Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 1054–1060
Food Chemistry
www.elsevier.com/locate/foodchem

Effects of an innovative dipping treatment on the cold storage of minimally processed *Annurca* apples

D. Albanese [a], L. Cinquanta [b], M. Di Matteo [a,*]

[a] *Department of Chemical and Food Engineering, University of Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy*
[b] *Department of Agricultural, Food, Environmental and Microbiological Science and Technology, University of Molise, Via F. De Sanctis, 86100 Campobasso, Italy*

Received 15 November 2006; accepted 2 May 2007

Abstract

The effect of trehalose as an edible coating on minimally processed *Annurca* apple slices was studied during cold storage. The edible coating was prepared by dipping the fruit in a solution containing trehalose at 0.8%, sucrose at 1.0% and sodium chloride at 0.1%. During storage at 6 °C the following parameters were monitored: weight loss, colour (hue angle (h°) and whitening index (WI)), firmness, malic and ascorbic acids, polyphenol content, microstructure by scanning electron microscopy (SEM) and microbial count. The results showed that such a coating reduced the browning phenomena; in fact the WI and h° values were significantly lower in coated samples than untreated ones. Moreover, decreases in weight loss and in the reduction of organic acids were observed in coated samples. Electron microscopy slides of the cut tissue showed how the coating worked.

Keywords: Minimally processed apples; Browning; Cold storage; Trehalose

1. Introduction

Minimally-processed (m.p.) fruits are defined as fruits that have been trimmed, peeled, cut and packaged. The demand for such products has increased significantly, although there are hurdles in the production of these commodities, due to the difficulty in preserving their freshness during storage. The short shelf life of fresh-cut fruit slices is mainly because of the cutting operations that cause damage and wounds to the fruit cell membrane, as a result of which, the vegetable tissues react by increasing respiration rate, ethylene production and tissue softening, causing a drop in fresh product quality. Moreover, mechanical injury or wounding causes leakage of polyphenol oxidases (PPO) into the vacuole, resulting in contact of PPO with phenolic compounds, causing cell browning and enhanced bacteria, yeast and mould growth (Ahvenainen, 1996). To extend the shelf life of m.p. apples, many methods have been proposed, including modified atmosphere packaging (MAP), dipping in antibrowning and antibacterial solutions, and edible coatings. For instance, low O_2 concentrations and CO_2, ranging from 2% to 5%, caused reduction in respiration rate, ethylene production and fruit browning (Ahvenainen, 1996); high O_2 concentration, alone or with an Ar and N_2O gas mixture was effective in inhibiting enzymatic discoloration, preventing anaerobic fermentation and inhibiting microbial growth (Day, 2003). The control of enzymatic browning in fresh-cut apples has also been achieved through different pre-treatments, based on mixtures of different compounds: 1% ascorbic acid + 0.2% citric acid (Pizzocaro, Torregiani, & Gilardi, 1993), 1% ascorbic acid + 0.5% $CaCl_2$ (Soliva-Fortuny, Grigelmo-Miguel, Odriozola-Serrano, Gorinsten, & Martin-Belloso, 2001), and 1% citric acid + 0.4–3.2% ascorbic acid (Sapers & Douglas, 1987). Dipping with other browning inhibitors, such as 4-hexylresorcinol or cysteine, has been effective in

* Corresponding author. Tel./fax: +39 089964131.
E-mail address: mdimatteo@unisa.it (M. Di Matteo).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.05.009

retarding cut surface browning in apple slices (Monsalve-Gonzales, Barbosa-Canovas, McEvily, & Iyengar, 1995). Moreover, some edible coatings can reduce respiration, thus prolonging product shelf life (Ahvenainen, 1996; Baldwin, Nisperos-Carriedo, & Baker, 1995). Edible coatings provide a semipermeable barrier against oxygen, carbon dioxide, moisture and solute movement, thereby reducing respiration, water loss and oxidation reaction rates (Park, 1999).

Coatings based on sucrose polyesters of fatty acids or cellulose derivatives (carboxymethylcellulose) delay water loss and browning. The use of a cellulose-based edible coating on fresh-cut apple cylinders stored in over-wrapped trays at 4 °C increased shelf life by about 1 week and retarded discoloration in cut mushroom (Baldwin et al., 1995). Another natural GRAS (Generally Recognized As Safe) compound used as an edible coating is trehalose, used mainly to preserve the aroma and colour of dried fruits (Albanese, Cinquanta, & Del Vaglio, 2001; Komes, Lovrić, Ganić, Kljusurić, & Banovic, 2005; Roser, 1991). The role of trehalose in decreasing biological activity in vegetables is well known (Albanese, Del Genio, Del Vaglio, & Di Matteo, 2003; Richards et al., 2002), owing to its properties, of water replacement, glass transformation and chemical stability (Colaco & Roser, 1995). Water replacement theory proposes that all biological macromolecules are normally stabilised by water, which forms hydrogen bonds around these molecules. Trehalose appears to have greater flexibility in the glycosidic bond between its two D-glucose molecules as compared to other disaccharides. Accordingly, trehalose fits more closely to the irregular surface of macromolecules, thus forming a coating on the fruit surface.

