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ScienceDirect
Food Chemistry 101 (2007) 274–280
Food Chemistry
www.elsevier.com/locate/foodchem

Physical and chemical changes in minimally processed green asparagus during cold-storage

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Received 12 September 2005; received in revised form 16 January 2006; accepted 16 January 2006

Abstract

The purpose of this research is to study effects of an innovative packaging method on the shelf-life of minimally processed green asparagus. The physical–chemical parameters analyzed (weight loss, colour, texture, chlorophyll and citric acid), and monitored by untrained panellists during the cold storage period at 6 °C, showed that pairing of a semi-permeable film with an adsorbent material and immersion in ascorbic acid solution were able to extend the shelf-life of green asparagus. Also, on the basis of the results obtained, a study of the chlorophyll and toughness degradation kinetics of green asparagus was conducted during cold storage. These data showed the toughening of asparagus spears to be faster than chlorophyll degradation. Thus, the former can be used as a reliable quality index to predict the shelf life of such products.

Keywords: Chlorophyll; Green asparagus; Kinetics degradation; Toughness

1. Introduction

In recent decades, the presence in the market of minimally processed vegetables is continuously increasing. In particular, minimally processed green asparagus (washed and cut at the base) is gaining popularity among consumers. The perishable nature of the product poses the challenge to develop effective methods of storage, so to extend its post-harvest use. The very short shelf-life of asparagus (*Asparagus officinalis*, L.) is mainly related to its high respiratory activity which continues after harvesting. Indeed, Kader (1992) found that its respiration rate was about 60 mg CO_2/kg/h at 5 °C, which quickly leads to the maturation and the senescence of the vegetable. The loss of quality is mainly perceived by consumers in the wrinkling of stems, hardness, loss of the green colour and brightness. The texture is determined by a range of mechanical properties which are mainly influenced by the cell wall components (Rodriguez et al., 2004). In green asparagus tissues, the development of ''woodiness'' is associated with the presence of a secondary cell wall.

After harvest, the green asparagus vascular system and tissues involved in the mechanical support of the stem tissues continue to develop as a consequence of cell growth, producing a natural wall thickening (secondary wall development), with associated lignification (Waldron, Parker, & Smith, 2003). The toughening of asparagus is mainly related to the degree of lignification of the pericyclic fibres (Bhowmik, Matsui, Kawada, & Suzuki, 2001; Siomos, Sfakiotakis, & Dogras, 2000), which is enhanced by enzymes such as phenylalanine ammonia-liase (PAL), peroxidases, and isoperoxidases (Chang, 1987; Goldstein, Jennings, & Marsh, 1972). While the quality of white asparagus is mainly affected by the process of toughening that takes place during post-harvest storage (Chang, 1987), the acceptability of green asparagus spears is hampered by undesirable colour

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doi:10.1016/j.foodchem.2006.01.048

