Original article

Influence of edible coatings on physiological and biochemical attributes of Japanese plum (*Prunus salicina* Lindell cv. Santa Rosa)

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Summary

Introduction – **'Santa Rosa' plum is a very delicate fruit with a limited shelf life owing to decay and mechanical injury. Hence, edible coatings were attempted (on-farm and off-farm) in 'Santa Rosa' plum to increase fruit shelf life and marketability.** *Materials and methods* – **'Santa Rosa' fruits were treated with edible coatings, namely, Semperfresh™ (1:3), vegetable wax (1:5), shellac-based wax (2:3) and water dip (control) at two different locations, *viz.*, on-farm and off-farm (in the lab) and then stored at 20 ± 2 °C. Quality evaluation of stored plums was carried out for changes in the firmness, physiological (loss in weight, respiration and ethylene evolution rates) and biochemical attributes (total antioxidant activity, malondialdehyde content and pectin methylesterase activity) at 3-days interval for a period of 15 days.** *Results and discussion* – **On-farm treated fruits showed maximum retention in firmness as compared to the untreated fruits. Respiration rate and ethylene evolution rates were also delayed significantly in coated fruits. Amongst the different coatings applied, shellac-based coating was found to be the best in maintaining maximum firmness (~67% higher) and overall fruit quality followed by Semperfresh™ and vegetable wax coatings. Off-farm coated plums were inferior in quality to on-farm treated ones.** *Conclusion* – **On-farm treatment of plum fruit with shellac-based coating showed the best results in maintaining the firmness, quality and postharvest life of plum up to 15 days.**

Keywords
India, Japanese plum, *Prunus salicina*, natural wax, fruit quality, shelf life, microstructure

Résumé

Influence d'enrobages comestibles sur les caractéristiques physiologiques et biochimiques de la prune du Japon (*Prunus salicina* Lindell cv. Santa Rosa).

Introduction – **La prune 'Santa Rosa' est un fruit très délicat avec une aptitude au stockage limitée par les pourritures et blessures mécaniques. C'est pourquoi les traitements post-récolte sont nécessaires pour augmenter la durée de vie des fruits.** *Matériel et méthodes* – **Les fruits de la variété 'Santa Rosa' ont été traités avec des enrobages comestibles, à savoir le Semperfresh™ (1:3), une cire végétale (1:5), une cire à base de gomme-laque (2:3), et un simple trempage dans l'eau (contrôle) sur deux sites distincts, *i.e.* à la ferme et en laboratoire, puis conservés à 20 ± 2 °C. L'évaluation de la qualité des prunes stockées a été réalisée pour la fermeté, des critères physiologiques (perte de poids, taux de respiration et évolution de dégagement d'éthylène) et des attributs biochimiques (activité anti-oxydante totale, teneur en malondialdéhyde et activité pectine-méthylesterase), tous les 3 jours pendant une période de 15 jours.** *Résultats et discussion* – **Les fruits traités à la ferme ont montré une rétention maximale de fermeté par rapport aux fruits non traités. Les taux de respiration et de production d'éthylène ont également été retardés de façon significative dans les fruits enrobés. Parmi les différents revêtements appliqués, l'enrobage à base de gomme-laque s'est montré le meilleur dans le maintien d'une fermeté maximale (67%) et de la qualité globale du fruit, suivi par le revêtement Semperfresh™ et la cire végétale. Les prunes enrobées au laboratoire étaient de qualité inférieure à celles traitées à la ferme.** *Conclusion* – **Le traitement à la ferme des prunes du Japon à base de gomme-laque a montré les meilleurs résultats en maintenant la fermeté, la qualité et une longévité post-récolte jusqu'à 15 jours.**

Significance of this study

What is already known on this subject?
- Plum is a climacteric fruit with a shelf life of 3–4 days only. Various approaches have previously been attempted to extend its life but not the edible coatings.

What are the new findings?
- On-farm application of shellac-based coating on the 'Santa Rosa' plum fruits proved beneficial to extend postharvest life up to 15 days at 20 ± 2 °C.

What is the expected impact on horticulture?
- This work would be useful to reduce postharvest losses of plums during transportation and extend their market availability considerably.

