

Liposomal and edible coating as control release delivery systems for essential oils: comparison of application on storage life of fresh-cut banana

M. Alikhani-Koupaei

Higher Educational Complex of Saravan, P.O. Box 9951634145, Saravan, Iran; mjd_alikhani@yahoo.com

Received: 1 June 2013 / Accepted: 13 November 2013

© 2014 Wageningen Academic Publishers

RESEARCH ARTICLE

Abstract

Bananas processed as fresh-cut products are highly sensitive to browning and softening. New post-harvest methods, such as the use of essential oils, are imperfect for preserving banana slices long enough to be marketable. To enable control release of oil and increases in antioxidant and antimicrobial activities, we successfully incorporated oil into liposome to form liposomal oil. The effects of oil and liposomal oil of rosemary combined with mucilage on the quality of fresh-cut banana were examined. Banana slices were subjected to a 5 min dip into solutions (1% rosemary oil (Ro), 1% rosemary oil plus 2% mucilage (Mu+Ro) and 1% rosemary liposomal oil plus 2% mucilage (Mu+LipoRo)) before being placed in a plastic tray and covered with a 0.04-mm thick plastic bag with six small pores in each bag. Physicochemical qualities were evaluated during 9 days storage at 5 °C. All treatments prevented product weight loss and the decrease of polyphenoloxidase and lipoxygenase activities during storage. There were small changes in colour, firmness, pH, titratable acidity, total soluble solids values and total phenolic content. Sensory analyses showed that minimally processed bananas were within the acceptable limits during 9 days of storage at 5 °C and Mu+LipoRo treatment had the greatest effect. The use of Mu+LipoRo preserves fresh-cut bananas for up to 9 days after processing and the results suggest that Mu+LipoRo treatment has the potential to improve the quality of fresh-cut bananas and extend their shelf life.

Keywords: banana, edible coating, essential oil, liposomal, storage life

1. Introduction

Newly cut fruits are attractive to consumers because they are fresh, nutritious, low priced, and ready-to-eat. Banana is highly perishable and does not freeze, hence minimal processing of fresh cut fruits is an important area in restricting decay. Minimally processed fruits are characterised by a shorter shelf life than their whole counterparts, because of their greater susceptibility to microbial spoilage, increased respiration rate and ethylene production, which is stimulated by wounding of the tissue (Chien *et al.*, 2007). In the last two decades, food scientists have attempted to develop new technologies that improve the quality and quantity of fresh-cut products, with the main aim of increasing their production without affecting quality and the environment, and at the same time fulfilling consumer expectations. At the same time, consumers have

become more critical to the use of synthetic additives to preserve foods or enhance characteristics such as colour and flavour (Corbo *et al.*, 2009). As a response to consumer pressure to reduce or eliminate chemically synthesised additives, foods preserved 'as natural as possible' are gaining more and more attention and numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth in fresh-cut fruits.

Essential oils are an ethereal fraction obtained by physical means from a plant. More recently, many applications of essential oils as natural antimicrobial and antioxidant agents have been described. Essential oils of coriander, mint, rosemary, parsley and citrus fruit peels, carbonyl compounds, or isothiocyanates obtained from cruciferous vegetables have been tested (González-Aguilar *et al.*, 2010). Essential oils are sensitive materials which can easily suffer

degradation by the action of oxygen, light and moderate temperatures. Furthermore, they are insoluble in water and for certain applications a controlled release is required. Essential oil formulations to achieve a controlled release include different coating forms as edible coating and liposomes.

An edible coating is a thin layer of edible material (hydrocolloid or lipid) applied on the surface of a food product with the purpose of generating a semi-permeable barrier to gases, water vapour, and volatile compounds. Edible coatings extend the shelf life of fresh-cut products by decreasing respiration, senescence and protecting aroma, texture and colour (Olivas and Barbosa-Canovas, 2005). Compounds most commonly are used to form edible coatings include chitosan, starch, cellulose, alginates, carrageenan, zein, gluten, whey, carnauba, mucilage and fatty acids (Allende *et al.*, 2006). Naturally occurring compounds with antimicrobial capacity, such as phenols, chitosan and organic acids have been tested to prove their effectiveness in food products (Lanciotti *et al.*, 2004).

In most cases, some additives are added to the coating formulation to help the preservation of the quality of fresh-cut produce (Ayala-Zavala *et al.*, 2008) and, in particular, the functionality of edible coatings can be expanded by incorporating antimicrobial compounds (Oussalah *et al.*, 2006; Rojas-Graü *et al.*, 2007).

Different antimicrobial agents can be used as dipping or filling solutions and treatments with edible coatings and have been found to extend the shelf life of fresh-cut fruits (Campaniello *et al.*, 2008; Rico *et al.*, 2007). The addition of antimicrobial agents, such as essential oils as part of edible coatings can extend fruit shelf life through slow release of the effective ingredients onto the surface of the product and by maintaining their high concentration during the packaging process (Ouattara *et al.*, 2000).

Currently, some novel methods have been introduced to improve stability and bioavailability of essential oils using liposomal encapsulation (Shoji and Nakashima, 2004), which reduces reactivity with the environment (water, oxygen, light), decreases the evaporation or the transfer rate to the outside environment, promotes handling ability, masks taste and enhances dilution to achieve a uniform distribution in the final product when used in very small amounts (Gibbs *et al.*, 1999). Liposome can be constructed of natural constituents such as lecithin from egg yolk or soy beans and cholesterol so it is non-toxic. The use of liposomal incorporation as an antimicrobial agent could aid in the protection of foods against the growth of spoilage and pathogenic microorganisms (Taylor and Davidson, 2005).

Since liposomes have already demonstrated the capability to control deliver various antibacterial agents and to protect

their contents from environmental exposure. We have carried out research into the comparison of essential oil and liposomal oil of rosemary combined with a mucilage edible coating to evaluate the influence of the control release delivery systems on physico-chemical qualities of fresh-cut banana cv. Dwarf Cavendish.

2. Materials and methods

Essential oil and mucilage extraction and purification

For essential oil extraction, dry plant materials of rosemary were distilled within 24 h in a steam distiller with an aqueous phase recycling system, using a plant material to water ratio of 2:1. The distillation time was about 2 h and the oil obtained was separated from the aqueous solution and dried by treating with anhydrous Na₂SO₄. Each essential oil was transferred into a dark glass flask filled to the top and kept at a temperature of 4 °C until used (Meepagala *et al.*, 2002). Also for mucilage extraction, the skins of cactus stems were removed and the remaining material cubed (1 cm³). Samples were homogenised (20% w/v) in distilled water. The slurry was centrifuged for 10 min at 4,500 rpm and the supernatant precipitated in ethanol and finally dried (Sáenz *et al.*, 1992).