changes due to chlorophyll degradation. Indeed, the degreening of spears during the storage period is the result of chloropyll (Chl) breakdown, which is, in turn, regulated by different pathways (Amir-Shapira, Goldschimdt, & Alman, 1987), such as: chlorophyllase activity (Sabater & Rodrisol, 1978), and the peroxidase-hydrogen system. It was shown that the presence, in the latter, of phenolic compounds, degrades Chl in spinach leaves and in parsley (Yamauchi & Minamide, 1985; Yamauchi & Watada, 1991). Other important factors in determining asparagus freshness are the moisture level and the organic acid content, which reflect the vegetable metabolic modification after harvest (Day, 1995; Lill, King, & O'Donoghue, 1990). Modified technology packaging (MAP), using semi-permeable film, was studied for shelf-life extension of minimally processed asparagus (Villanueva, Tenorio, Sagardoy, Renondo, & Saco, 2005). In fact, respiration rate is strongly affected by the composition of the surrounding atmosphere, and asparagus quality greatly benefits from storage in low O_2 and high CO_2 concentration environments, achieved in a passive modified atmosphere packaging, using semi-permeable film. Moreover, the retention of freshness in minimally processed vegetables under MAP can be improved by means of dipping in an antioxidant solution. While a variety of vegetable products commonly undergo this procedure to protect readily oxidative compounds, this type of preventive-treatment has not yet been tested on green asparagus. In order to predict the shelf life of vegetable, many studies are currently applying a kinetic and modelling approach, based on the degradation of the main quality index of each product (Taoukis, Labuza, & Saguy, 1997). Previous studies have also used this approach to predict the shelf-life of asparagus, modeling the kinetics of degradation of the ascorbic acid (Esteve, Farrè, & Frigola, 1995) or the evolution of spoilage organisms (Garcia-Gimeno, Castillejo-Rodriguez, Barco-Alcala, & Zurera-Cosano, 1998). This approach has also been used to model the kinetics of textural and colour degradation as a consequence of high temperature treatments (Lau, Tang, & Swanson, 2000). The purpose of the present study was to evaluate the shelf-life of asparagus spears dipped in an ascorbic acid solution and packaged in a semi-permeable film coupled with an adsorbent material®. The quality evaluation of asparagus was assessed by measuring changes in the main physical–chemical parameters and by visually monitoring the product, during a period of 21 days of cold storage.

Moreover, a model of the asparagus degradation kinetics, adopting chlorophyll content and tissue toughness as quality indices was developed.

2. Materials and methods

2.1. Plant material

Green asparagus spears, (*Asparagus officinalis* cv. *Desto*), after being collected from a commercial plantation in the Campania region of Italy, were immediately precooled in water, then selected by diameter (1.5 ± 0.1 cm) and cut at a length of about 20 cm. Three kinds of samples (each samples about 200 g) were compared during the cold storage period at 6 ± 0.5 °C:

U, untreated asparagus spears;
U + film, untreated asparagus spears packaged with semi-permeable film;
C, asparagus spears dipped in ascorbic acid solution (2% w:w) for 1 min, before packaging with an adsorbent/desorbent material® in a semi-permeable film.

The semi-permeable film used in the trials U + film and C was a Cryovac MRX: thickness 15 μm, O_2 transmission rates = 10.000 $cm^3/24$ h m^2 bar, CO_2 transmission rates = 41.000 $cm^3/24$ m^2 bar, moisture vapour transmission rates = g/24 h m^2 at 38 °C and 100% UR.

A total of 63 asparagus spears packages were prepared (for three treatments by three replicates by seven moments in time) and stored for three weeks at 6 °C; such a temperature was different from the temperature recommended (2 °C), in order to reproduce the transport and storage condition.

At 0, 3, 5, 7, 10, 12, 17 and 21 days, the product quality was evaluated by measuring the following parameters: weight loss, colour, chlorophyll content, texture, citric acid; at 0, 5, 7, 10, 17 and 21 days product quality was evaluated by a visual quality assessment.

2.2. Weight loss

The weight loss of the samples, which is due mainly to loss of water, was determined with a precision balance (Gibertini mod. E 42, Italy) during the entire storage time.

2.3. Colour

Colour was assessed with a CR-200 Chromameter (Minolta, Japan) having an aperture size of 10 mm. The Hunter values (L^*, a^*, b^*) were monitored on the surface of stored asparagus samples; 10 spear readings of the asparagus bud segments were used for the colour measurements. The results were expressed with the hue angle, defined as $\tan^{-1}(b^*/a^*)$, that is well correlated with chlorophyll content (Lau et al., 2000). The more the hue angle approached 90°, the more the yellowness increased.