The objective of this work was to evaluate the effectiveness of dipping with a novel solution, containing trehalose, sucrose, and sodium chloride, on the quality of m.p. apple slices. For this purpose, the main chemical, physical and microbiological parameters during cold storage at 6 °C were monitored.

2. Materials and methods

2.1. Plant material

Annurca Rossa del sud apples [protection system PGI: Protected Geographical Indication; Council Regulation (EC) No. 510/2006], harvested in October 2004, were provided from Istituto Sperimentale di Frutticoltura Caserta, Italy, after treatment to obtain redness (Lo Scalzo, Testoni, & Genna, 2001). Subsequently, the apples were washed in distilled water, peeled and cut into slices of a thickness of about 1.5 cm using a clean stainless-steel knife. Dipping solutions of trehalose, NaCl and sucrose, and their combinations were tested on apple slices in a preliminary experiment and the following concentrations were used for this work: trehalose (0.8%) + NaCl (0.1%) + sucrose (1.0%; all w/w). NaCl and sucrose were combined with trehalose, in order to slow down water activity on the apple slices' surface. Two kinds of samples (3 replicates for each sample, of about 200 g) were compared during the cold storage period at 6 ± 0.5 °C

U: untreated apple slices packaged with a semipermeable film;
A: apple slices dipped in trehalose/NaCl/sucrose solution for 5 min, before packaging with a semipermeable film.

The semipermeable film used in the trials was Cryovac MRX: thickness 15 μm, O_2 transmission rates = 10 l per day m^2 bar, CO_2 transmission rates = 41 l per day m^2 bar, moisture vapour transmission rates = g/24 h m^2 at 38 °C and 100% UR.

Just prior to packaging and at regular intervals, the product quality was evaluated by measuring the following parameters: total soluble solids (°Brix), weight loss, colour, pH, firmness, malic acid, ascorbic acid, and polyphenol content, microstructure by scanning electron microscopy (SEM) and microbial count. The results were expressed as a mean of three replicates.

2.2. Analyses

Total soluble solid (°Brix) content was determined at 20 °C using a digital refractometer (PR1, Atago, Japan) on the juice obtained from apple slices, after filtering through Whatman #1 filter paper.

pH was determined using an electronic pH meter (Crison, model Micro pH 2002, S.A., Barcelona, Spain).

Weight loss percentage was estimated after each period of storage by weighing the apple slices after they were removed from packaging (Gibertini, Model E 42, Italy).

Colour was assessed with a CR-200 Chromometer (Minolta, Japan) having an aperture size of 10 mm. During each storage time, Hunter values (L^*, a^*, b^*) were monitored on the surface of stored apple slice samples; nine readings were obtained from the three replicates with three measurements for each replicate. Browning of the apple surface was analysed by conversion of measured Hunter values into whiteness index (WI) values.

$$\mathrm{WI} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}},\ \text{and hue angle}$$

$$h^\circ = \tan^{-1}\frac{b^*}{a^*}.$$

Apple firmness was measured by compression tests using a texturometer (Ametek Lloyd Instruments LRX plus, United Kingdom), with specific software (Nexygen batch 4.1). The size of apple samples analysed was standardised with a cylinder cutter into pieces 2.0 cm in diameter and 1 cm in height. Firmness of apple slices was obtained from load and strain curves recorded during the compression of cylindrical samples to 50% of initial height using two horizontal parallel plates with the sample placed on the centre of the lower plate. The crosshead speed was 50 mm per min

with a cell load of 50 N. During the storage period, for each treatment, five apple samples were tested.

2.3. Organic acids

About 5 g of apple were homogenised in an Ultraturrax T25 (Janke & Kunkel Labortechnick, Germany) at 13 500 g. The homogenate was put in a flask (50 ml) with distilled water and centrifuged (Biofuge Primo, Italy) at 10 000*g* for 10 min; the supernatant was then filtered (Millipore. millex gv, 0.22 μm pore size filters, Millipore, USA). Malic acid and ascorbic acid concentrations were analysed by an ion chromatograph (Dionex Corporation, USA), consisting of a GP 50 gradient pump, LC 50 oven, ED 50 electrochemical detector, Ionpac AS11 column (250 × 4 mm) and Ionpac AS11 Guard (50 × 4 mm). Acquisition and integration of chromatograms were performed with Peaknet G4G1T0 Dionex Corp. software. The mobile phase used was double distilled water (E_1) and NaOH 100 mM (E_2) for a total running time of 25 min, using the following gradient: 93% E_1, at time 0 to 65% E_1, at 20 min, then to 93% E_1, in 4 min. The flow rate was 0.5 ml min^{-1}.

