30 mL of an acetone/ethanol (50:50 v:v) solution, containing 200 mg L-1 of butylated hydroxytoluene (BHT). The homogenate was filtered under suction in a buchner funnel and washed with acetone/ethanol solvent until colourless. Filtrate was adjusted to 100 mL volume with acetone/ethanol. An aliquot was placed in a 1 cm cuvette and its absorbance was measured at 470 nm. Total carotenoids (mg/g of sample) were calculated as described by Gross (1991).

2.5. Statistical analysis

Analysis of ANOVA (Multifactor and one-way) was used to find differences between treatments, storage and interaction of both factors for each one of the variables studied. Means were compared by significant difference (LSD) test, at a significance level p = 0.05 using the Statgraphics software (version 2.1; Statistical Graphics Co., Rockville, USA). Three independent trials were carried out.

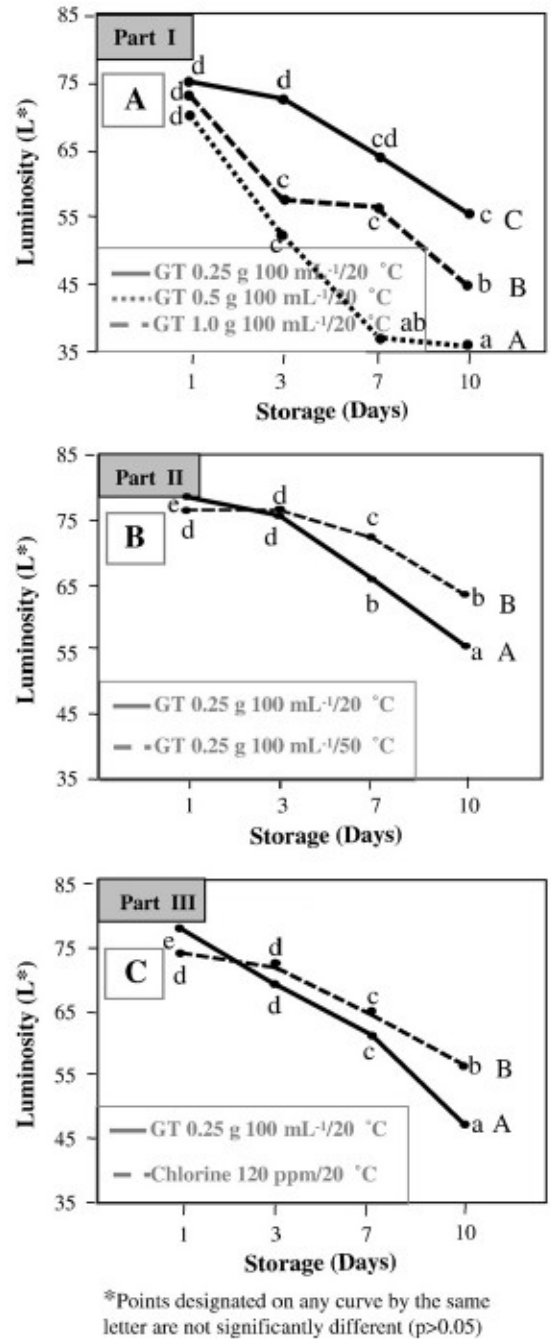

Fig. 4. Changes in luminosity (CIE L□a□b□ parameters) in fresh-cut lettuce stored at 4 °C for 10 days. Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL-1 at 20 °C). Part II: 0.25 g 100 mL-1 GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL-1 GT at 20 °C and 120 ppm chlorine at 20 °C.

3. Results and discussion

3.1. Headspace

The package headspace was monitored over 10 days of storage. Carbon dioxide and oxygen content, starting at atmospheric values, significantly ($p < 0.05$) changed regardless of the treatment. GT concentration (Fig. 2A,B) affected the respiration rate of the samples. Significantly (p b 0.05) lower oxygen and higher carbon dioxide levels were observed in samples treated with GT concentrations (0.5 and 1.0 g L^{-1}) compared to those treated with the lowest concentration (0.25 g L^{-1}). Higher GT concentrations produced an increase in the respiration rate, maybe related to a higher stress caused by the more concentrated treatments (Surjadinata & Cisneros-Zevallos, 2003). As indicated by Saltveit (2001) for fresh produce subjected to heat-shock for less than 2 min, treatment temperature (Fig. 2C,D) did not show any effect on the oxygen and carbon dioxide concentrations, results in agreement with previous work of the authors (Martin-Diana et al., 2005b). Headspace composition was significantly ($p < 0.05$) different in samples treated with the optimised GT treatment (0.25 g 100 m L^{-1}, 20 °C) and those treated with chlorine (Fig. 2E,F). These indicated that the GT treatment increased respiration of the produce in comparison with chlorine, probably indicating a higher stress caused by the GT treatment.