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Mots-clés
Inde, prunier du Japon, *Prunus salicina*, cires naturelles, qualité des fruits, durée de conservation, microstructure

Introduction

In India, Japanese plum varieties like 'Beauty', 'Santa Rosa' and 'Mariposa' are grown commercially under sub-temperate climatic conditions, but 'Santa Rosa' dominates because of its self-fruitfulness and its characteristic flavour (Chattopadhyay, 2008). It is a climacteric fruit with a short shelf life of 3–4 days under ambient conditions. The perishable nature of the ripe fruits poses a serious problem for their storage, transportation and marketing from far-flung areas located in different terrains and at high altitudes to the plains. Rough handling and lack of proper storage conditions further reduce the postharvest life of the fruits drastically, which limits the availability of the fruits. Due to sensitivity of plums to chilling injury, it limits the exploitation of cold storage for extending their shelf life. Thus, there is an urgent need to extend its availability through proper postharvest management practices. Among different postharvest management strategies of fresh fruit handling, use of edible coatings has been reported to be very successful to preserve fruit quality (Navarro-Tarazaga *et al.*, 2011) with additional benefit of reducing the volume of non-biodegradable packaging materials (Olivas *et al.*, 2008; Campos *et al.*, 2011). The coatings may contribute to extend the shelf life of fresh fruits by reducing moisture migration, gas exchange and oxidative reaction rates (Baldwin *et al.*, 1996; Park, 1999). The beneficial effect of these coatings may be more pronounced if they are applied on the farm, immediately after the fruit is harvested. This may help curb the metabolic processes and maintain the fruit quality. Since the information of the on-farm application of these surface coatings was very scanty, the major aim of our study was to investigate, the effect of on-farm and off-farm (after transportation) application of edible coatings on physiological and biochemical attributes of plum fruit, which have an effect on fruit softening, ripening and overall fruit quality for wholesale marketing.

Materials and methods

Fruit material, treatments and observations recorded

The plum (*Prunus salicina* Lindell) cv. Santa Rosa fruit were harvested at climacteric stage of maturity in the month of July from a private orchard at Kullu, Himachal Pradesh, India. The sorted and graded fruits were divided into two lots. One lot of plums was subjected to application of different surface coatings, namely, Semperfresh™ (Mantrose UK Ltd., Berkshire, England), vegetable wax coating (Nipro Technologies Ltd., Panchkula, India), shellac-based coating (from resinous secretion of lac insects, Indian Institute of Natural Resins and Gums, Ranchi, India) in the ratio of coating:water to be as 1:3, 1:5 and 2:3, respectively. The untreated or control fruits were dipped in distilled water on the farm with each treatment comprising of 120 fruits, with five replications.

The treated fruits were air-dried for 1 h and then transported to New Delhi within 15 h. In the lab the coated plums were packed in punnets having ventilation and stored at supermarket conditions (20 ± 2 °C and 90 ± 2% relative humidity) for further study. Fruits of the second lot were treated with edible coatings in the laboratory after transportation within 15 h and stored under similar environmental conditions as the on-farm treated fruits. Observations were recorded at 3-days interval on fruit firmness, respiration rate, ethylene evolution rate, physiological loss in weight, total antioxidant activity, malondialdehyde content and pectin methylesterase activity.

Fruit characteristic measurements

Fruit firmness

Fruit firmness was determined using a texture analyzer (model: TA+Di, Stable Micro Systems, UK) using compression test. Firmness was defined as maximum force (kgf) during the puncture, which was expressed in Newton (N) (Sharma *et al.*, 2011).

Physiological loss in weight

Physiological loss in weight of the stored fruits was calculated by using the method of Mahajan *et al.* (2011) and expressed in percentage.

Respiration and ethylene evolution rate

Respiration rate in the plums was measured by static headspace technique using auto gas analyzer (Model: Checkmate 9900 O_2/CO_2, PBI Dansensor, Denmark) and expressed as mL CO_2 kg^{-1} h^{-1}. Ethylene evolution rate was measured by Hewlett Packard gas chromatograph (model 5890 Series II) and expressed in µL kg^{-1} h^{-1} (Jhalegar *et al.*, 2012).

Microstructure of the fruit peel

The surface microstructure of peel from both coated and a control sample was analyzed using SEM technique and viewed on EVO/MA10 scanning electron microscope at an accelerating voltage of 15 kV. Samples were mounted with conductive adhesive and coated with 24 µm palladium. Comparable magnifications and positions of samples were photographed. The scanning electron microscope Zeiss EVO/MA10, available at Division of Entomology, IARI was used for viewing the specimens with different magnifications.

Total antioxidant activity

Total antioxidant activity (expressed as µmol Trolox g^{-1}) in the plums was determined by the CUPRAC method of Apak *et al.* (2004). One mL each of copper (II) chloride, neocuproine and ammonium acetate buffer (pH 7) were mixed with the alcoholic extract of the sample (0.1 mL) and the developed color was measured after 30 minutes of incubation in a spectrophotometer (Spectra Max M2, Molecular Devices, USA) at 450 nm.