2.4. Texture

To evaluate the mechanical properties of asparagus spears, flexural tests were performed by a texturometer fitted with a cone probe (10 cm × 1 cm) (Ametek Lloyd Instruments LRX plus, UK) provided with specific software (Nexygen batch 4.1). The crosshead speed was 50 mm/min with a load cell of 50 N. Specimens for three-point bending tests were used (Fig. 1) and the distance

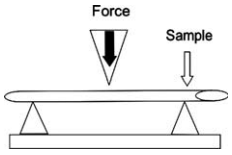

Fig. 1. Flexural test with three-point loading used for evaluation of spears asparagus mechanical properties.

between two knife edges was 4 cm. During the storage period, for each experiment, five asparagus samples (medium section of about 8 cm length) were tested. Mechanical properties (toughness) of asparagus were obtained from load and deformation curves.

2.5. Chlorophyll content

About 2 g of samples were homogenized in 10 ml of 80% acetone at 4 °C with an Ultra Turrax T 25 homogenizer (Janke & Kunkel Labortechnick, Germany) for 2 min and centrifuged at 13,500*g* (Biofuge-Primo, Italy) at 10,000*g* for 15 min at 4 °C. The supernatant was filtered and diluted to 10 ml with 80% acetone. Absorbance (A) was measured at 645 and 663 nm using a Perkin–Elmer lambda-Bio 40 (USA) spectrophotometer. Chlorophyll *a* and *b* concentrations were calculated as follows (Arnon, 1949):

$$\text{Chla [mg/g dry matter]} = \frac{[(12.7 \times \text{Abs}_{663}) - (2.6 \times \text{Abs}_{645})] \times \text{ml Acetone}}{\text{mg dry matter}}$$

$$\text{Chlb [mg/g dry matter]} = \frac{[(22.9 \times \text{Abs}_{645}) - (4.68 \times \text{Abs}_{663})] \times \text{ml Acetone}}{\text{mg dry matter}}$$

2.6. Citric acid

About 5 g of asparagus spears were homogenized in an Ultra Turrax T 25 homogenizer (Janke & Kunkel Labortechnick, Germany). The homogenate was put in a flask (50 ml) with distilled water and it was centrifuged (Biofuge Primo, Italy) at 10,000*g* 10 min; the supernatant was then filtered (Millipore. millex gv, 0.22 μm pore size, USA). Citric acid concentration was analyzed by an ion-exchange chromatograph (Dionex Corp., USA), consisting of: GP 50 gradient pump; LC 50 chromatography enclosure; ED 50 electrochemical detector; Ionpac AS11 column (250 × 4) mm; Ionpac AS11 Guard (50 × 4) mm. Acquisition (and integration) of chromatograms was performed with the Peaknet G4G1T0 Dionex Corp. software. The mobile phase used was bi-distilled water (E1) and, 100 mN NaOH (E2) for a total running time of 25 min by using the following gradient 93%E1–7%E2 at time 0 to 65%E1–35%E2 in 20 min; 93%E1–7%E2 in 4 min. The flow rate was 0.5 ml/min.

2.7. Visual assessment of quality

The asparagus visual characteristics were evaluated over the storage time placid by a 10 member untrained panel. The observed main characteristics were: colour changes and tissue toughening, strictly related to the two principal phenomena (chlorophyll degradation and lignification) responsible for asparagus spears loss of freshness and presence of off-odours. A four-point scoring scale was employed (1, unacceptable; 2, acceptable; 3, good; 4, very good).

2.8. Kinetics modelling

To develop a systematic kinetic and modelling approach the first step is to detect the main quality indices of the food product (Taoukis et al., 1997). The most important parameters connected with green asparagus quality loss are certainly dependent on the degreening and the lignification phenomena (Chang, 1987; Siomos et al., 2000). Therefore, a modelling kinetics approach of these phenomena can be developed to predict the shelf life after a minimum value of the quality index is chosen. In this work, the chlorophyll content was chosen as quality index to measure the degradation of green colour, while toughness degree was chosen for the lignification process. The minimum value of both chlorophyll content and toughness was established by visual assessment.