2.4. Total phenol content

Total polyphenols was determined for lyophilised sample (1 g), by adding 10 ml of solvent (methanol–water–acetic acid 30:69:1 V/V/V), and homogenising the sample (Ultra-Turrax) for 1 min. Tubes were centrifuged (3000*g* for 15 min), and the clear supernatant was collected. The extraction was repeated with another 10 ml of solvent. Supernatants were combined and then dried. The solid residue was dissolved in methanol. Total phenolic content was determined according to the Folin–Ciocalteu method (Singleton & Rossi, 1965) by reading the absorbances at 760 nm. Results were expressed as grams of gallic acid equivalents (GAE) per 100 g of apple sample.

2.5. Scanning electron microscopy

Apple slice samples were prepared and examined with a scanning electron microscope (LEO 420, Model 2.04, Assing, Italy) (Tu, Nicolai, & De Baerdemaeker, 2000).

Microstructure changes of A and U apple samples during the storage period were evaluated by image analysis. With image analysis software (Image Pro-Plus 5.1, Media Cybernetic, USA), apple SEM images were evaluated as V = intercellular space area and M = cellular area, to calculate V/M ratio.

2.6. Microbial count

Microbiological analyses were performed by homogenising the edible part of each sample. For microbiological analysis, apple slices (10 g) were removed aseptically from each package and transferred into sterile bags with an appropriate amount of sterile Ringer's solution (ratio 1:10). The sample and Ringer's solution were blended for 5 min in a homogeniser (Stomacher Lab-Blender 400, Seward, UK). Serial dilutions were made using Ringer's solution and the microbial status was evaluated by plate counts. Mesophilic aerobic counts were enumerated using Plate Count Agar (Oxoid) and incubation at 30 °C for 3 days; yeast and mould counts by using Rose-Bengal Chloramphenicol Agar (Oxoid) and incubating at 25 °C for 5 days.

2.7. Sensorial assessment of quality

Quality attributes of the apple slices were evaluated over the storage time period by a 10-member untrained panel. Each panelist was asked to rate two apple quality attributes, colour and firmness. Firmness evaluation was conducted by crushing apple slices between fingertips. A four-point scoring scale was employed (1: unacceptable, 2: acceptable, 3: good and 4: very good).

2.8. 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.

3. Results and discussion

Apples were harvested at an acceptable maturity for commercial use (Table 1). During the storage period, the dipping treatment was effective in slowing down weight loss (Fig. 1). In U samples, the weight loss detected after 8 days of storage was 2.11% of the initial weight, whereas the loss detected for A apple samples was 1.51%. Significant, differences between U and A samples were detected at each data point. The lower weight loss obtained for sample A suggested a positive action of the pre-treatment in slowing down transpiration phenomena, probably due to the development of an amorphous glass on the apple surface that retarded water evaporation. In both apple samples, a slight increase in pH values (from 3.3 to 3.5) was observed after 8 days. This phenomenon was linked to the malic acid decrease (Fig. 2a), due to the increase in respiration rate following peeling and cutting. The A samples showed a higher content ($P < 0.05$) in malic acid after 6 storage days

Table 1
Weight, soluble solids, peel colour, malic acid, and pH of *Annurca* apples

Weight (g)	95.5 ± 7.2
Dry weight (g/per 100 g)	15.4 ± 1.2
°Brix	13.5 ± 1.5
L	58.5 ± 8.24
a^*	12.4 ± 5.16
b^*	26.6 ± 7.31
Malic acid (g/per 100 g)	0.55 ± 0.01
pH	3.3 ± 0.2

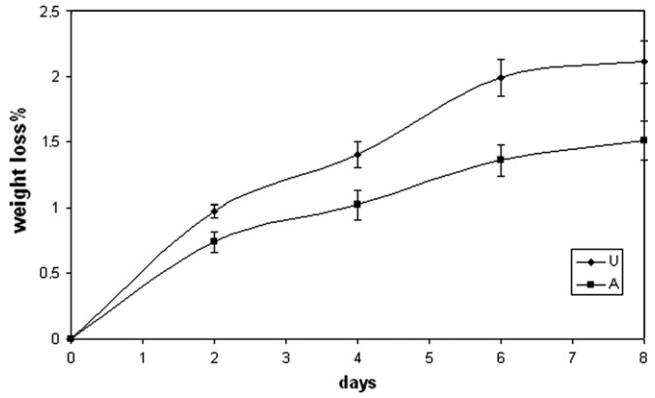

Fig. 1. Weight loss evolution in apple samples during cold storage period (U: untreated samples; A: pre-treated samples).