Preparation of liposomal oil

Multi-lamellar vesicles were prepared according to the thin film hydration method of Gortzi *et al.* (2006). A lipid solution was prepared by dissolving 5 mg/ml of phosphatidylcholine, 1 mg/ml cholesterol and 0.1 mg/ml essential oil in 3 mg/ml chloroform. Phosphatidylcholine from fresh egg yolk and chloroform were obtained from Sigma Chemicals Company Ltd. (St. Louis, MO, USA). Cholesterol was purchased from Fluka (Buchs, Switzerland). 5.0 ml of the lipid solution was introduced in a 100 ml round-bottomed flask. The solvent was evaporated in a Heidolph Laborota rotary evaporator (model 4000; Heidolph Laborota, Schwabach, Germany), at 35-40 °C, under reduced pressure (13-14 mm Hg). The dry lipid film so obtained was hydrated with 5 ml distilled water. Mechanical stirring of the lipids in aqueous medium was performed with the rotary evaporator equipment at 37 °C followed by manual stirring in the water bath, for 2 h, at the same temperature. This suspension was allowed to hydrate for 2 h in order to anneal any structural defects. The compound incorporated vesicles were separated from the unincorporated compounds by centrifugation. Vesicular dispersions were spun in a laboratory centrifuge (Hettich Universal 320 R; London, UK), at 10 °C, 10,000 rpm, for 60 min. The supernatant was removed and the liposomes were reconstituted with distilled water.

Preparation of coating solutions

The method employed for preparing the coating solutions was developed by Ojagh *et al.* (2010) with minor modifications. The mucilage solution (2.0%) containing 1.0% acetic acid as a solvent and glycerol plasticiser (0.75%) was stirred by a magnetic stirrer at room temperature for 1 h to obtain complete dispersion. Then the rosemary oil with 0.1 mg/ml concentration (1.0%), mixed with Tween 80 (0.2%), to help distribute and completely incorporate the rosemary oil, or liposomal rosemary oil prepared as described in the previous paragraph (1.0%), were added to the mucilage solution and then stirred using a magnetic stirrer for 30 min. The final solution was centrifuged for 5 min at 4,500 rpm and the supernatant obtained was used to prepare the edible coating.

Sample preparation

Bananas (*Musa acuminata* AAA Group cv. Dwarf Cavendish) at ripeness stage 4 (peel more yellow than green) and free from visual defects were directly obtained from the local plantation in Chabahar seaport, Iran. The bananas were peeled and cut into ten 15 mm thick slices. Three replicates of 10 slices of different bananas per treatment and per day were analysed for the effect of different treatments. The samples were placed in an ice bath immediately after cutting. Four different treatments were used: (1) slices dipped in distilled water (control); (2) rosemary oil with 0.1 mg/ml concentration alone (Ro); (3) rosemary oil plus mucilage (Mu+Ro); and (4) liposomal rosemary oil plus mucilage (Mu+LipoRo). For coating treatments, the slices were submerged for 5 min in the coating solution. Then a tissue paper was used to absorb excess solution from the surface, and the slices were placed in a plastic tray and covered with a 0.04-mm thick plastic bag, and six small pores were made per bag. All samples were stored at 5 ± 0.5 °C and 55% RH and effects of treatments after intervals of 3 days for 9 days storage in total period were studied.

Quality evaluation of fresh-cut banana

Colour

The colour on opposite sides of each slice was measured with a Minolta CR-300 colorimeter (Minolta Co. Ltd., Osaka, Japan) in the CIE $L^*a^*b^*$ mode CIELAB colour space. The parameters determined where L^* ($L^* = 0 =$ black, and $L^* = 100 =$ white), a^* ($-a^* =$ greenness and $+a^* =$ redness), b^* ($-b^* =$ blueness and $+b^* =$ yellowness), chroma (C) (intensity or colour saturation). C was calculated using the formula $C = (a^2 + b^2)^{1/2}$.

Firmness

Firmness of each slice was measured using a TA-XT2 penetrometer (Stable Microsystems Texture Technologies Inc., Godalming, UK) by measuring the force required for a plunger of 2 mm in diameter to penetrate 10 mm into the cut surface at a rate of 5 mm/s. Each slice was punctured twice in each opposite side.

pH, titratable acidity and total soluble solid

10 g of pulp from each replicate were pureed and homogenised with 100 ml distilled water. The pH was measured by using a Quick 31314 pH meter (Hanna Instruments Inc., Woonsocket, RI, USA). The suspension was titrated with 0.1 N sodium hydroxide and the results were expressed as mg malic acid per 100 g sample, the most common acid in this fruit (Cemeroglu, 1992). The total soluble solids content was measured by Atago digital refractometer (Model PAL-1; Tokyo, Japan), after diluting 10 g of the banana puree in 30 ml of deionised water and blending with a homogenised for 2 min.

Weight loss

For the weight loss measurement, a banana slice from each replicate was oven dried at 70 °C for 24 h, and then put in a desiccator to cool to room temperature. Weight losses were detected by considering the differences between initial dry weight and final dry weight of currently tested slices divided by their initial dry weight.

Sensory analyses

A panel of six trained persons, aged between 20 and 40 years old, carried out sensory analysis at days 3, 6 and 9 of storage. The panellists received three samples of four treatments per evaluation in random order. The samples were presented to the panellists in individual rooms. The attributes evaluated were: firmness, colour, juiciness, flavour (including sweetness, acidity and odour), taste and visual appearance based on a nine-point scale, scoring from one (lowest) to nine (highest), where five means acceptable (limit of marketability). The panellists average response was calculated for each attribute (Deng *et al.*, 2005).

Determination of the polyphenoloxidase and lipoxygenase enzymes activity

For the polyphenoloxidase (PPO) assay 10 g of banana slices from the ice bath were homogenised at a 1:2 with 0.5 mol/l phosphate buffer (pH 7.0) in the presence of 50 g/l polyvinylpyrrolidone (PVPP) using a commercial blender (Stomacher 400 Circulator Homogeniser; Seward Ltd., Worthing, UK) and centrifuged at 5,010 rpm and 4 °C for 15 min. The supernatant, which contained PPO

activity, was used as the experimental enzyme source. The substrate mixture contained 20 mmol/l catechol as substrate in 5 mmol/l sodium phosphate buffer (pH 7). The reaction cuvette contained 2.9 ml substrate mixture and 0.1 ml the supernatant. The rate of catechol oxidation was followed at 25 °C at 400 nm (Shimadzu UV-Vis 1601 spectrophotometer; Shimadzu, Tokyo, Japan) for 60 and 120 s. The enzyme activity unit was defined as a 0.001 change in absorbance between 60 and 120 s under the assay conditions (Ihl *et al.*, 2003).