In particular, the increases in time of the toughness parameter was assumed to obey the following kinetic law:

$$\frac{dB}{dt} = k_b B^n \tag{1}$$

where k_b and n are the apparent kinetic rate and the apparent kinetic order, while B is the toughness parameter. It is worth noting that Eq. (1) is not the true kinetic mechanism of the lignification process which instead depends on a complex system of biological reactions. In fact, it is rather an apparent kinetic law which just gives an estimation of the degradation rate due to the lignification phenomena. On the basis of experimental measures of the toughness parameter, it is possible to estimate kinetic parameters, k and n, with a regression analysis. Once a critical value of the toughness parameter is chosen (B_{max}), the asparagus shelf-life can be predicted by integrating, in time, Eq. (1) until the critical condition is reached. In this way, the following expressions are obtained for different reaction orders:

$$\text{Se } n \neq 1 \quad t_{sl} = \frac{B_{max}^{1-n} - B_0^{1-n}}{k(1-n)} \tag{2}$$

$$\text{Se } n = 1 \quad t_{sl} = \frac{1}{k} \cdot \ln\left(\frac{B_{max}}{B_0}\right) \tag{3}$$

On the other hand, the quality loss related to the degreening of asparagus spears can be measured by the chlorophyll content decrease in time. A similar analysis can be conducted by simply changing the sign in Eq. (1).

2.9. Statistical analysis of results

The results were analysed using one-way analyses of variance. Differences ($p < 0.05$) between means were studied with the Student–Newman–Keuls test.

Apparent reaction orders and kinetics in the mathematical models were found through non-linear regression of data. Results were validated by residuals analysis and *F*-test.

3. Results and discussion

3.1. Weight loss

The use of a semi-permeable film was effective in slowing down the asparagus weight loss process during the storage period (Table 1). In unpacked samples, in fact, the weight loss detected over the 21 days, was 27.2% of the initial weight with statistical differences at each sampling date. In contrast, the loss detected for the asparagus samples packaged in a semi-permeable film, with a low water permeability, was about 6.8% for U + film and 5.2% for C samples. Significant differences between U + film and C samples were detected at each sampling point. The lower weight loss obtained for sample C pointed to the positive action of the adsorbent material on the slow transpiration phenomena. This behaviour can be explained by the adsorbent capacity to retain ethylene and is the object of an ongoing study.

3.2. Citric acid

In fresh asparagus, citric acid content was higher in the top of the spears than in the bottom portion (Figs. 2(a) and (b)) (Bhowmik, Matsui, IKeuchi, & Suzuki, 2002). During the first five days of the storage period, a slight drop in the citric acid content was detected in the top portion of sample C. The latter showed, after 21 days, a value of about 25 mg citric acid/100 g dry matter, corresponding to a loss of about 37% of the initial amount. The rate of citric acid decrease during the storage period was slower in dipped samples, both in the bottom and top portions of the asparagus. The untreated samples, with and without semi-permeable film, showed similar behaviour in citric acid evolution during storage, with a more significant decrease in unpackaged samples.

Table 1
Weight loss percentage evolution in asparagus samples during cold storage period (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples)

Time days	U	U + film	C
0	0 ± 0.00 aa*	0 ± 0.00 b	0 ± 0.00 c
3	2.63 ± 0.15 ab*	0.73 ± 0.15 b	0.66 ± 0.03 c
5	5.10 ± 0.07 ac*	2.11 ± 0.045 b	1.45 ± 0.04 c
7	7.85 ± 0.04 ad*	2.45 ± 0.04 b	1.74 ± 0.02 c
10	10.11 ± 0.02 ae*	3.04 ± 0.08 b	2.00 ± 0.02 c
12	10.41 ± 0.045 af*	4.01 ± 0.035 b	2.45 ± 0.03 c
17	22.51 ± 0.035 ag*	4.74 ± 0.035 b	3.09 ± 0.035 c
21	27.2 ± 0.165 ah*	6.78 ± 0.03 b	3.48 ± 0.02 c

Data are the averages of three replicates ± standard deviation.
Different letters (a, b, c, ...) in the same column indicate significant differences ($p < 0.05$).
Different letters (a*, b*, c*, ...) in the same row indicate significant differences ($p < 0.05$).