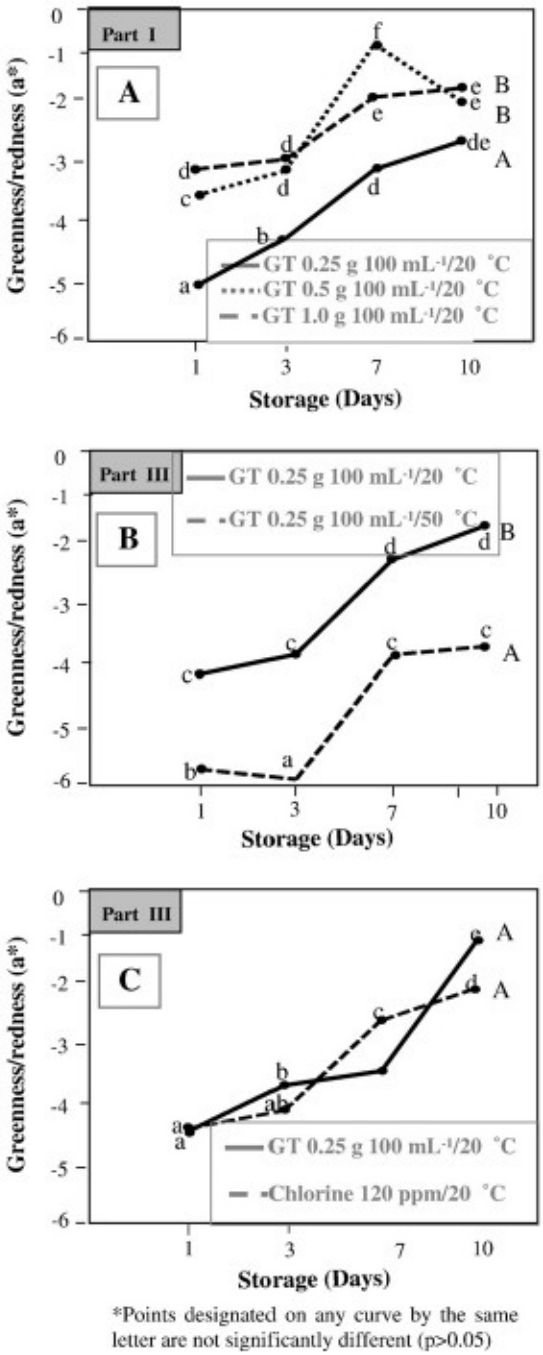

Fig. 5. Changes in a□ (CIE L□a□b□ parameters) in fresh-cut lettuce stored at 4 °C for 10 days. Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