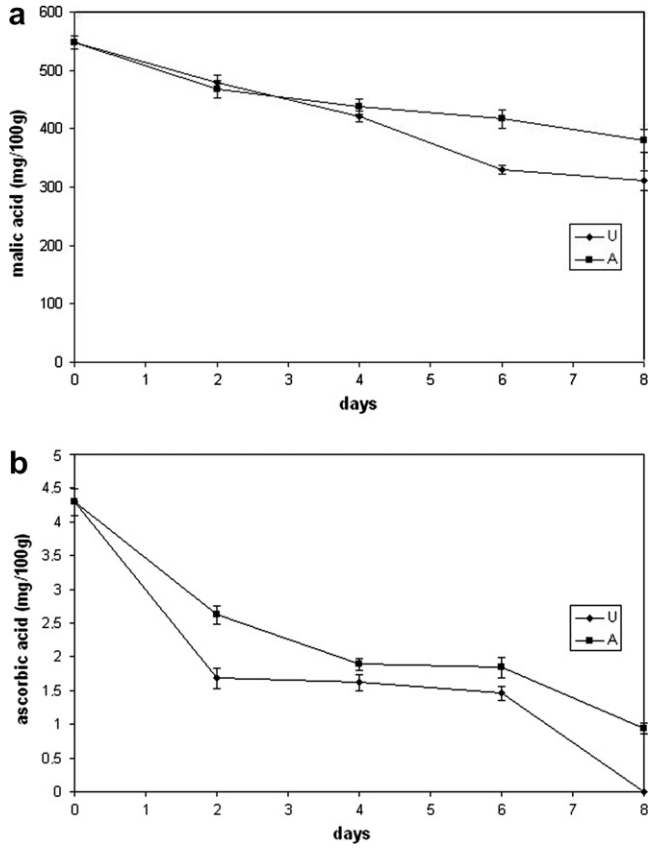

Fig. 2. Malic acid (a) and ascorbic acid (b) content in apple samples during cold storage period (U: untreated samples; A: pre-treated samples).

compared to the U samples, a similar trend was shown for ascorbic acid (Fig. 2b).

The *Annurca* apples were characterised by a high polyphenol content: 287 ± 11.8 mg (GAE) per 100 g (Fig. 3), as compared to a range of 11–230 mg (GAE) per 100 g measured in 13 different apple cultivars (Boyer & Liu, 2004). A higher polyphenol content could lead to the formation of dark pigments due to PPO activity. For both apple samples, a significant decrease in polyphenols during the first 2 storage days was observed, owing to the effects of

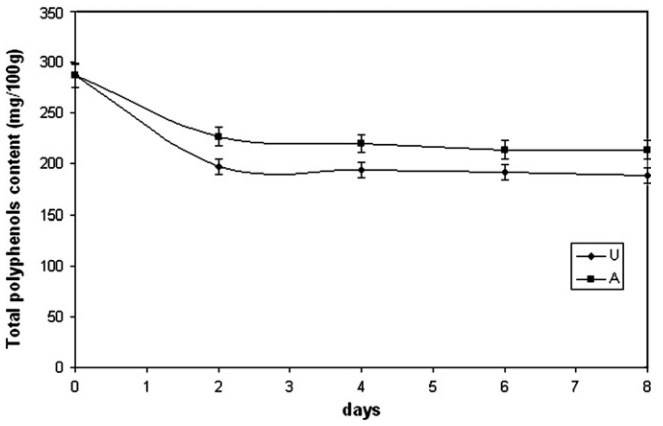

Fig. 3. Total phenolic content in apple samples during cold storage period (U: untreated samples; A: pre-treated samples).

mechanical injury on m.p. apples. During the remaining storage period, a more stable phenol content in both samples was detected, with a higher polyphenol content ($P < 0.05$) in apple sample A probably due to the trehalose properties. Trehalose is able to conform with the irregular polar groups of macromolecules, such as polyphenol compounds, thus slowing down the browning reactions with