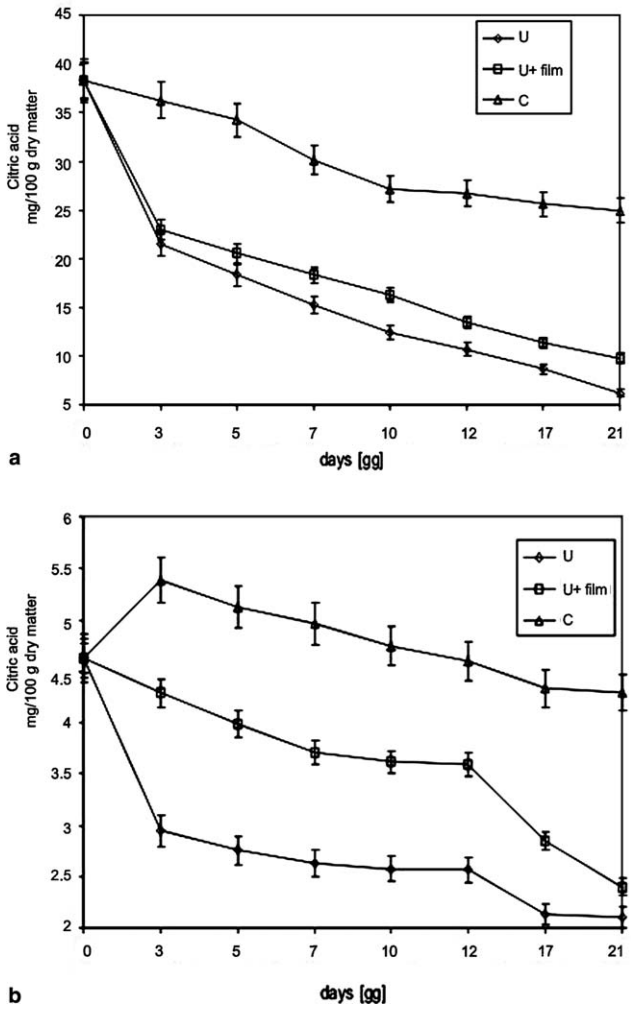

Fig. 2. Citric acid content (mg/100 g dry matter) in asparagus sample (top portion: a; bottom portion: b) during cold storage period (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples).

3.3. Visual assessment of quality

The untrained panel detected a rapid degradation of quality parameters in asparagus samples stored unpackaged, first by appearance of an undesirable texture degree, and second by loss of green colour (Table 2). The unacceptable texture degree was reported by the panel after 10 storage days, while the storage time for green colour change was deemed still acceptable at the 21 days mark. Although packaging of the product with semi-permeable film slowed down the appearance of these degradation phenomena, the

adsorbent and dipping were far more effective in preserving the asparagus quality during storage.

3.4. Texture

An increase of breaking force was detected for all samples, with no significant differences until 5 days of storage (Fig. 3). Afterwards, the toughness recorded for spears asparagus C was significantly lower than those of the other samples, with a breaking force value, after 21 days of storage, of about 4.2 N/cm^2. The value was less than half of the values obtained in untreated samples (9.9 N/cm^2). The toughness data obtained during the storage tests pointed to greater and significant phenomena for untreated asparagus samples, while the dipping in antioxidant solution was effective in slowing it down. In fact, oxygen stimulates some of the enzymes responsible for toughness with a significant increase of lignocellulose content under open air conditions (Gomez, Lopez Camelo, & Cacace, 1995). Choosing, as quality index, the toughness parameter, the shelf-time can be estimated by integrating, in time, Eq. (1), once the kinetic parameters, k_b and n, estimated. Regression curves, corresponding to different apparent orders, were compared and the curve that better fits the experimental data was chosen on the basis of residual analysis and F-tests.