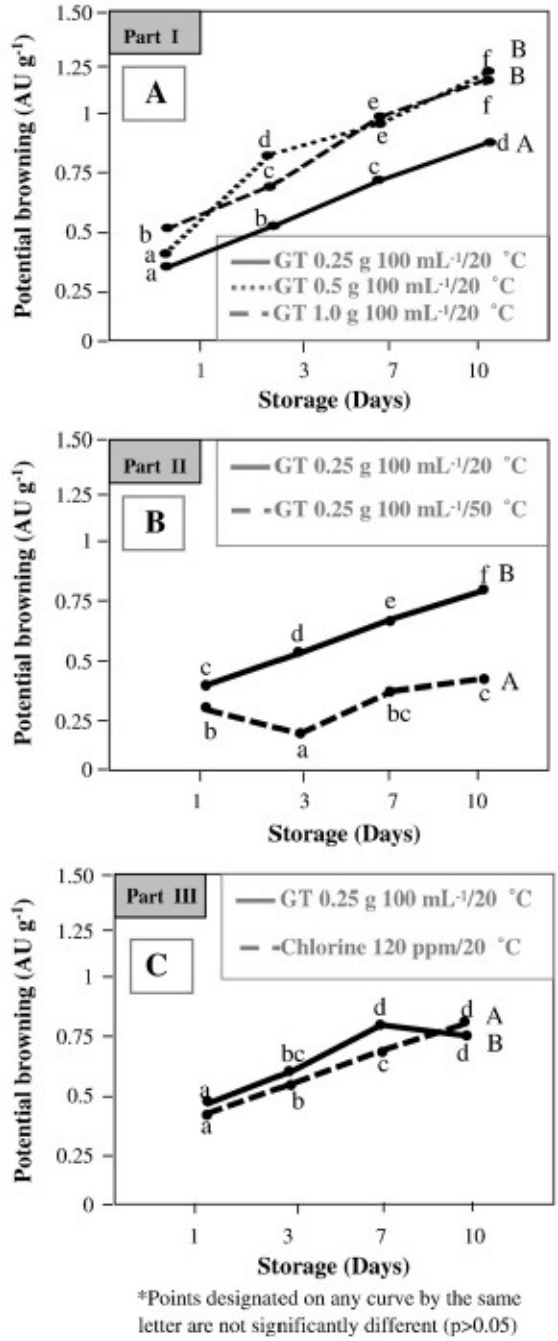

Fig. 6. Changes in potential browning (AU g^{-1}) in fresh-cut lettuce stored at 4 °C for 10 days. Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

In all cases, the levels of oxygen were above 6%, avoiding hypoxic atmosphere conditions which favour the fermentative processes that cause the formation of acetaldehyde and the appearance of off-flavours compounds (Kays, 1991; Beaudry, 1999). In general, the highest respiration rates were observed in samples treated with the intermediate and high concentrations (0.5 and 1.0 g 100 mL^{-1}) GT, indicating a possible negative effect of increased stress with the use of an excessively high concentration of Green tea.

3.2. pH

Changes in pH were monitored in all the samples through the 10-day storage (Fig. 3, Part I, Part II and Part III). A significant increase in pH was observed for all the samples, with a peak at day 7 followed by a decrease at day 10. Different authors (King & Bolin, 1989; Jacxsens, Devlieghere, Ragaert, Vanneste & Debevere, 2003; Gomez-Lopez, Devlieghere, Bonduelle & Debevere, 2005) have explained this pH increase with the growth of gram-negative microorganisms, which play an important role in the spoilage of fresh-cut lettuce. Lower GT concentrations kept significantly ($p < 0.05$) lower pH values during the storage than higher concentrations (Fig. 3A). The lower pH values at day 10 could respond to an increase in lactic acid bacteria. The samples treated with GT and heat-shock maintained significantly lower produce pH, with no peak observed at day 7 (Fig. 3B). This could be due to the inhibitory effect of temperature on bacterial growth. Delaquis et al. (1999) described a reduction in bacterial counts in lettuce treated with warm water. Similar behaviour was observed in samples treated with chlorine compared with those treated with GT (Fig. 3C).