Malondialdehyde content

The malondialdehyde (MDA) content was measured using thiobarbituric acid as reactive reagent by following the method of Eum *et al.* (2009) and expressed as µmol g^{-1}. A 2-g sample was homogenized in 0.2 M phosphate buffer and centrifuged. One mL extract was mixed with 4 mL of 20% trichloroacetic acid and 0.5% thiobarbituric acid and heated. Absorbance of the extracts were recorded at 440, 532, and 600 nm, using a spectrophotometer (Spectra Max M2, Molecular Devices, USA).

Pectin methyl esterase activity

Pectin methyl esterase (PME) activity in plums was measured following the method of Sharma *et al.* (2011) and expressed as µmol min^{-1} g^{-1}. Five gram of plum sample was homogenised in 15 mL cold (4 °C) 8.8% sodium chloride using a pestle and mortar. The homogenate was centrifuged at 15,000 × *g* for 15 min and aliquot was collected and pH was adjusted to 7.5 with sodium hydroxide, after which it was used for enzyme assay. For enzyme assay, 2 mL pectin was mixed with 0.15 mL bromothymol blue and 0.83 mL distilled water. The absorbance of the mixture was measured at 620 nm.

Statistical analysis

A two-way analysis of variance was performed on the data sets using SAS 9.3 software and significant effects ($P < 0.05$) were noted. Significant difference amongst the means was determined by Duncan's Multiple Range Test (Base SAS®, 2014).

Results and discussion

Effect on physical and physiological attributes

Effect of edible coatings on fruit firmness

Softening is a major factor limiting the shelf-life of plum and affecting its acceptability by the consumers. In this study, the fruit firmness was found to be significantly influenced by the location and the surface coating applied. Fruit firmness showed a progressive decline under all the treatment conditions with the advancement in storage period. The on-farm treated fruits had higher firmness (13.87 N) as compared to fruits that were treated in the lab (off-farm) after transportation (13.75 N). Coated fruits displayed a slower rate of decline in firmness than control fruits (water dipped). At the end of storage period (15 days), shellac-based coated fruits showed ~69% higher fruit firmness than control fruits in both on-farm and off-farm treatments (Figure 1A), followed by Semperfresh™ coated (~66% and 65.5%, respectively) and vegetable wax coated fruits (~35% and 34%, respectively). Higher retention of firmness by the coated fruits indicated that coatings were effective in retarding the metabolic and enzymatic activities and also the degradation of cell wall components in plum. Previous studies have also reported a