For the lipoxygenase (LOX) assay, 10 g of banana slices in an ice bath were homogenised in 20 ml Tris-HCl buffer (100 mM, pH 8.0) containing 0.5 g of PVPP at 4 °C. Then the mixture was centrifuged for 15 min at 19,000 rpm at 4 °C. The supernatant, which contained LOX activity, was used as the experimental enzyme source. The substrate mixture contained 157.2 µl linoleic acid as substrate in 157.2 µl Tween 80 and 10 ml distilled water that diluted to 200 ml with 0.2 M phosphate buffer (pH 7.0). The reaction cuvette contained 2.7 ml substrate mixture and 0.3 ml the supernatant. The rate of linoleic acid catalysis was followed at 25 °C at 234 nm (Shimadzu UV-Vis 1601 spectrophotometer). The enzyme activity unit was defined as a 0.001 change in absorbance under the assay conditions at 3 min period (González-Aguilar *et al.*, 2004).

Total phenolic determination

The total free phenolic content was estimated spectrophotometrically using the method described by Singleton and Rossi (1965). Briefly, banana slices (5 g) were pureed and homogenised with 25 ml water. The mixture was centrifuged at 5010 rpm and 4 °C for 15 min. 4 ml of sodium carbonate solution (75 g/l) was added to 1 ml of the centrifugate, then the reaction mixture was allowed to react with 5 ml Folin-Ciocalteu solution (0.2 N). The absorbance of the solution at 765 nm was measured after

2 h with a Shimadzu UV-Vis 1601 spectrophotometer. The standard curve was established using gallic acid.

Microbiology

For the microbiological analysis, banana slices (10 g) were removed aseptically from each package and were transferred into a sterile plastic package to which 90 ml Ringer's sterile solution was added. The sample and the Ringer's solution were blended for 60 s by using a stomacher, 400 Circulator (Seward Ltd., Thetford, UK). The Portuguese standard methods, EN ISO 4833 (ISO, 2003), was followed for counting total aerobic plate count (TPC).

Statistical analysis

A randomised design with three replicates was used. All data obtained from the trials were analysed using analysis of variance (ANOVA) and using the computer software SPSS, version 15.0 (SPSS Inc., Woking, UK). Means were compared using the LSD test at the $P < 0.05$ level.

3. Results and discussion

Quality evaluation of fresh-cut banana

Colour

The colour coordinates of the control samples were $L^* = 65.2 \pm 1.2$ and $C = 31.4 \pm 2$. The colour of fresh-cut banana was modified as a result of storage time, and both L^* and C were significantly reduced ($P < 0.05$) after 9 days storage (Figures 1 and 2). The results suggested that, after a short immersion in the coating solutions, banana slices were able to maintain their colour quality. In fact the C parameter indicates the degree of saturation of colour and a decrease in C is as the result of enzymatic browning. On the third day of storage, treated samples did not significantly differ

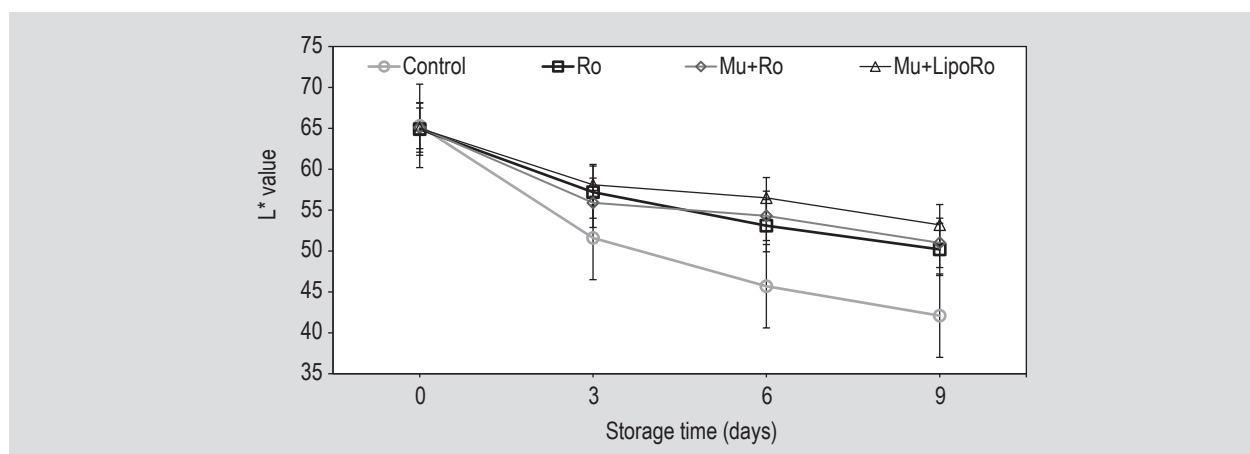


Figure 1. Effect of different treatments on L^* values of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

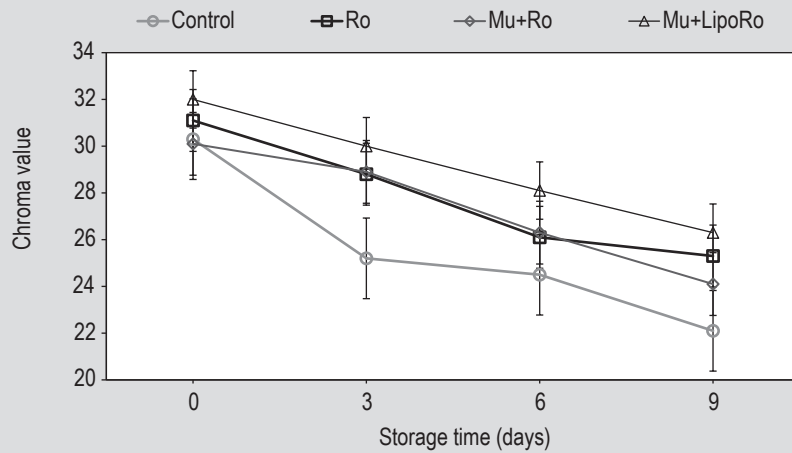


Figure 2. Effect of different treatments on chroma values of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

from each other in terms of L^* values (Figure 1). After 9 days of storage, Mu+LipoRo showed the least changes in L^* and C values, revealing a lower browning incidence. Bico *et al.* (2009) observed least change in L^* and C values in banana slices treated by immersion in a solution of 1% calcium chloride, 0.75% cysteine and 0.75% ascorbic acid plus storage in a controlled atmosphere.