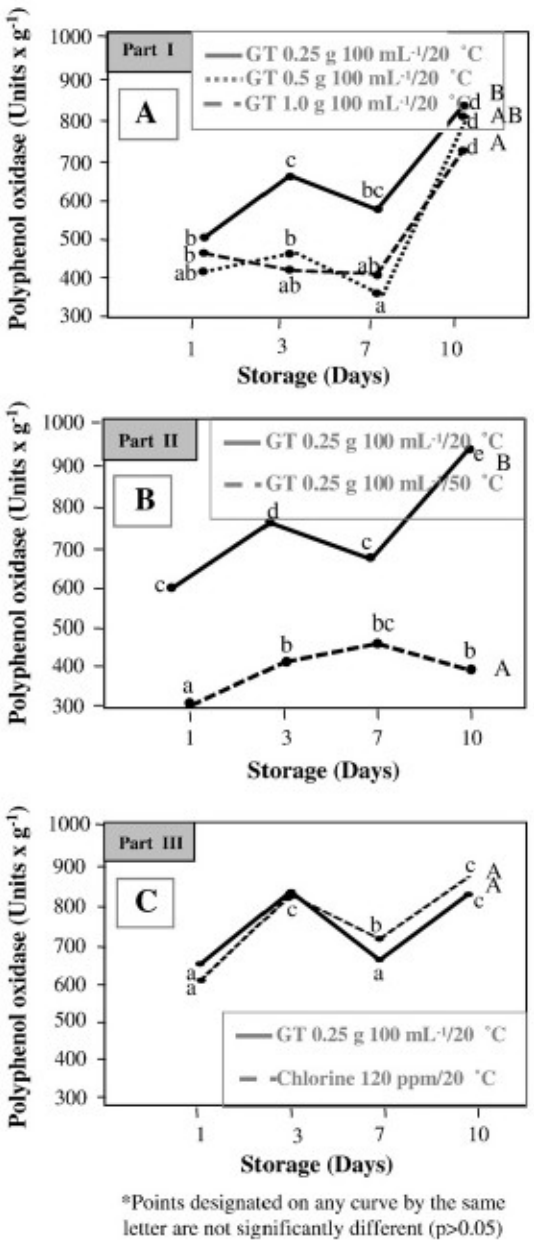

Fig. 7. Changes in polyphenol oxidase enzymatic activity (PPO, units g^{-1}) in fresh-cut lettuce stored at 4 °C for 10 days. Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

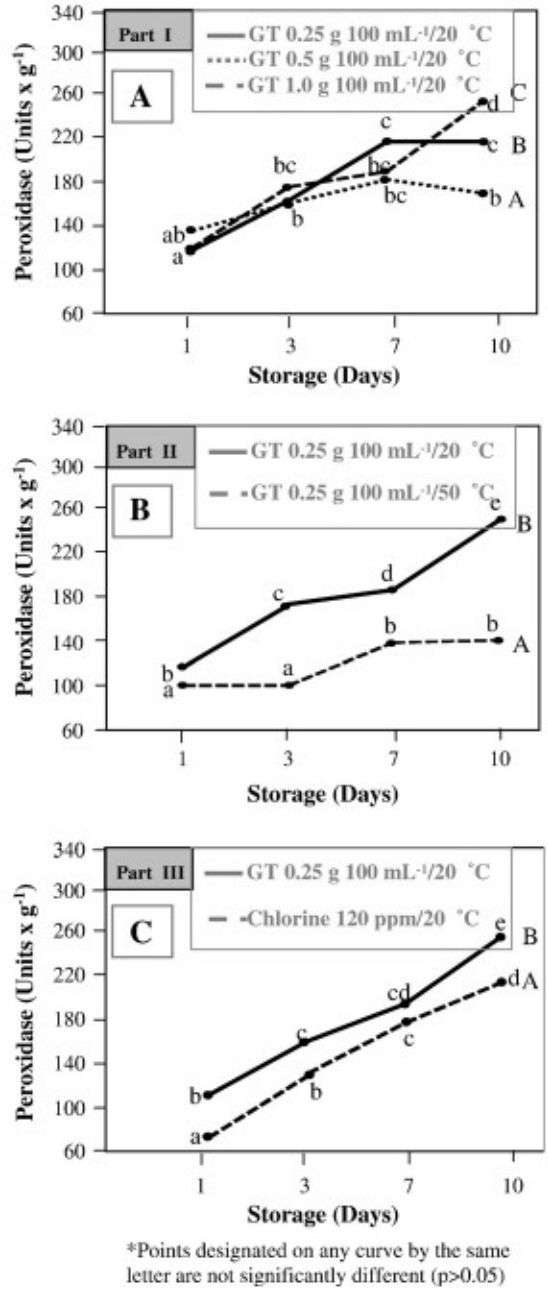

Fig. 8. Changes in peroxidase enzymatic activity (POD, units g^{-1}) in fresh-cut lettuce stored at 4 °C for 10 days. Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

Table 1
Sensory evaluation of fresh-cut lettuce using a rating score (1–10) in fresh-cut lettuce stored at 4 °C for 10 days