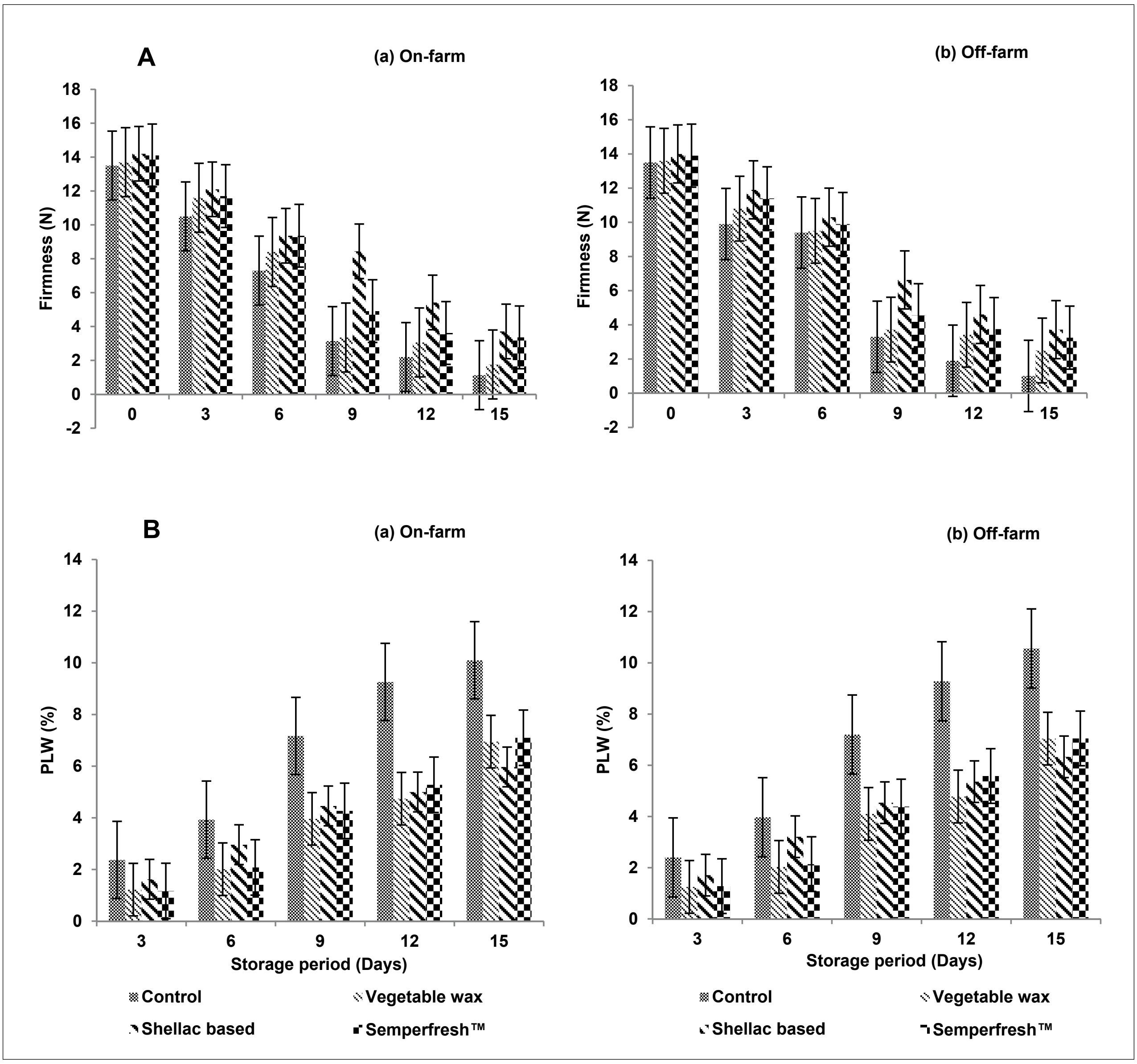

FIGURE 1. Effects of (a) On-farm; and (b) Off-farm application of edible coatings on (A) Firmness; and (B) Physiological weight loss of plums cv. Santa Rosa. Data are means and SD bars of three replications (n = 5 fruits) recorded at 3-day intervals. Values with same alphabets do not differ significantly.

similar performance of delaying softness in chitosan coated citrus fruit (Chien *et al.*, 2007) and Semperfresh™ coated cherry (Yaman and Bayoundurlc, 2002). Firmness of coated and uncoated fruits decreased progressively during storage for both treatment locations, primarily because of the increase in the enzyme activity rendering them much softer. Fruit treated after transportation reported lower values of firmness because of the absence of any physical barrier to restrict the physiological activities during transportation.

Effect on physiological loss in weight

Both coated and uncoated plum fruits continuously lost weight during storage, with the control (water dipped) fruits losing weight more rapidly than the coated fruits. Fruits coated with edible films after transportation showed a higher percent weight loss as compared to the on-farm treated samples (Figure 1B). For on-farm treated control fruits, the PLW was 10.10% after 15th day of storage, while it was 10.56% for off-farm treated ones. Overall, amongst the coatings applied, shellac-based and Semperfresh™ displayed better efficacy in reducing weight loss, followed by vegetable wax. Increase in PLW with the increase in storage period may be due to increase in moisture loss from the fruits. The fruits treated with shellac-based and Semperfresh™ coatings had lowest weight loss followed by vegetable wax coated fruits that may be as a result of the different moisture permeability of the coatings. In addition, edible coatings act as an extra layer which also coats the stomata leading to a decrease in transpiration and in turn, a reduction in the weight loss. Our results were consistent with previous studies of Zhou *et al.* (2008) on pear.