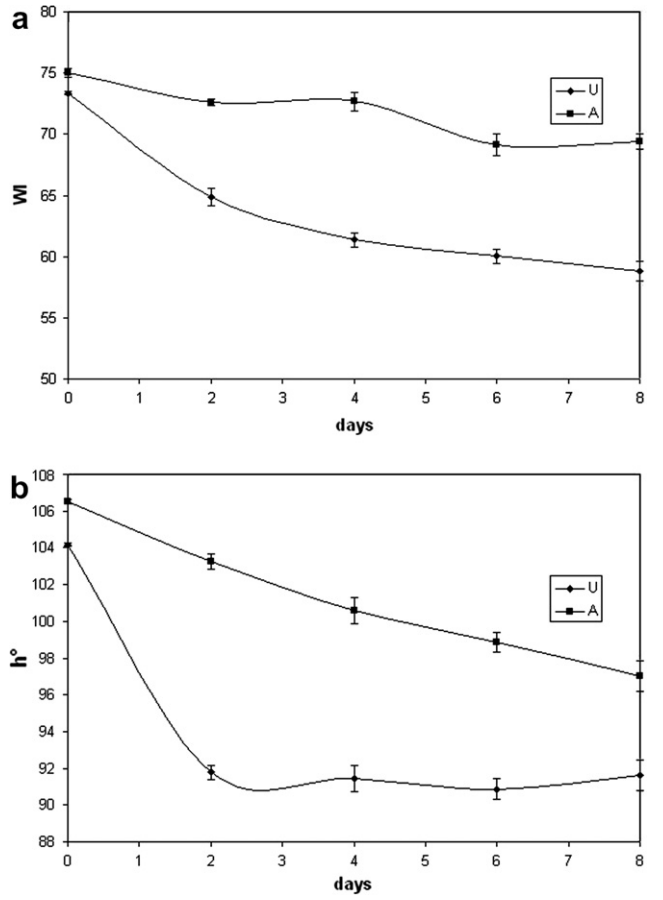

Fig. 4. Colour parameters whiteness index (a) and hue angle (b) of apple samples during cold storage period (U: untreated samples; A: pre-treated samples).

PPO. The outbreak of browning on the surface of the apples was accompanied by a decrease of WI and h° (Fig. 4a and b); these values were in agreement with other experimental results obtained with different apple cultivars (Rocculli, Romani, & Della Rosa, 2004; Rocha & Morais, 2003). The rate of decrease of WI and h° values, may be divided into two periods; in the first period lasting until the 2nd day of storage, the browning increased sharply, which could be attributed to the consumption of substrates by PPO. In the second period, between the second and the 8th day of storage, browning approached a plateau. The pre-treatment allowed a significant ($P < 0.05$) slowdown of the colour change.

Table 2
Firmness values (N) of untreated (U) and pre-treated (A) apple samples in cold storage

Days	U	A
0	50.4 ± 0.08 a	50.4 ± 0.08 a
2	50.4 ± 0.07 a	50.6 ± 0.11 b
4	50.4 ± 0.01 a	50.4 ± 0.09 b
6	50.6 ± 0.12 a	50.6 ± 0.18 b
8	49.5 ± 0.15 b	49.8 ± 0.25 b

Data are the average of five replicates ± standard deviation.
Different letters (a, b) in the same column indicate significant differences ($p < 0.05$).

3.1. Texture measurement

Both apple samples showed a good maintenance of initial firmness (Table 2). On the contrary, a decrease in firmness was reported in m.p. *Jonagored* apples in cold storage (Rocha & Morais, 2003). These differences could be attributed to the pectin content; that of *Annurca* was found to be quantitatively and qualitatively important for its contribution to the high tissue firmness (Lo Scalzo et al., 2001). The natural porosity observed by SEM in U apple tissue showed the capability of such structure to allow mass transport, highlighted by the weight loss (Figs. 5a and 6a). The tissue of the A sample showed a significant decrease of porosity during storage, owing to the ability of trehalose molecules to bind protectively onto the surface of molecular structures by means of hydrogen bonds (Figs. 5b and 6b).

With image analysis software apple SEM images, selected in pixel and using a grey scale, in a threshold range from 84 to 109 pixels, were evaluated. The data obtained