Sensory analysis						
Part I	Storage (days)	Treatment	General Browning	Photosynthetic Browning	Vascular Browning	Fresh appearance
ANOVA treatments			GT 0.25/20 °C A	GT 0.25/20 °C A	GT 0.25/20 °C A	GT 0.25/20 °C A
			GT 0.5/20 °C B	GT 0.5/20 °C B	GT 0.5/20 °C B	GT 0.5/20 °C B
			GT 1.0/20 °C C	GT 1.0/20 °C C	GT 1.0/20 °C C	GT 1.0/20 °C C
	1	GT 0.25/20 °C	1.90 ± 0.55a	0.90 ± 0.14a	0.95 ± 0.37a	7.30 ± 0.45f
		GT 0.5/20 °C	3.05 ± 0.70b	1.40 ± 0.29b	2.11 ± 0.69b	4.14 ± 0.47d
		GT 1.0/20 °C	4.20 ± 0.20c	5.75 ± 0.67d	4.00 ± 0.56d	2.36 ± 0.55b
		GT 0.25/20 °C	3.65 ± 0.22b	2.30 ± 0.67b	3.14 ± 0.59c	4.80 ± 0.27d
	3	GT 0.5/20 °C	5.55 ± 2.08c	3.61 ± 0.90c	4.95 ± 0.84d	3.66 ± 0.52c
		GT 1.0/20 °C	8.00 ± 0.72d	7.58 ± 0.10e	7.21 ± 0.62e	1.38 ± 0.48a
		GT 0.25/20 °C	4.46 ± 0.39c	3.38 ± 0.91c	3.32 ± 2.06c	5.50 ± 0.35e
		GT 0.5/20 °C	8.70 ± 0.98d	8.28 ± 0.70g	8.86 ± 0.92f	0.98 ± 0.13a
	7	GT 1.0/20 °C	8.80 ± 0.91d	7.72 ± 0.40f	8.55 ± 0.94f	2.30 ± 0.52b
		GT 0.25/20 °C	7.66 ± 0.50d	4.90 ± 0.89d	4.70 ± 0.66d	5.80 ± 0.27e
		GT 0.5/20 °C	8.90 ± 0.82d	7.60 ± 0.66f	8.61 ± 0.99f	1.38 ± 0.21a
		GT 1.0/20 °C	8.50 ± 0.55d	7.60 ± 0.65f	6.71 ± 0.97f	1.17 ± 0.64a
	10					
Part II	Storage (days)	Treatment	General Browning	Photosynthetic Browning	Vascular Browning	Fresh appearance
ANOVA treatments			GT 0.25/20 °C B	GT 0.25/20 °C B	GT 0.25/20 °C A	GT 0.25/20 °C A
			GT 0.25/50 °C A	GT 0.25/50 °C A	GT 0.25/50 °C B	GT 0.25/50 °C B
	1	GT 0.25/20 °C	1.90 ± 0.55ab	0.90 ± 0.14a	0.95 ± 0.37a	7.30 ± 0.45c
		GT 0.25/50 °C	1.00 ± 0.41a	0.69 ± 0.47a	0.87 ± 0.48a	8.86 ± 0.72e
		GT 0.25/20 °C	3.65 ± 0.22c	2.30 ± 0.37c	3.14 ± 0.58d	4.80 ± 0.27a
	3	GT 0.25/50 °C	1.12 ± 0.25a	1.62 ± 0.48b	1.87 ± 0.25b	8.31 ± 0.19d
		GT 0.22/20 °C	4.46 ± 0.38d	3.38 ± 0.61c	3.32 ± 0.96d	5.50 ± 0.35b
		GT 0.25/50 °C	2.30 ± 0.76 b	2.50 ± 0.51c	2.80 ± 0.27c	7.30 ± 0.67c
	7	GT 0.25/20 °C	7.66 ± 0.50e	4.90 ± 0.49e	4.70 ± 0.25e	5.80 ± 0.27b
		GT 0.25/50 °C	3.62 ± 0.63c	3.12 ± 0.25d	3.25 ± 0.29d	6.50 ± 0.41b
	10					
Part III	Storage (days)	Treatment	General Browning	Photosynthetic Browning	Vascular Browning	Fresh appearance
ANOVA treatments			GT 0.25/20 °C A	GT 0.25/20 °C A	GT 0.25/20 °C A	GT 0.25/20 °C A
			Chlorine/20 °C A	Chlorine/20 °C A	Chlorine/20 °C B	Chlorine/20 °C A
	1	GT 0.25/20 °C	1.95 ± 0.27a	0.95 ± 0.11a	0.85 ± 0.42a	7.35 ± 0.65d
		Chlorine/20 °C	2.02 ± 0.25a	0.87 ± 0.14a	0.94 ± 0.12a	7.25 ± 0.50d
		GT 0.25/20 °C	3.50 ± 0.45b	3.20 ± 0.57b	4.04 ± 0.50c	5.40 ± 0.12c
	3	Chlorine/20 °C	3.65 ± 0.22b	2.30 ± 0.67b	3.14 ± 0.58b	4.80 ± 0.27b
		GT 0.25/20 °C	4.40 ± 0.19c	3.85 ± 0.24c	5.30 ± 0.54d	5.55 ± 0.77c
		Chlorine/20 °C	4.46 ± 0.38c	3.38 ± 0.11c	3.32 ± 0.26b	5.50 ± 0.35c
	7	GT 0.25/20 °C	7.50 ± 0.42d	3.95 ± 0.37c	5.55 ± 0.37d	5.10 ± 0.59c
		Chlorine/20 °C	7.66 ± 0.50d	4.40 ± 0.39d	4.70 ± 0.25c	5.80 ± 0.27c
	10					

Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

3.3. Browning

3.3.1. Colour measurement

Colour was monitored in all the samples during the entire storage (Figs. 4 and 5). Luminosity values (L*) decreased during storage (Fig. 4A), which can be related to the appearance of browning. The concentration of the treatment (Part I) significantly affected luminosity and increasing GT concentration decreased luminosity values. This might indicate higher browning levels in the lettuce treated with higher GT concentrations. Increasing the temperature of the treatment from 20 °C to 50 °C (Part II) resulted in higher luminosity values of the samples (Part II, Fig. 4B), probably due to the inhibitory effect of heat-shock on enzymes involved in the enzymatic browning (Saltveit, 2001). Compared with a control treatment (chlorine), GT samples showed lower values in luminosity during the storage (Part III, Fig. 4C). The amount of polyphenols present in the Green tea might have favoured a quicker appearance of browning. Another colour parameter related to browning (Castaner, Gil, Ruiz & Artes, 1999) and to the breakdown of chlorophyll (Bolin & Huxsoll, 1991) is a* value. A significant increase in a□, indicating a shift from greenness to redness, was observed in all the samples during storage (Fig. 5). GT concentration showed an effect from day 1 on this parameter, increasing a□ values with increasing concentration (Part I, Fig. 5A). As observed from luminosity results, heat-shock (50 °C) reduced the appearance of browning, which was reflected in lower a* values during storage (Part II, Fig. 5B). No difference between GT and chlorine samples was observed (Part III, Fig. 5C).

3.3.2. Potential browning

Potential browning was monitored in all the samples during storage (Fig. 6). A significant increase of potential browning during storage was observed ($p < 0.05$). This increase in the concentration of phenolic compounds is caused by the wounding stress from the treatment (Hanotel, Fleuriet & Boisseau, 1995).

Lower potential browning was found in samples treated with the lowest GT concentration (GT 0.25 g L^{-1}) compared with the other two concentrations (0.5 g 100 mL^{-1} and 1.0 g L^{-1}) (Part I, Fig. 6A). The combination of GT and heat-shock kept lower potential browning values than GT treatment at room temperature (Part II, Fig. 6B) those results are in agreement with findings from Murata, Tanaka, Minoura and Homma (2004) and Saltveit (2001). Samples treated with chlorine showed higher levels of potential browning than samples treated with

GT (Part III, Fig. 6C). GT is known for the high antioxidant capacity; however this resulted in a negative effect applied to a commodity affected by browning such as fresh-cut lettuce is, mainly due to the nature of this natural antioxidant. Green tea is rich in polyphenols, which act as substrate for browning-related enzymes.

3.3.3. Browning-related enzymes (peroxidase (POD) E.C.1.11.1.7 and polyphenol oxidase (PPO) E.C.1.10.3.1.)

Browning-related enzymes were measured as indicators of browning in all the samples after treatment and during the 10 days of storage (Fig. 7). A significant increase in PPO was observed during the storage. Samples treated with the highest concentrations of GT (0.05 and 1.0 g 100 mL^{-1}) showed lower levels of PPO activity (Part I, Fig. 7A) than samples treated with 0.25 g 100 mL^{-1} GT. These results contrast with those obtained for the other browning markers (Figs. 4A, 5A, 6A and Table 1), showing higher browning incidence in the samples treated with increasing concentrations of GT. Samples treated with the GT at 50 °C (heat-shock) showed significantly (p b 0.05) lower PPO activity than those treated with GT at 20 °C (Part II, Fig. 7B). The inhibition of PPO activity due to heat-shock treatment has been previously reported (Saltveit, 2001). No significant percentage of PPO or POD activity is lost due to the direct effect of the treatment (Martin-Diana et al., 2005c). The reduction in the activity of PPO and POD might be due to an indirect effect caused by feedback inhibition by the lack of phenolic compounds (substrate) or due to a direct effect on a receptor (unknown) implicated in the synthesis of PPO and POD. No differences between samples treated with chlorine and GT 0.25 g L^{-1} (Part III, Fig. 6C) were found.