Firmness

Significant decreases in firmness took place during storage period and after 9 days storage. The Mu+LipoRo treatment and control showed the lowest and highest rates of firmness loss, respectively (Figure 3). Mu+Ro and Mu+LipoRo had a similar effect on preventing firmness loss in banana slices during the first 3 days of storage. Despite the hydrophilic character of polysaccharides, they can act as a barrier to water transfer, delaying dehydration and, therefore

extending the firmness of the coated fruit. A softening process in fruits has been reported to be dependent on the increase in polygalacturonase, β -galactoxidase and pectinmethylesterase activities (Barret and Gonzalez, 1994). Essential oils from antioxidant phenolics could reduce the action of cell-wall degrading enzymes.

pH, titratable acidity and total soluble sugars

The results revealed that pH increased as titratable acidity decreased with storage time. This was expected because malic and citric acids, which are the prevalent organic acids in banana, were used as substrates for the enzymatic reactions of respiration. The results presented in Table 1 indicate that the application of (Mu+LipoRo) and (Mu+Ro) treatment significantly delays losses in titratable acidity (TA) of banana slices ($P < 0.05$). The application of (Mu+LipoRo) resulted in lowest average pH in fresh cut

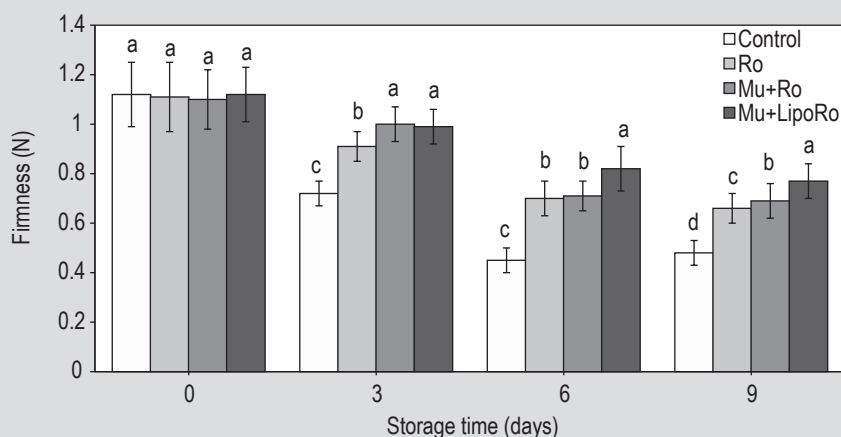


Figure 3. Effect of different treatments on firmness of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

Table 1. Effect of different treatments on total soluble solids (TSS), titrable acidity (TA), pH of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C.

Quality parameters	Storage periods (day)	Control	Ro	Mu+Ro	Mu+LipoRo
TSS (%)	0	11.8 ^a	11.8 ^a	11.7 ^a	11.8 ^a
	3	15.25 ^b	15.30 ^b	13.40 ^a	13.45 ^a
	6	19.76 ^d	16.48 ^b	15.41 ^a	17.34 ^c
	9	21.77 ^d	18.49 ^b	17.39 ^a	19.35 ^c
TA (mg/100 g)	0	0.38 ^a	0.38 ^a	0.38 ^a	0.38 ^a
	3	0.34 ^b	0.35 ^b	0.36 ^a	0.36 ^a
	6	0.31 ^b	0.31 ^b	0.33 ^a	0.32 ^a
	9	0.30 ^b	0.30 ^b	0.32 ^a	0.32 ^a
pH	0	4.4 ^a	4.4 ^a	4.4 ^a	4.4 ^a
	3	4.5 ^a	4.5 ^a	4.5 ^a	4.5 ^a
	6	4.6 ^b	4.6 ^b	4.6 ^b	4.5 ^a
	9	4.8 ^b	4.8 ^b	4.8 ^b	4.7 ^a

Means with the same letters within a period of storage are not significantly different at $P < 0.05$ using least significant difference.

Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

banana, 4.7, compared with 4.8 in the control slices at day 9. Liposomal oil could inhibit TA loss may be due to the protection of antioxidant phenolics in essential oils. Previous studies showed that the edible coating functioned as a self-controlling atmosphere and selectively permeated C_2H_4 , CO_2 and O_2 inside and out of the fruit, thus reducing fruit respiration metabolism (Hagenmaier, 2005). Reduced respiration rate may be reflected in lower changes in pH, TA and total soluble sugars (TSS) (Beaulieu and Gorny, 2002). There was no direct relationship between pH and TA due to variations of buffer capacity of the organic acids present in the sample, although in general, higher acid levels in fruit are often associated with lower pH values and vice versa (El Ghaouth *et al.*, 1991).

TSS contents in control banana slices gradually increased during the storage, and this was inhibited by (Mu+Ro) and (Mu+LipoRo) treatments. The TSS of the control showed a significantly higher value during storage, while Mu+Ro showed the lowest increase in TSS with storage time. The inhibition of TSS increase with these treatments was probably due to slowing down of respiration and metabolic activity, hence retarding the senescence process.

Weight loss

Water loss is factor that affects fresh-cut fruit quality. The control samples had the highest weight loss (3.1%) and Mu+LipoRo the lowest weight loss (1.27%), followed by Mu+Ro (1.31%), (Figure 4), possibly due to decreased respiration rates and reduced moisture transfer, and a

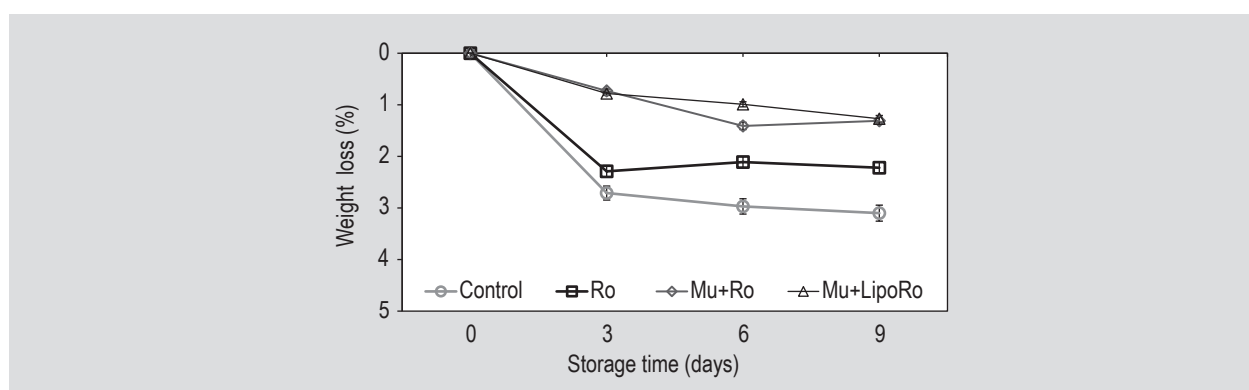


Figure 4. Effect of different treatments on weight loss of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