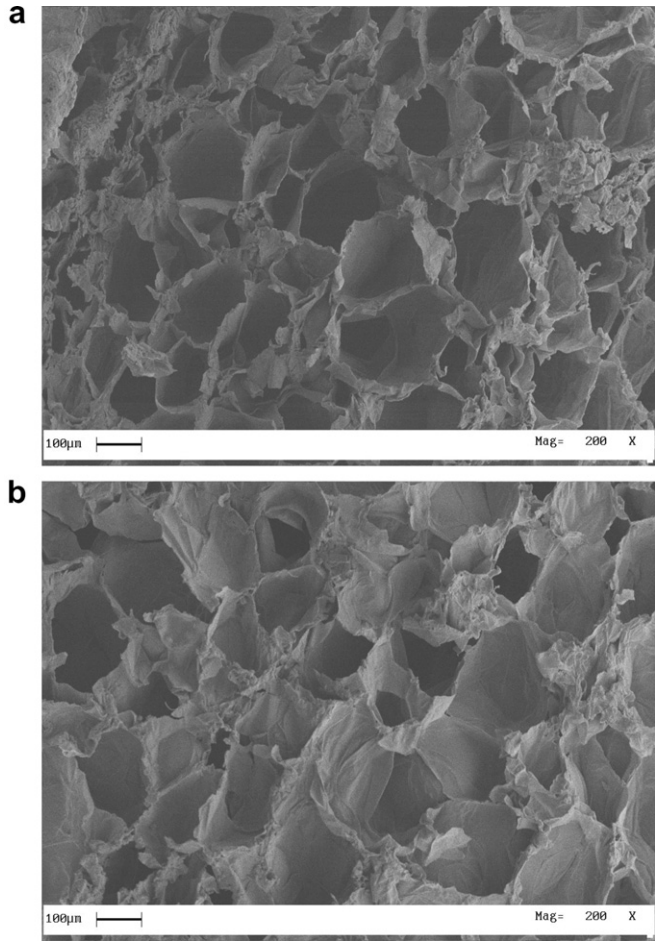

Fig. 5. SEM of untreated (a) and pre-treated (b) apple tissue at time 0.

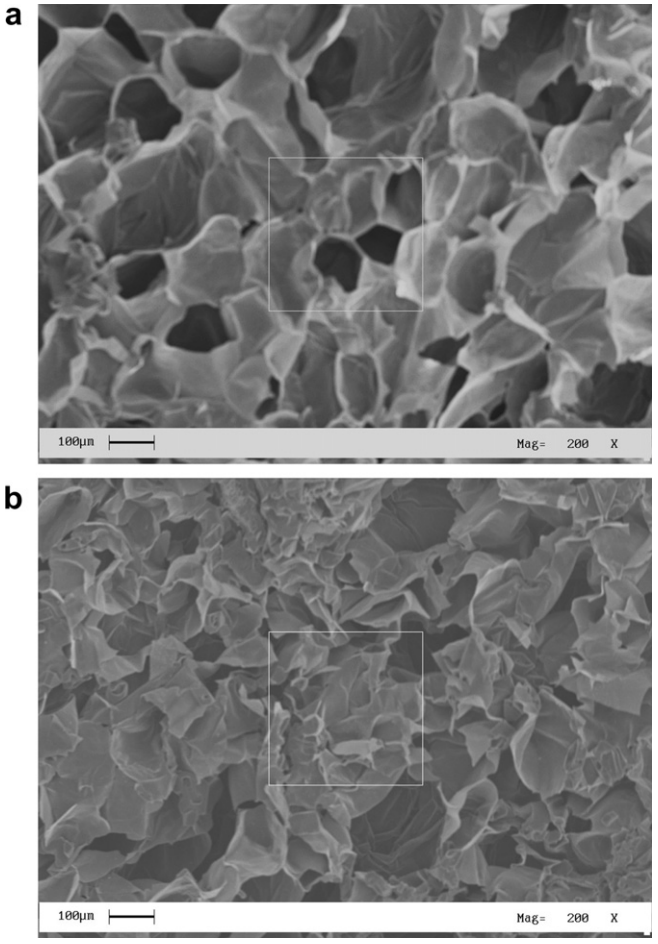

Fig. 6. SEM of untreated (a) and pre-treated (b) apple tissue after eight cold storage days.

(Table 3) showed a slow down of inter-tissue spaces area (V) for both samples that, for the U apple sample, was due to the effect of storage period and for A apple sample was caused by the action of trehalose.

3.2. Microbial growth

The initial count of aerobic mesophilic micro-organisms on fresh apple slices was about 2.85 log cfu per g, whereas the yeast and mould loads were about 1.48 log cfu per g. Although the micro-organisms of fruit are mainly represented by yeast and moulds, the higher aerobic mesophilic load found in the apple samples was probably due to the fruit processing (washing, peeling and cutting operations) that causes the substitution of the yeast and mould population by bacteria (Soliva-Fortuny, Elez-Martinez, & Martin-Belloso, 2004). During the storage period, for both U and A samples, an increase in mesophilic aerobic counts was observed (Fig. 7), but the growth rate observed in U apple samples was higher than in A ones. A similar trend was observed in yeast and mould growth during storage (Fig. 8), with a more marked difference between the A and U samples. An explanation of this phenomenon is related to the hydrophilic properties of trehalose and its ability to form an amorphous glass film that allows a reduction in water activity (Sussich, Urbani, Princevalle, & Cesaro, 1998) on the surface of the apple slices. Many authors, in fact, suggest superficial water removal by draining, to avoid microbial spoilage on the fruit surface (Bett et al., 2001; Soliva-Fortuny et al., 2004).