The GT controlled significantly (p b 0.05) the POD activity (Fig. 8, Part A). Higher levels of GT inhibited better the POD than lower GT levels. The temperature increase produced an inhibitory effect on this enzyme when the treatment was combined with GT 0.25 g 100 mL^{-1} (Part II, Fig. 8B). Lower levels of POD activity were observed in samples treated with GT than in chlorinated samples (Part III, Fig. 8C). This inhibitory effect responds to the antioxidant capacity of the catechines, the main polyphenol compounds in GT, which can act as free radical-scavengers, quenching hydroxyl radicals (-OH) or superoxide anion radicals (O-) (Hanasaki, Ogawa & Fukui, 1994; Sichel,$_2$ Corsaro, Scalia, Bilio & Bonomo, 1991; Aoshima, & Ayabe, 2007). In contrast with PPO, the polyphenols are not specific subtracts of the POD enzyme.

Table 2
Changes in ascorbic acid (mg g^{-1}) in fresh-cut lettuce stored at 4 °C for 10 days

Part I	Storage (days)	Treatment	Ascorbic acid (mg g^{-1})
ANOVA treatments	GT 0.25/20 °C A	GT 0.5/20 °C B	GT 1.0/20 °C B
	1	GT 0.25/20 °C	0.07 ± 0.001d
		GT 0.5/20 °C	0.07 ± 0.006d
		GT 1.0/20 °C	0.8 ± 0.001e
		GT 0.25/20 °C	0.06 ± 0.002c
	3	GT 0.5/20 °C	0.06 ± 0.001c
		GT 1.0/20 °C	0.07 ± 0.001d
		GT 0.25/20 °C	0.05 ± 0.003b
		GT 0.5/20 °C	0.06 ± 0.001c
	7	GT 1.0/20 °C	0.06 ± 0.001c
		GT 0.25/20 °C	0.04 ± 0.002a
		GT 0.5/20 °C	0.05 ± 0.001b
		GT 1.0/20 °C	0.05 ± 0.001b
	10		
Part II	**Storage (days)**	**Treatment**	**Ascorbic acid (mg g^{-1})**
ANOVA treatments		GT 0.25/20 °C B	GT 0.20/50 °C A
	1	GT 0.25/20 °C	0.07 ± 0.001f
		GT 0.25/50 °C	0.02 ± 0.001b
		GT 0.25/20 °C	0.06 ± 0.002e
	3	GT 0.25/50 °C	0.02 ± 0.000b
		GT 0.25/20 °C	0.05 ± 0.003d
	7	GT 0.25/50 °C	0.02 ± 0.001b
		GT 0.25/20 °C	0.04 ± 0.002c
		GT 0.25/50 °C	0.01 ± 0.001a
	10		
Part III	**Storage (days)**	**Treatment**	**Ascorbic acid (mg g^{-1})**
ANOVA treatments		GT 0.25/20 °C B	Chlorine/20 °C A
	1	GT 0.25/20 °C	0.07 ± 0.001e
		Chlorine/20 °C	0.05 ± 0.000c
		GT 0.25/20 °C	0.06 ± 0.002d
	3	Chlorine/20 °C	0.04 ± 0.000b
		GT 0.25/20 °C	0.05 ± 0.003c
	7	Chlorine/20 °C	0.03 ± 0.002a
		GT 0.25/20 °C	0.04 ± 0.002b
		Chlorine/20 °C	0.03 ± 0.000a
	10		

Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

Table 3
Changes in carotenoids (mg g^{-1}) in fresh-cut lettuce stored at 4 °C for 10 days