For the degradation rate based on the toughness parameter increase, the statistical analysis suggests a first-order kinetic model. The analysis was conducted for all samples and the results showed a kinetic order independent of the analysed sample.

The values of kinetic constants and the confidence intervals for each considered sample are shown in Table 3.

For all the samples, first-order kinetic model curves are shown in Fig. 4. It is apparent that the packaging with only the semi-permeable film (U + film) does not affect the increase rate of the toughness parameter, while immersion and packaging with the adsorbent material (C) decreases the rate of the lignification phenomena.

A maximum level of the toughness parameter has to be fixed in order to estimate the shelf-life time. This maximum

Table 2
Visual score[a] in asparagus samples during cold storage period (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples)

Sample	Days	Texture	Green colour	Opened bracts	Off-odours
U	0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	5	3.3 ± 0.3	3.3 ± 0.7	3.6 ± 0.5	3.9 ± 0.3
	10	2.1 ± 0.3	3.2 ± 0.4	2.7 ± 0.5	3.5 ± 0.5
	17	1.3 ± 0.5	2.6 ± 0.5	1.5 ± 0.5	3.0 ± 0.5
	21	1.0 ± 0.0	2.1 ± 0.3	1.0 ± 0.0	2.4 ± 0.5
U + film	0	4.0 ± 0.0	4 ± 0.0	4 ± 0.0	4 ± 0.0
	5	3.5 ± 0.5	4.0 ± 0.0	3.8 ± 0.4	4.0 ± 0.0
	10	2.7 ± 0.5	3.8 ± 0.4	2.9 ± 0.6	3.9 ± 0.3
	17	2.0 ± 0.5	3.1 ± 0.3	1.7 ± 0.5	3.2 ± 0.4
	21	1.0 ± 0.0	2.9 ± 0.3	1.4 ± 0.5	2.8 ± 0.6
C	0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	5	3.9 ± 0.3	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
	10	3.7 ± 0.5	3.9 ± 0.3	3.6 ± 0.5	3.8 ± 0.4
	17	3.1 ± 0.6	3.7 ± 0.5	3.2 ± 0.6	3.5 ± 0.5
	21	2.8 ± 0.4	3.3 ± 0.5	3.0 ± 0.5	3.0 ± 0.5

[a] Scoring scale employed: 1, unacceptable; 2, acceptable; 3, good; 4, very good.

Table 3
Kinetics constants and confidence intervals calculated for asparagus toughness (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples)

Sample	Toughness (N/cm^2)	
	K	CI
U	0.0682	±0.00157
U + film	0.0535	±0.00147
C	0.0355	±0.00139

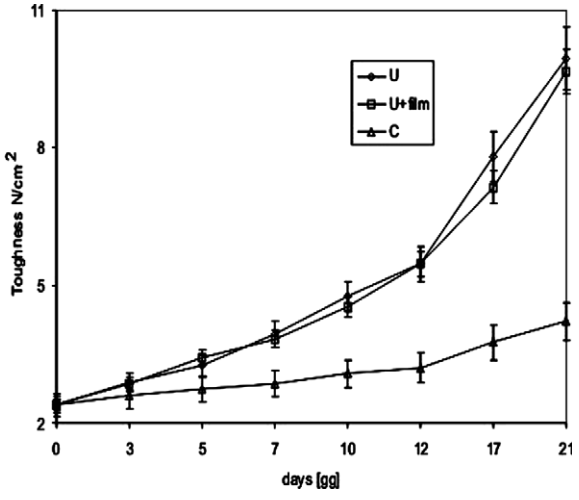

Fig. 3. Toughness evolution in asparagus sample during cold storage period (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples).