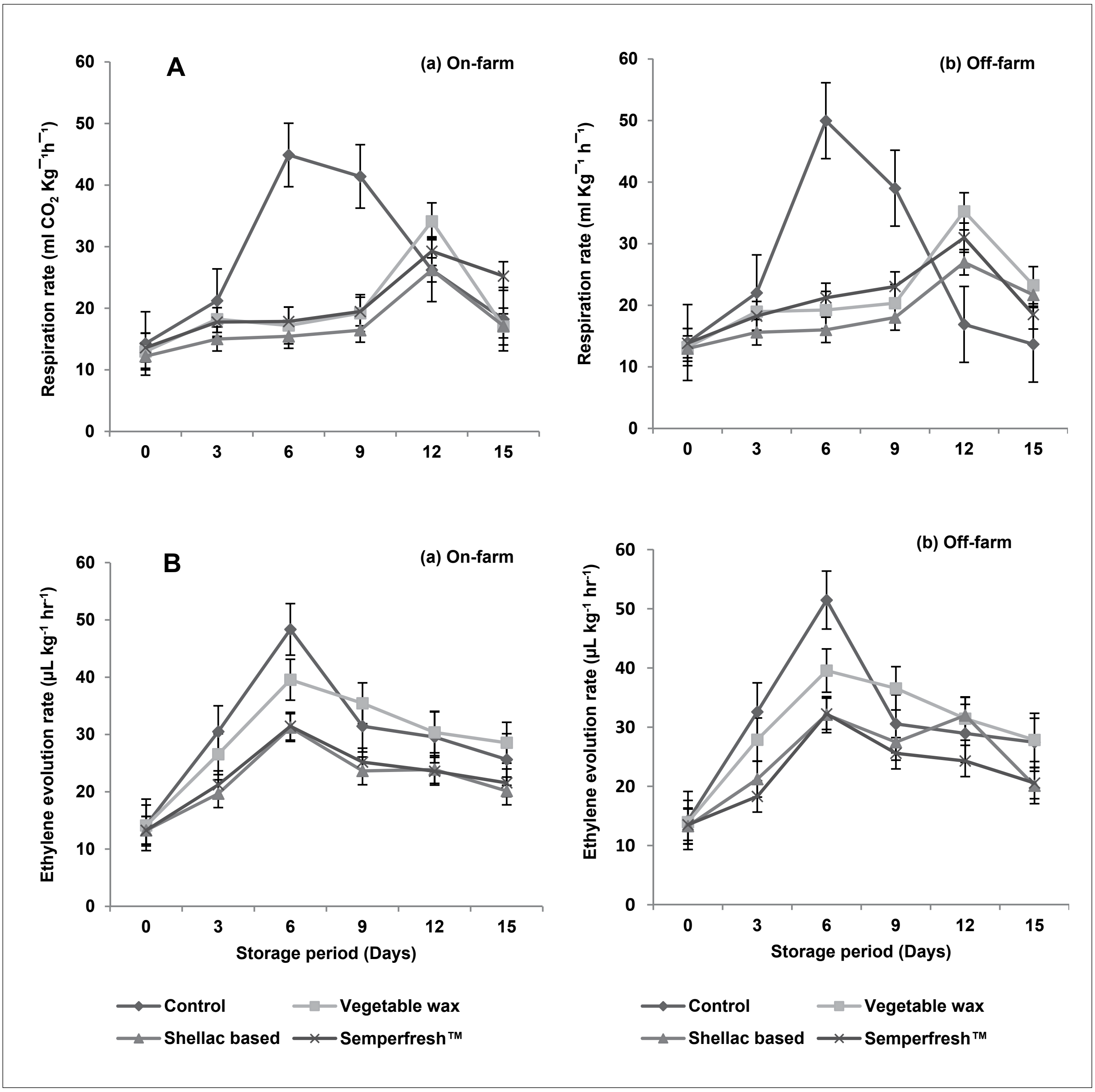

FIGURE 2. Effects of (a) On-farm; and (b) Off-farm application of edible coatings on (A) The respiration rate (in mL CO_2 kg^{-1} h^{-1}) and (B) The ethylene evolution rate (in µL kg^{-1} h^{-1}) in plums cv. Santa Rosa. Data are means and SD bars of three replications (*n* = 5 fruits) recorded at 3-day intervals.

Effect on respiration rate

Respiration rate is a major metabolic process which gives an indication of the storage life of a produce. Coatings had a significant influence on the respiration rate of the plums. The rate of respiration steadily increased initially followed by a gradual decline in all the fruits irrespective of the treatment (Figure 2A). The rate of CO_2 production (mL CO_2 kg^{-1} h^{-1}) increased in control (water dipped) fruits and the climacteric peak was observed after 6 days of storage. In case of coated fruits, the climacteric upsurge was shifted to 12th day both for on-farm and off-farm treated fruits. In all treatments, the attainment of respiratory peak was followed by a decline in the rate of respiration. Control (water dipped) fruits recorded ~62% and ~72% higher respiration rate, respectively for on-farm and off-farm treated fruits as compared to shellac-based coated samples. Amongst the coatings applied, shellac-based coated fruits maintained significantly the lowest respiration rate throughout the storage period followed by Semperfresh™ and vegetable wax coated ones. The suppression of respiration rate by the coating may be due to partial blockage of the pores on fruits by them. Significant reduction in respiration rate by shellac-based wax may be because of the fact that it is more efficient in restricting the gas exchange between fruit and the surrounding atmosphere during storage. Similar findings were reported earlier by Eum *et al.* (2009), Zhou *et al.* (2008) and Velickova *et al.* (2013) on plum, pear and strawberry, respectively.