Table 3
V/M ratio of apple samples tissue (U: untreated; A: pre-treated) at start and end of storage period

	U		A	
	0 days	8 days	0 days	8 days
M (μm^2)	$1.17E \times 10^6$	$1.17E \times 10^6$	$1.17E \times 10^6$	$1.177E \times 10^6$
V (μm^2)	$2.76E \times 10^5$	$1.46E \times 10^5$	$2.21E \times 10^5$	$1.12E \times 10^5$
E (V/M)	0.235	0.125	0.189	0.0961

V = inter-tissue spaces area, and M = tissue area.

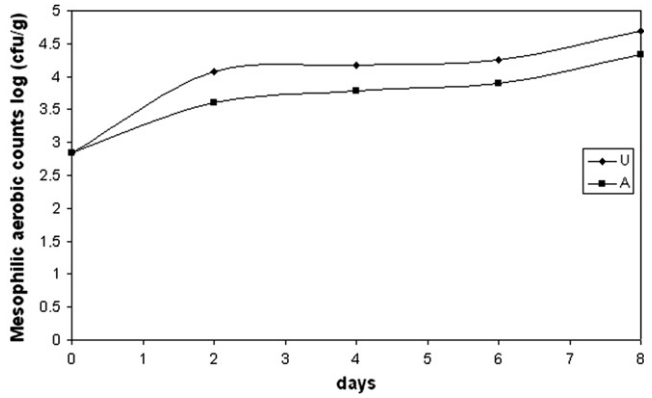

Fig. 7. Aerobic mesophilic microbial growth in apple samples during cold storage (U: untreated samples; A: pre-treated samples).

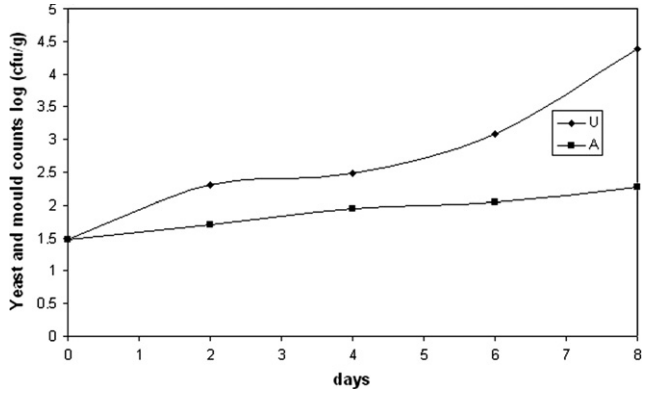

Fig. 8. Yeast and mould growth in apple samples during cold storage (U: untreated samples; A: pre-treated samples).

Table 4
Colour and firmness score[a] in apple samples during cold storage period (U: untreated samples; A: pre-treated samples)

Days	U		A	
	Colour	Firmness	Colour	Firmness
0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
2	1.5 ± 0.5	3.6 ± 0.7	3.5 ± 0.7	3.8 ± 0.4
4	1.3 ± 0.5	3.6 ± 0.5	3.0 ± 0.7	3.7 ± 0.5
6	1.0 ± 0.0	3.3 ± 0.5	3.0 ± 0.5	3.4 ± 0.5
8	1.0 ± 0.0	3.2 ± 0.4	2.7 ± 0.7	3.4 ± 0.5

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

3.3. Sensory quality assessment

The untrained panel detected the rapid degradation of colour in control apple samples stored unpackaged, mainly by the appearance of undesirable brown pigments on the apple slices' surface (Table 4). The degree of unacceptable colour, was reported by the panel soon after 2 storage days, whereas the colour of A apple samples was deemed acceptable even on the 8th day mark.

Small differences in texture changes for both samples were detected by the panel, who judged firmness to be acceptable across all 8 days.

4. Conclusions

The present work studied the effects of dipping in an innovative solution on m.p. apples in cold storage. The collected data showed that in trehalose-coated apple samples, the changes in physical and chemical parameters (weight loss, colour, malic acid, ascorbic acid and polyphenol content) were lower than in uncoated samples. Moreover, the pre-treatment was effective in retarding the growth of yeast, moulds and aerobic mesophilic bacteria. No differences were detected in firmness values between

both samples during storage. The ability of trehalose to slow down the quality changes of apple samples could be related to the flexibility in the glycosidic bond between the two D-glucose molecules of trehalose and to the property of conforming its shape to the irregular polar groups of polyphenol compounds, thus forming a coating film on the apple surface that slows down browning reactions and weight loss.

Acknowledgements

This work was financed by "VATIPICI" 2002–2005 project supported by the Ministry for University and Scientific Research of Italy and the Ministry for Agricultural and Forestal Policies of Italy.