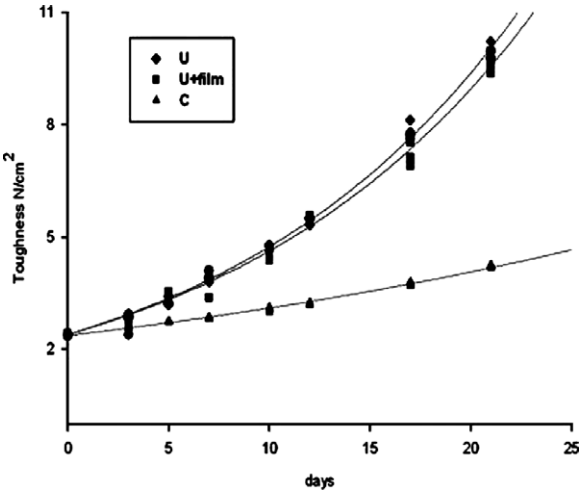

Fig. 4. Nonlinear regression curves for the decrease in time of the toughness parameter for all samples (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples).

value was chosen on the basis of the visual quality assessment. The shelf life time untrained judges' panel confirmed that texture was acceptable within 10 days (Table 2) for the untreated sample. Therefore, with the kinetic parameters of the untreated sample and with a shelf-life equal to 10 days, the maximum toughness value was calculated using Eq. (3). This value was 4.7361 N/cm^2 (B_{max}). Successively, the B_{max} was used to estimate shelf-life times for the sample U + film and the sample C with the corresponding kinetic parameters shown in Table 3. With this procedure, shelf-life times for U + film and C samples were 12.8 days and 19.2 days, respectively. These values are slightly underestimated in comparison with the visual assessment results shown in Table 2. These differences can be explained by considering that an untrained panel could give an unreal evaluation of the sample, as shown by the wide standard deviations of scores listed Table 2.

3.5. Chlorophyll content

A significant loss of chlorophyll content was observed during the storage period in all asparagus samples (Fig. 5). The pre-treatment with ascorbic acid retarded the loss of chlorophyll content, slowing down oxidative phenomena that are responsible for the breakdown of the pigment (Amir-Shapira et al., 1987; Yamauchi & Minamide, 1985; Yamauchi & Watada, 1991), and leading to the undesirable yellowing of asparagus spears. The reduction in hue angle corresponded to a decrease in the intensity of greenness and an increase in yellowness (Little, 1975). The hue angle decrease detected during the cold storage period was consistent with the chlorophyll content trend. Asparagus samples C, in fact, showed a smaller change of green colour than did U and U + film asparagus samples (Fig. 6).

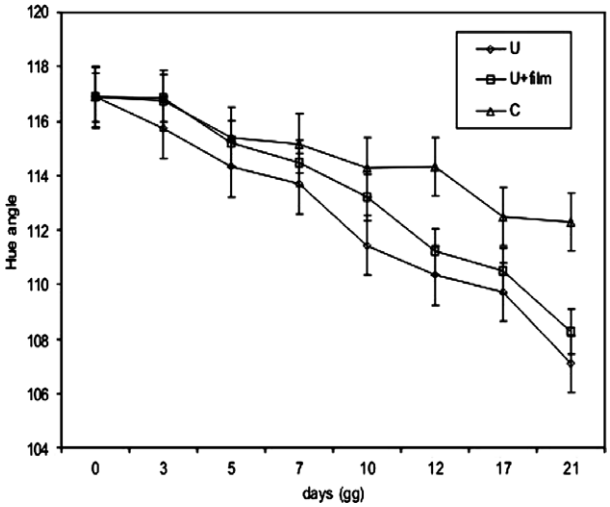

Fig. 6. Hue angle of different asparagus samples during cold storage period (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples).

Using the same toughness parameter modelling procedure, the rate of chlorophyll degradation showed first-order kinetics for all asparagus samples.

The values of kinetic constants and the confidence intervals for each considered sample are listed in Table 4, while regression curves are shown Fig. 7.