Effect on ethylene evolution rate

Coatings and the treatment site (on-farm/off-farm) had a significant effect on the ethylene evolution rate of the 'Santa Rosa' plum fruits. An initial increasing trend in the rate of ethylene evolution (Figure 2B) was observed with advancement in the storage period. In on-farm treated control (water dipped) fruits, ethylene evolution rate was 14.23 µL kg^{-1} h^{-1} at the beginning that showed a dramatic rise to 48.35 µL kg^{-1} h^{-1} by the 6th day, followed by a significant decline to 25.64 µL kg^{-1} h^{-1} by the 15th day. A similar trend was observed in the off-farm treated fruits. Nevertheless, coated fruits recorded significantly lower rates of ethylene evolution than the control fruits by the end of storage, thereby indicating the slowing down of the ripening process. Off-farm treated plums coated with shellac-based wax, showed ~27% lower ethylene evolution rate as compared to control counterparts whereas on-farm shellac-based coated fruits showed about ~26% reduction. Among the coatings, shellac-based and Semperfresh™ resulted in lowest rate of ethylene evolution, followed by vegetable wax coating. Compared with the control fruits, the coated plum fruits showed a significantly reduced ethylene evolution rate during storage. This may be ascribed to barrier properties of the coatings that changed the gas composition around and inside the fruits. On-farm application also contributed to the suppressed ethylene evolution rate thereby indicating the slowing down of the ripening process. Similar pattern was observed earlier by Valero

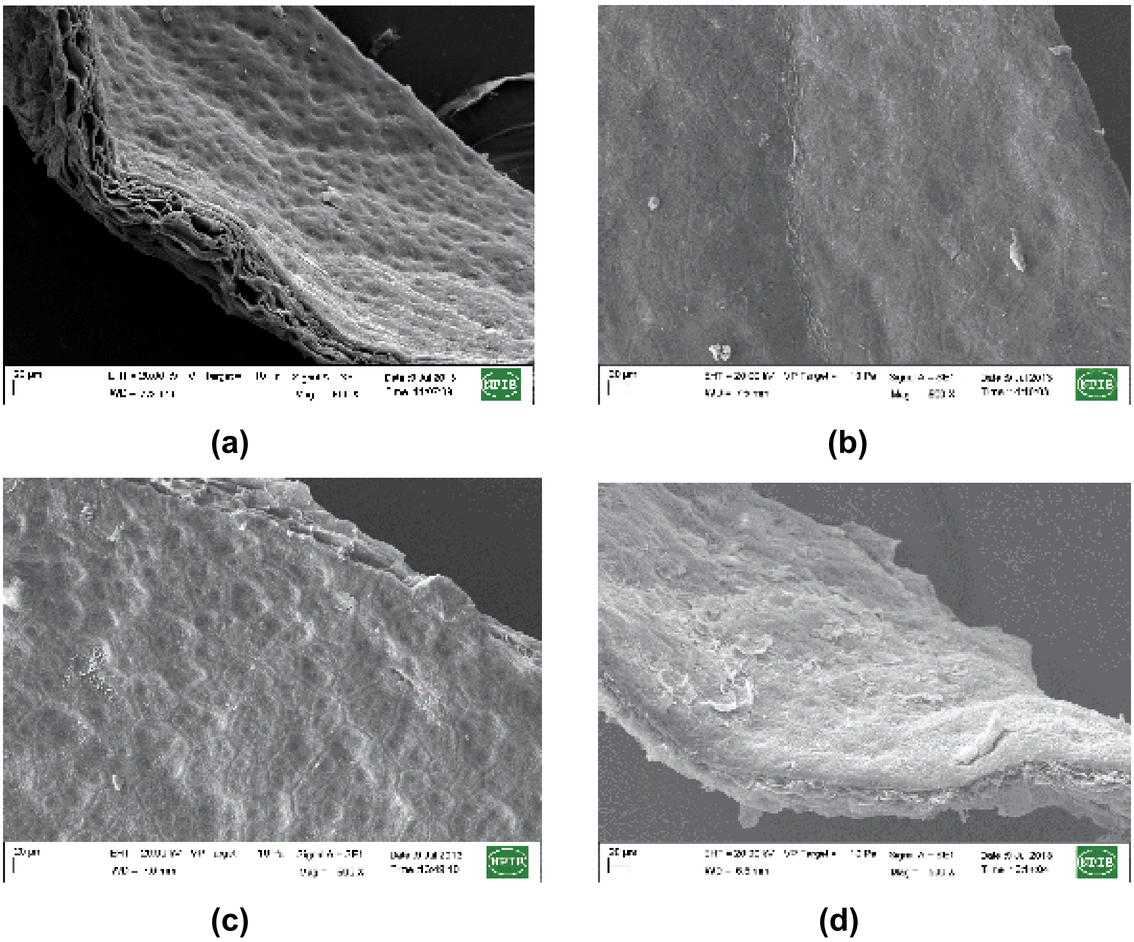

Figure 3. Scanning electron micrograph of the peel of (a) Water dipped (control); (b) Shellac-coated; (c) Semperfresh™ coated; and (d) Vegetable wax coated plums.