high selective gas permeability ratio CO_2/O_2 . Previous experiments using eugenol, thymol or menthol vapours revealed benefits due to reduced weight loss in cherries and grapes (Martinez-Romero *et al.*, 2005; Serrano *et al.*, 2005). Wong *et al.* (1994) reported apple slices coated with carbohydrate/lipid bilayer film presented reduced water loss between 12 and 14 times compared to uncoated apple slices in similar storage conditions. The loss of water decreased turgor and firmness of fresh-cut banana due to the absence of cuticle and exposure of internal tissues (Beaulieu and Gorny, 2002). The gradient of water vapour pressure at different locations is the primary reason for moisture loss from fresh fruits and vegetables. The refrigerator used in the research was running with around 60% RH and this could be another reason of high weight losses of the control and Ro.

Sensory analyses

Figure 5 shows the results of the sensory evaluation during storage. It can be seen that after 3, 6 and 9 days of storage, Mu+LipoRo had the highest sensory scores, while control had the lowest for the sensory attributes

evaluated. On day 3, unlike other scores, the highest scores were given for taste in the control treatment because the panellists preferred the natural taste of the cut fruit. For all treatments, sensory attributes decreased with storage time. Also, it can be observed that Ro coated fruits had lower scores than Mu+LipoRo and Mu+Ro, while there were no significant differences between Mu+LipoRo coating and Mu+Ro in most of the sensory attributes evaluated ($P < 0.05$). On the other hand, flavour and taste scores were highest for the Mu+Ro treatment on day 6. After 6 days of storage, better results for visual appearance and marketability were obtained for Mu+LipoRo and Mu+Ro treatments, while the control presented a very obvious decrease and had the lowest scores (3.4), to the extent of being unacceptable for consumers, largely due to significant shrivelling and browning. In conclusion, the Mu+LipoRo treatment seemed to best maintain sensory properties.

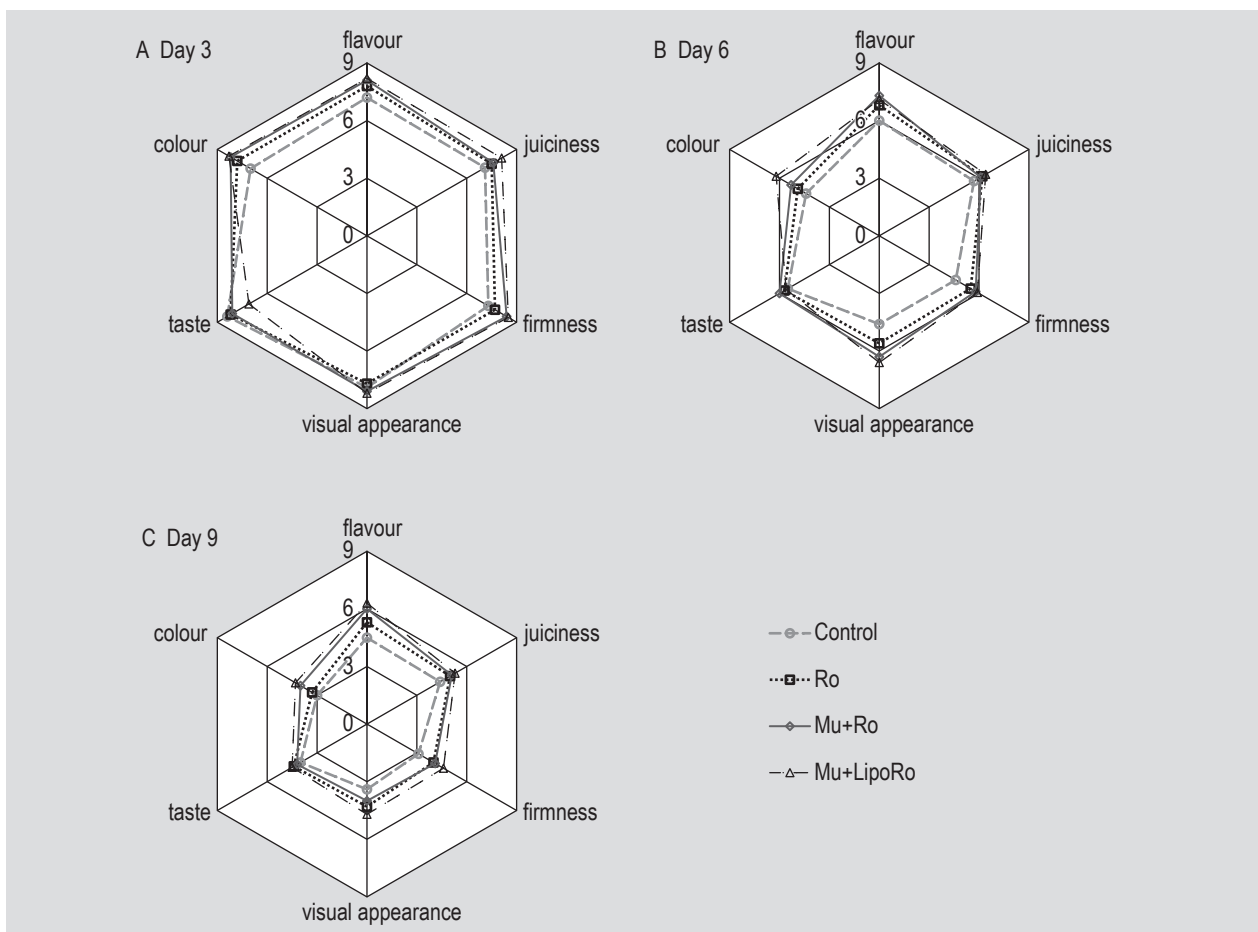


Figure 5. Effect of different treatments on sensory scores of fresh-cut banana variety Dwarf Cavendish during 3 (A), 6 (B) and 9 (C) days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

Activities of polyphenoloxidase and lipoxygenase enzymes and total phenolic content

PPO and LOX activities significantly increased with storage time. Control cut bananas showed a significant increase in PPO and LOX activities over time. All treatments significantly inhibited the increase of PPO and LOX activities. Mu+LipoRo-treated cut fruits had significantly lower PPO (37,400 U/min/g FW) and LOX (41,300 U/min/g FW) activities on day 9 (Figure 6 and 7).