Part I	Storage (days)	Treatment	Carotenoids (mg g^{-1})
ANOVA treatments	GT 0.25/20 °C A	GT 0.5/20 °C B	GT 1.0/20 °C B
	1	GT 0.25/20 °C	0.41 ± 0.11a
		GT 0.5/20 °C	0.99 ± 0.11c
		GT 1.0/20 °C	1.01 ± 0.13c
		GT 0.25/20 °C	0.47 ± 0.04a
	3	GT 0.5/20 °C	1.01 ± 0.17c
		GT 1.0/20 °C	0.97 ± 0.14c
		GT 0.25/20 °C	0.41 ± 0.11a
		GT 0.5/20 °C	0.70 ± 0.11b
	7	GT 1.0/20 °C	0.80 ± 0.13b
		GT 0.25/20 °C	0.49 ± 0.19a
		GT 0.5/20 °C	0.77 ± 0.07b
		GT 1.0/20 °C	0.68 ± 0.06b
	10		
Part II	**Storage (days)**	**Treatment**	**Carotenoids (mg g^{-1})**
ANOVA treatments		GT 0.25/20 °C B	GT 0.25/50 °C A
	1	GT 0.25/20 °C	0.41 ± 0.01c
		GT 0.25/50 °C	0.09 ± 0.02b
		GT 0.25/20 °C	0.47 ± 0.04c
	3	GT 0.25/50 °C	0.07 ± 0.02b
		GT 0.25/20 °C	0.41 ± 0.01c
	7	GT 0.25/50 °C	0.02 ± 0.00a
		GT 0.25/20 °C	0.49 ± 0.02c
		GT 0.25/50 °C	0.01 ± 0.01a
	10		
Part II	**Storage (days)**	**Treatment**	**Carotenoids (mg g^{-1})**
ANOVA treatments		GT 0.25/20 °C B	Chlorine/20 °C A
	1	GT 0.25/20 °C	0.41 ± 0.01d
		Chlorine/25 °C	0.31 ± 0.01b
		GT 0.25/20 °C	0.47 ± 0.04d
	3	Chlorine/25 °C	0.35 ± 0.03c
		GT 0.25/20 °C	0.41 ± 0.01d
	7	Chlorine/25 °C	0.36 ± 0.02c
		GT 0.25/20 °C	0.49 ± 0.01d
		Chlorine/25 °C	0.24 ± 0.04a
	10		

Part I: GT at different concentrations (0.25, 05 and 1 g 100 mL^{-1} at 20 °C). Part II: 0.25 g 100 mL^{-1} GT at 20 °C and 50 °C. Part III: 0.25 g 100 mL^{-1} GT at 20 °C and 120 ppm chlorine at 20 °C.

3.4. Sensory analysis

Browning in photosynthetic and vascular tissues, general levels of browning and fresh appearance of the samples were evaluated by the sensory panel. The panel found differences in browning between samples treated with different GT concentrations. Increasing GT concentrations produced more visual browning on the samples and differences between treatments were observed throughout the storage (Table 1, Part I). Scores for fresh appearance also showed the unsuitability of high GT concentrations (0.5 and 1.0 g 100 mL^{-1})

compared with 0.25 g 100 mL^{-1} GT. Samples treated with GT and heat-shock showed less browning (Table 1, Part II). No differences between chlorine and GT-treated samples were observed (Table 1, Part III). Fresh appearance of samples treated with GT at 0.25 g 100 mL^{-1} or chlorine received higher scores than samples treated with GT at higher concentrations, due mainly to the presence of browning.

3.5. Nutritional markers

3.5.1. Ascorbic acid

A significant (p b 0.05) decrease in ascorbic acid was observed during the storage. Increasing GT concentration showed a protective effect, avoiding ascorbic acid degradation (Table 2, Part I). This effect might be due to the antioxidant capacity of catechines; the aromatic rings with hydroxyl groups possess strong antioxidant properties (Peterson & Totlani, 2005). A significant ($p < 0.05$) decrease of the ascorbic acid content was observed after the treatment with heat-shock and GT (Table 2, Part II), probably due to the low stability of ascorbic acid at high temperatures (Nagy & Smooth, 1976; Dewanto, Wu, Adom & Liu, 2002). When small concentrations of GT (0.25 g 100 mL^{-1}) were compared with chlorine a larger protection effect was observed in the case of GT (Table 2, Part II).

3.5.2. Carotenoids

Similar behaviour as the ascorbic acid was observed for carotenoids (Table 3, Part I). Carotenoid concentration significantly (p b 0.05) dropped during the storage in all the treatments. The use of temperature (heat-shock) increased carotenoid degradation (Table 3, Part II). The use of GT (0.25 g 100 mL^{-1}) resulted in higher carotenoid levels, compared with the chlorine based treatment (Table 3, Part III).

4. Conclusion

The use of Green tea extracts, at the lowest concentration tested (0.25 g 100 mL^{-1}), is a feasible preservation method for minimally processed lettuce. Green tea treatment positively affected the preservation of ascorbic acid and carotenoid content of the samples. However, the use of high GT concentration (0.5 or 1.0 g 100 mL^{-1}) resulted in an unacceptable product when evaluated by the sensory panel. Browning appears to be an important factor involved in this negative effect.

Further research involving safety markers would be needed in order to study the efficacy of GT in controlling microorganism spoilage and pathogen growth. The use of GT in combination with other treatments, as for example heat-shock, is recommended by the authors.

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