et al. (2013) in coated plums treated with alginate. Among the coatings, shellac-based and Semperfresh™ resulted in lower rate of ethylene evolution followed by vegetable wax coating. Earlier, Perez-Gago *et al.* (2003) reported no effect on ethylene production of plums coated with hydroxypropyl methylcellulose incorporated with bee wax or shellac whereas Eum *et al.* (2009) reported a reduced ethylene efflux in plums coated with sorbitol incorporated Versasheen™.

Microstructure of the fruit peel

The surface microstructure of peel from both coated and control (water dipped) fruits was analyzed using SEM technique. As evidenced from the scanning electron micrographs, edible coatings applied on the plum fruits led to filling of the pores on the surface giving the peels a smoother texture which further helped in arresting the metabolic activities and curbing the bio-chemical changes taking place during storage (Figure 3).

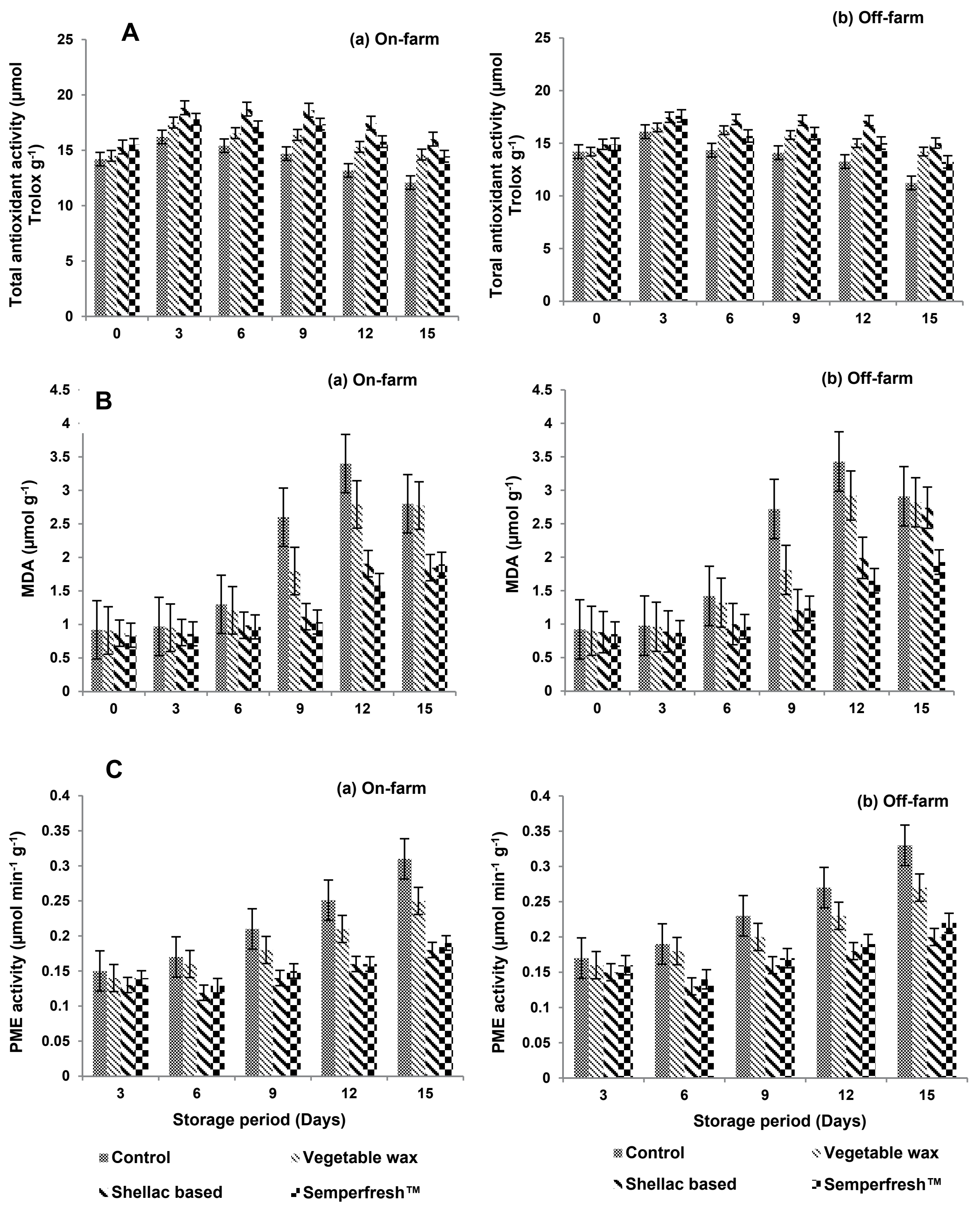

Figure 4. Effects of (a) On-farm; and (b) Off-farm application of edible coatings on (A) The total antioxidant; (B) The MDA content; and (C) The PME activity in plums cv. Santa Rosa. Data are means and SD bars of three replications (n = 5 fruits) recorded at 3-day intervals. Values with same alphabets do not differ significantly.

Effects on biochemical attributes

Effect on total antioxidant activity

The total antioxidant activity was significantly higher in fruits coated with edible coatings as compared to water dipped (control) fruits. However, maximum total antioxidant activity after 15 days of storage was recorded (Figure 4A) in on-farm treated fruits coated with shellac-based coating (mean value of 17.49 µmol Trolox g^{-1}), followed by Semperfresh™ (mean value of 16.34 µmol Trolox g^{-1}) and vegetable wax (mean value of 15.81 µmol Trolox g^{-1}). The antioxidant capacity increased initially followed by a progressive decline with the increase in storage duration. Such decline in antioxidant activity can be related to decline in phenolics and other related compounds which contribute to the overall antioxidant capacity. This trend indicated that edible coatings were able to delay the senescence and decay effectively for on-farm treated plums. The percent decrease was highest (20.90%) for off-farm treated plums. Similar trend was also reported by Sanchez-Gonzalez *et al.* (2011) in hydroxypropyl methylcellulose and chitosan coated grapes.

Effect on malondialdehyde content