The control showed a large decrease in total phenolic after 9 days, while Mu+LipoRo produced the lowest decrease (52.1 mg/100 g FW by day 9). The control had the lowest total phenolic content during storage. The total free phenolic level of control bananas (36.1 mg/100 g FW on day 9) also

decreased more rapidly than that with other treatments (Figure 8).

Banana is a highly perishable fruit that is extremely sensitive to low temperatures which increase lipid peroxidation in the cell membrane. LOX is a participator in fruits senescence, and carries out peroxidation reactions on plasma membrane lipids which could increase the level of lipid unsaturation and increase membrane fluidity (Lee *et al.*, 2005). Cold stress increased LOX activity in maize seedlings, which suggests that LOX may be included in the occurrence of chilling injury (Pinhero *et al.*, 1998). The reduction of chilling injury symptoms of cut bananas by different treatments played an important role in the inhibition of LOX activity and the resulting increase of storage life.

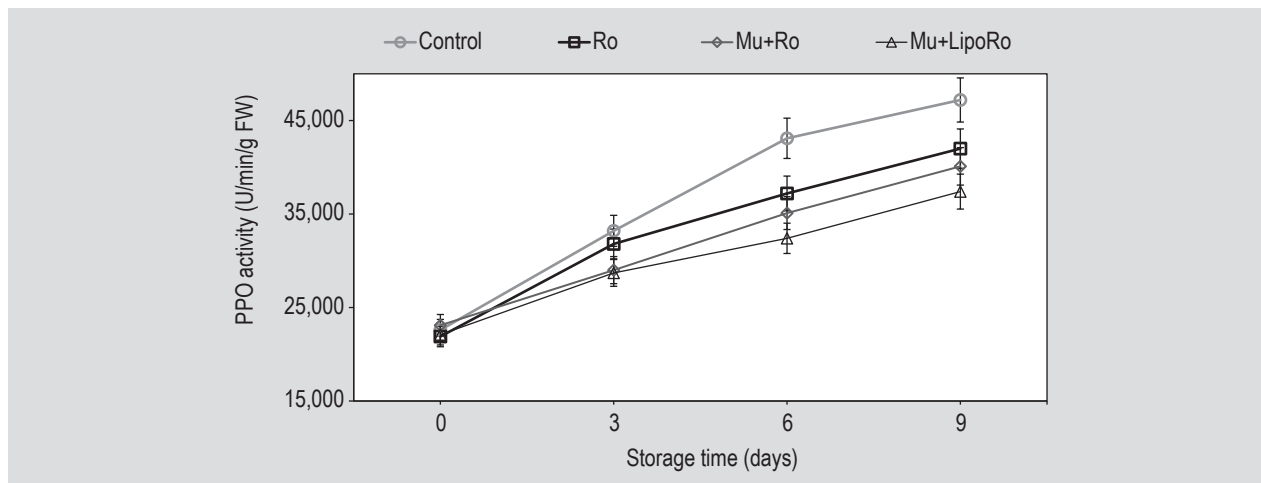


Figure 6. Effect of different treatments on polyphenoloxidase activity of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

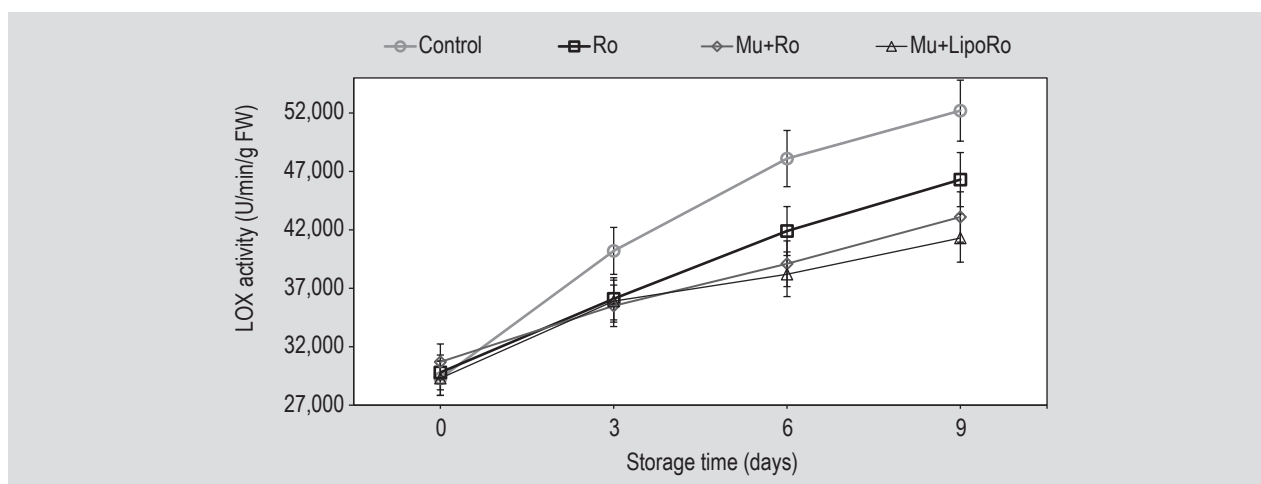


Figure 7. Effect of different treatments on lipoxygenase activity of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

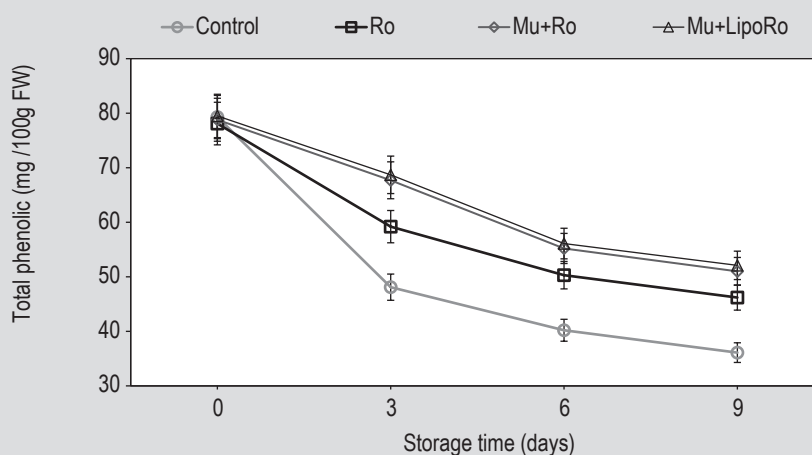


Figure 8. Effect of different treatments on total phenolic content of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C. Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

Oxidation of phenolic substrates by PPO is believed to be a major cause of the brown discoloration of many fruits and vegetables. The degree of browning in banana, after cutting, was correlated with PPO activity and the concentration of free phenolic substrates. The PPO reaction requires oxygen and the effect of low oxygen on browning in such systems is often attributed mainly to PPO (Vámos-Vigyázó, 1981).