References

Ahvenainen, R. (1996). New approaches in improving the shelf life of minimally processed fruit and vegetables. *Trend in Food Science & Technolgy, 7*, 179–187.

Albanese, D., Del Genio, F., Del Vaglio, M., & Di Matteo, M. (2003). Il trealosio come coadiuvante nell'essiccazione di vegetali. *Ingredienti Alimentari, 2*, 6–9.

Albanese, D., Cinquanta, L., Del Vaglio, M., & Di Matteo M. (2001) Drying processes for quality tomatoes. In *Proceeding of fifth italian conference on chemical and process engineering* (pp. 895–898) vol. 2.

Baldwin, E. A., Nisperos-Carriedo, M. O., & Baker, R. A. (1995). Edible coatings for lightly processed fruit and vegetables. *HortScience, 30*, 35–38.

Bett, K. L., Ingram, D. A., Grimm, C. C., Lloyd, S. W., Spanier, A. M., Miller, J. M., et al. (2001). Flavor of fresh cut gala apples in barrier film packaging as affected by storage time. *Journal of Food Quality, 24*, 141–156.

Boyer, L., & Liu, H. R. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal, 3*, 1–15.

Colaco, C. A. L. S., & Roser, B. (1995). Trehalose – A multifunction additive for food preservation. In M. Mathlouthi (Ed.), *Food packaging and preservation* (pp. 123–140). London: Blackie Professional.

Day, B. P. F. (2003). Novel map applications for fresh-prepared produce. In R. Ahvenainen (Ed.), *Novel Food packaging techniques*. CRC Press.

Komes, D., Lovrić, T., Ganić, K. K., Kljusurić, G. J., & Banovic, M. (2005). Trehalose improves flavour retention in dehydrated apricot puree. *International Journal of Food Science & Technology, 40*, 425–435.

Lo Scalzo, R., Testoni, A., & Genna, A. (2001). "*Annurca*" apple fruit, a southern Italy apple cultivar: textural properties and aroma composition. *Food Chemistry, 73*, 333–343.

Monsalve-Gonzales, A., Barbosa-Canovas, G. V., McEvily, A. J., & Iyengar, R. (1995). Inhibition of enzymatic browning in apple products by 4-hexyresorcinol. *Food Technology, 49*, 110–118.

Park, H. J. (1999). Development of advanced edible coatings for fruits. *Trends in Food Science and Technology, 10*, 254–260.

Pizzocaro, F., Torregiani, D., & Gilardi, G. (1993). Inhibition of apple polyphenoxidase (PPO) by ascorbic acid, citric and sodium chloride. *Journal of Food Processing Preservation, 17*, 21–30.

Richards, A. B., Krakowka, S., Dexter, L. B., Schmid, H., Wolterbeek, A. P. M., Waalkens-Berendsen, D. H., et al. (2002). Trehalose: A review of properties, history of use and human tolerance, and results of multiple safety studies. *Food and Chemical Toxicology, 40*, 871–898.

Rocculli, P., Romani, S., & Della Rosa, M. (2004). Evaluation of physico-chemical parameters of minimally processed apples packed in non-conventional modified atmosphere. *Food Research International, 37*, 329–335.

Rocha, A. M. C. N., & Morais, A. M. M. B. (2003). Shelf life of minimally processed apple (cv. Jonagored) determined by colour changes. *Food Control, 14*, 13–20.

Roser, B. (1991). Trehalose, a new approach to premium dried foods. *Trends in Food Science and Technology, 7*, 166–169.

Sapers, G. M., & Douglas, F. W. (1987). Measurement of enzymatic browning at cut surfaces and in juice of raw apple and peer fruits. *Journal of Food Science, 52*, 1258–1262.

Singleton, V. L., & Rossi, A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology Viticolture, 16*, 144–158.

Soliva-Fortuny, R. C., Elez-Martinez, P., & Martin-Belloso, O. (2004). Microbiological and biochemical stability of fresh cut apples preserved by modified atmosphere packaging. *Innovative Food Science and Emerging Technology, 5*, 215–224.

Soliva-Fortuny, R. C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinsten, S., & Martin-Belloso, O. (2001). Browning evaluation of ready-to-eat apples as affected by modified atmosphere packaging. *Journal of Agricultural and Food Chemistry, 49*, 3685–3690.

Sussich, F., Urbani, R., Princevalle, F., & Cesaro, A. (1998). Polymorphic amorphous and crystalline forms of trehalose. *Journal of the American Chemical Society, 120*, 7893–7899.

Tu, K., Nicolai, B., & De Baerdemaeker, J. (2000). Effects of relative humidity on apple quality under simulated shelf temperature storage. *Scientia Horticulture, 85*, 217–229.