Malondialdehyde (MDA), a product of lipid peroxidation, is often used as an indicator of oxidative damage of the cells. The MDA content was found to be significantly influenced by the type of coating and the treatment location. With the progress in storage period, a continuous increase in MDA content was observed, both in control and coated fruits (Figure 4B). The control samples generally had a higher level of MDA than the coated plums. Further, Semperfresh™ coating was more efficient in reducing MDA production with advancement of storage period in the fruits treated off-farm whereas, both shellac-based and Semperfresh™ coatings performed equally better in on-farm treated plums. With regard to treatment site, fruits treated on the farm with edible coatings showed lower mean values for MDA content as compared to off-farm treated samples which were significant only after 12 days of storage. The increase in MDA during storage is attributed to the progressive increase in fruit senescence. The application of edible coatings delayed the increase in MDA, Semperfresh™ coating being the most efficient. Similar increasing trend in MDA content has been reported by Eum *et al.* (2009) in Versasheen™ coated plums.

Effect on pectin methyl esterase (PME) activity

Irrespective of the treatment, PME activity in the 'Santa Rosa' plum fruits showed a continuous increase with the advancement in storage period (Figure 4C). In general, the PME activity was significantly higher for control (water dipped) fruits with mean values of 0.218 µmol min^{-1} g^{-1} for on-farm treated fruits and 0.238 µmol min^{-1} g^{-1} for fruits treated after transportation. However, amongst the coatings, the minimum PME activity was recorded for shellac-based coated fruits for both on-farm and off-farm treatments (0.180 or 0.200 µmol min^{-1} g^{-1}, respectively). On-farm treated fruits showed comparatively less PME activity due to inhibition of enzyme activity at earlier stages of treatment. Overall, coating treatments led to about 82–86% reduction in PME activity over uncoated plums with the minimum PME activity in shellac-based coated fruits. This can be attributed to the fact that all coatings form a physical barrier around the fruit leading to depletion of oxygen and subsequently decrease in the enzyme activity (Velickova *et al.*, 2013). Earlier, Khan *et al.* (2004) also reported similar results in 1-MCP treated 'Tegan Blue' plums.

Conclusion

All the edible coatings experimented during this work had a positive effect on retarding the senescence related activities of 'Santa Rosa' plums without affecting the quality of the fruit. Of the coatings tested, the shellac-based coating was the most effective in retarding the changes associated with quality over untreated fruit. Further, on-farm treatment of the plums with edible coatings proved to be better than off-farm treatment (after transportation) with regard to the retention of firmness and overall fruit quality.

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