Inactivation of these enzymes is considered necessary to minimise deterioration. Essential oils are a good source of antioxidant and antimutagenic phenolic (Kitazuru *et al.*, 2004). Also edible coatings have a high selective gas permeability ratio CO_2/O_2 compared with conventional synthetic films. They can also act as carriers of product additives such as antioxidants and/or antimicrobial agents and can improve the mechanical integrity or handling characteristics of the product (Gontard *et al.*, 1996). Therefore, all treatments have positive effects on the reduction of these enzymes activities.

Microbiology

TPC showed that, on day 0, the total number of aerobic mesophilic microorganisms contained in all banana samples were below the detection limit, 10 cfu/g (Table 2). However, on day 3 and 6 there were a significantly higher number of counts in the untreated sample (control) compared to the other treatments. On day 9, control and RO had the highest microbial count, while Mu+LipoRo and Mu+Ro treatments had a lower count of <400 cfu/g. Jacxsens *et al.* (2002) reported that the critical limit for TPC for vegetables is 10^8 cfu/g. Olivas and Barbosa-Canovas (2005) contend that the modified atmosphere created by coating may change the growth rate of spoilage and pathogenic bacteria. The modified atmosphere may inhibit the growth of organisms usually responsible for spoilage, while encouraging the growth of pathogens.

Table 2. Effect of different treatments on total aerobic plate count of microorganisms of fresh-cut banana variety Dwarf Cavendish during 9 days of storage at 5 ± 0.5 °C.

Microbiology parameter	Storage periods (day)	Control	Ro	Mu+Ro	Mu+LipoRo
Total plate count (cfu/g)	0	<10 ^a	<10 ^a	<10 ^a	<10 ^a
	3	2.3×10^{2b}	<200 ^a	<200 ^a	<200 ^a
	6	4.1×10^{3b}	<400 ^a	<400 ^a	<400 ^a
	9	6.0×10^{5c}	4.2×10^{4b}	<400 ^a	<400 ^a

Means with the same letters within a period of storage are not significantly different at $P < 0.05$ using least significant difference.

Ro = 1% rosemary oil; Mu+Ro = 1% rosemary oil plus 2% mucilage; Mu+LipoRo = 1% rosemary liposomal oil plus 2% mucilage.

4. Conclusions

In the last two decades, food scientists have attempted to improve technologies that maintain the quality and quantity of fresh-cut products, with the main aim of increasing their production without affecting quality and the environment, while fulfilling consumer expectations. At the same time, consumers have become more critical to the use of synthetic additives to make food safe or enhance characteristics such as colour and flavour. The growing demand for lightly processed products with the same safety guarantees as those treated by traditional methods of preservation has urged researchers to focus most of their efforts on studying new ways of extending the shelf life of fresh-cut produce. Thus we evaluated the effects of essential oil and liposomal oil of rosemary combined with a mucilage edible coating on the physico-chemical qualities of fresh-cut banana cv. Dwarf Cavendish. To resolve the poor water solubility and to control release of the oil, we successfully incorporated the oil into liposome to form Liposomal oil. The liposomal oil combined with mucilage inhibited PPO, LOX activities, fruit softening and weight-loss, and retained higher firmness and soluble solids content. Chroma, L^* value, and pH also showed smaller changes compared to the control, while free rosemary oil dissolved in distilled water near the same concentration, had lesser effects on the physico-chemical attributes. The data presented suggest that Mu+LipoRo has potential for improving the quality of fresh-cut bananas. Further study disclosed that liposomal encapsulation of essential oils at optimal formulation can result in an increased availability of oils for extending product shelf-life.

Acknowledgements

The author wishes to thank Dr. Safabakhsh for the English editing. The author is also grateful to the Department of Agriculture (Esfahan University and Technology, Iran), and the Laboratory of Post Harvest Technology.

References

- Allende, A., Tomás-Barberán, F.A. and Gil, M.I., 2006. Minimal processing for healthy traditional foods. *Trends in Food Science & Technology* 17: 513-519.
- Ayala-Zavala, J.F., del Toro-Sanchez, L., Alvarez-Parrilla, E., Soto-Valdez, H., Martin-Belloso, O., Ruiz-Cruz, S. and Gonzalez-Aguilar, G., 2008. Natural antimicrobial agents incorporated in active packaging to preserve the quality of fresh fruits and vegetables. *Stewart Postharvest Review* 4: 1-9.
- Barrett, D.M. and Gonzalez, C., 1994. Activity of softening enzymes during cherry maturation. *Journal of Food Science* 59: 574-577.
- Beaulieu, J.C., Gorny, J.R. 2002. Fresh-cut fruits. In: Gross, K.C., Wang, C.Y. and Saltveit, M. (eds.) *The commercial storage of fruits, vegetables, and florist and nursery stocks*. Agricultural handbook number 66. Available at: <http://www.ba.ars.usda.gov/hb66/146freshcutfruits.pdf>.
- Bico, S., Raposo, M., Morais, R. and Morais, A., 2009. Combined effects of chemical dip and/or carrageenan coating and/or controlled atmosphere on quality of fresh-cut banana. *Food Control* 20: 508-514.
- Campaniello, D., Bevilacqua, A., Sinigaglia, M. and Corbo, M., 2008. Chitosan: antimicrobial activity and potential applications for preserving minimally processed strawberries. *Food Microbiology* 25: 992-1000.
- Cemeroglu, B., 1992. *Basic analysis methods in fruit and vegetable processing industry* [in Turkish]. Arsu Press, Ankara, Turkey.
- Chien, P.-J., Sheu, F. and Yang, F.-H., 2007. Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering* 78: 225-229.
- Corbo, M.R., Bevilacqua, A., Campaniello, D., D'Amato, D., Speranza, B. and Sinigaglia, M., 2009. Prolonging microbial shelf life of foods through the use of natural compounds and non-thermal approaches – a review. *International Journal of Food Science & Technology* 44: 223-241.
- Deng, Y., Wu, Y. and Li, Y., 2005. Effects of high O₂ levels on post-harvest quality and shelf life of table grapes during long-term storage. *European Food Research and Technology* 221: 392-397.
- El Ghaouth, A., Arul, J., Ponnampalam, R. and Boulet, M., 1991. Use of chitosan coating to reduce water loss and maintain quality of cucumber and bell pepper fruits. *Journal of Food Processing and Preservation* 15: 359-368.
- Gibbs, B.F., Kermasha, S., Alli, I. and Mulligan, C.N., 1999. Encapsulation in the food industry: a review. *International Journal of Food Sciences and Nutrition* 50: 213-224.
- Gontard, N., Thibault, R., Cuq, B. and Guilbert, S., 1996. Influence of relative humidity and film composition on oxygen and carbon dioxide permeabilities of edible films. *Journal of Agricultural and Food Chemistry* 44: 1064-1069.
- González-Aguilar, G., Tiznado-Hernandez, M., Zavaleta-Gatica, R. and Martinez-Téllez, M., 2004. Methyl jasmonate treatments reduce chilling injury and activate the defense response of guava fruits. *Biochemical and Biophysical Research Communications* 313: 694-701.
- González-Aguilar, G.A., Ayala-Zavala, J., Olivas, G., de la Rosa, L. and Álvarez-Parrilla, E., 2010. Preserving quality of fresh-cut products using safe technologies. *Journal für Verbraucherschutz und Lebensmittelsicherheit* 5: 65-72.
- Gortzi, O., Lalas, S., Chinou, I. and Tsaknis, J., 2006. Reevaluation of antimicrobial and antioxidant activity of Thymus spp. extracts before and after encapsulation in liposomes. *Journal of Food Protection* 69: 2998-3005.
- Hagenmaier, R.D., 2005. A comparison of ethane, ethylene and CO₂ peel permeance for fruit with different coatings. *Postharvest Biology and Technology* 37: 56-64.
- Ihl, M., Aravena, L., Scheuermann, E., Uquiche, E. and Bifani, V., 2003. Effect of immersion solutions on shelf-life of minimally processed lettuce. *LWT-Food Science and Technology* 36: 591-599.
- International Organization for Standardization (ISO), 2003. ISO 4833:2003. Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of microorganisms – colony-count technique at 30 °C. ISO, Geneva, Switzerland.