Table 4
Kinetics constants and confidence intervals calculated for asparagus chlorophyll degradation (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples)

Sample	Chlorophyll (mg/100 g DM)	
	K	CI
U	−0.0641	±0.00208
U + film	−0.0535	±0.00148
C	−0.0355	±0.00139

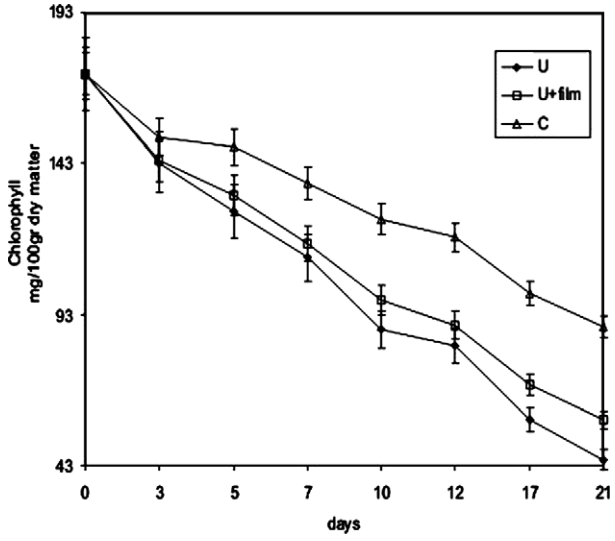

Fig. 5. Chlorophyll content(mg/100 g dry matter) in asparagus samples during cold storage period (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples).

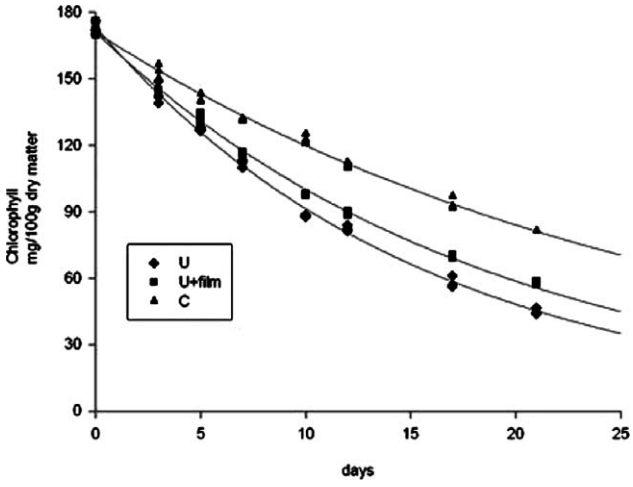

Fig. 7. Nonlinear regression curves for the decrease in time of the chlorophyll content for all samples (U, untreated samples; U + film, packaged samples; C, dipped and packaged ® samples).

From the green colour score of the visual quality assessment, the untreated asparagus sample showed a shelf-life time of 21 days. With the same procedure as described for the calculation of the maximum level of acceptability for the toughness parameter, a chlorophyll minimum value was fixed. This value was 44.9 mg/100 g dry matter, while shelf-life times for U + film and C samples were 25.2 days and 37.9 days, respectively.

4. Conclusions

The present work studied the effects produced on the shelf life of minimally processed green asparagus by dipping it in an ascorbic acid solution and wrapping in semipermeable film. The collected data showed that, in dipped and packaged ® samples (C), the loss of quality detectable by visual evaluation and the changes in physical–chemical parameters (weight loss, colour, texture, chlorophyll and citric acid) in dipped and packaged ® samples (C) were lower than those in untreated (U) and packaged film (U + film) samples.

Moreover, on the basis of the results obtained, a study of the chlorophyll and toughness degradation kinetics of green asparagus was conducted during cold storage. From a nonlinear regression analysis, first-order kinetics were elucidated for both chlorophyll and toughness parameters and for all asparagus samples. Moreover, the texture degradation rate, estimated by toughness parameter, was much higher than was the chlorophyll loss. This suggests that the toughness parameter could be used as a good quality index to predict the shelf life of minimally processed asparagus.

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