- Jacxsens, L., Devlieghere, F. and Debevere, J., 2002. Predictive modelling for packaging design: equilibrium modified atmosphere packages of fresh-cut vegetables subjected to a simulated distribution chain. *International Journal of Food Microbiology* 73: 331-341.
- Kitazuru, E., Moreira, A., Mancini-Filho, J., Delincee, H. and Villavicencio, A.L.C.H., 2004. Effects of irradiation on natural antioxidants of cinnamon (*Cinnamomum zeylanicum* N.). *Radiation Physics and Chemistry* 71: 39-41.
- Lanciotti, R., Gianotti, A., Patrignani, F., Belletti, N., Guerzoni, M. and Gardini, F., 2004. Use of natural aroma compounds to improve shelf-life and safety of minimally processed fruits. *Trends in Food Science & Technology* 15: 201-208.
- Lee, S.H., Ahn, S.J., Im, Y.J., Cho, K., Chung, G.-C., Cho, B.-H. and Han, O., 2005. Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in figleaf gourd and cucumber roots. *Biochemical and Biophysical Research Communications* 330: 1194-1198.
- Martinez-Romero, D., Castillo, S., Valverde, J., Guillen, F., Valero, D. and Serrano, M., 2005. The use of natural aromatic essential oils helps to maintain post-harvest quality of 'Crimson' table grapes. *Acta horticulturae* 682: 1723-1729.
- Meepagala, K.M., Sturtz, G. and Wedge, D.E., 2002. Antifungal constituents of the essential oil fraction of *Artemisia dracunculus* L. var. *dracunculus*. *Journal of Agricultural and Food Chemistry* 50: 6989-6992.
- Ojagh, S.M., Rezaei, M., Razavi, S.H. and Hosseini, S.M.H., 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry* 120: 193-198.
- Olivas, G. and Barbosa-Cánovas, G., 2005. Edible coatings for fresh-cut fruits. *Critical Reviews in Food Science and Nutrition* 45: 657-670.
- Ouattara, B., Simard, R., Piette, G., Begin, A. and Holley, R., 2000. Diffusion of acetic and propionic acids from chitosan-based antimicrobial packaging films. *Journal of Food Science* 65: 768-773.
- Oussalah, M., Caillet, S., Saucier, L. and Lacroix, M., 2006. Antimicrobial effects of alginate-based film containing essential oils for the preservation of whole beef muscle. *Journal of Food Protection* 69: 2364-2369.
- Pinhero, R.G., Paliyath, G., Yada, R.Y. and Murr, D.P., 1998. Modulation of phospholipase D and lipoxygenase activities during chilling. Relation to chilling tolerance of maize seedlings. *Plant Physiology and Biochemistry* 36: 213-224.
- Rico, D., Martin-Diana, A.B., Barat, J. and Barry-Ryan, C., 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: a review. *Trends in Food Science & Technology* 18: 373-386.
- Rojas-Graü, M., Tapia, M., Rodríguez, F., Carmona, A. and Martin-Belloso, O., 2007. Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. *Food Hydrocolloids* 21: 118-127.
- Sáenz, C., Vásquez, M., Trumper, S. and Fluxá, C., 1992. Extracción y composición química de mucílago de tuna (*Opuntia ficus indica*). In: *Proceedings of the 2nd International Congress of Prickly Pear and Cochineal*. September 1991, Universidad de Chile, Santiago, Chile, pp. 93-96.
- Serrano, M., Martinez-Romero, D., Castillo, S., Guillén, F. and Valero, D., 2005. The use of natural antifungal compounds improves the beneficial effect of MAP in sweet cherry storage. *Innovative Food Science & Emerging Technologies* 6: 115-123.
- Shoji, Y. and Nakashima, H., 2004. Nutraceuticals and delivery systems. *Journal of Drug Targeting* 12: 385-391.
- Singleton, V. and Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144-158.
- Taylor, T.M., Weiss, J., Davidson, P.M. and Bruce, B.D., 2005. Liposomal nanocapsules in food science and agriculture. *Critical Reviews in Food Science and Nutrition* 45: 587-605.
- Vámos-Vigyázó, L. and Haard, N.F., 1981. Polyphenol oxidases and peroxidases in fruits and vegetables. *Critical Reviews in Food Science & Nutrition* 15: 49-127.
- Wong, D.W., Tillin, S.J., Hudson, J.S. and Pavlath, A.E., 1994. Gas exchange in cut apples with bilayer coatings. *Journal of Agricultural and Food Chemistry* 42: 2278-2285.

