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RESEARCHARTICLE

Effect of sesame protein and lotus starch based bioactive coatings with the incorporation of *Garcinia indica* extract on the shelf-life extension of sapodilla

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ABSTRACT

Sapodilla (*Manilkara zapota* L.), a climacteric fruit is having high rate of ethylene production which leads to early senescence followed by spoilage. There is need to increase the shelf life of sapodilla after harvesting. Different coating solutions were prepared from sesame protein isolate (SP) and lotus seed starch (LS) with the addition of *Garcinia indica* extract (GE). Two coats were applied on the fruit with manual drying after an interval of 20 min. Fruits were stored at 70-75 % RH and 20 ±1 ºC temperature in plastic mesh trays in an environment chamber to store all coated and non-coated fruits. *Garcinia indica* (GE) extract with the combination of sesame protein and lotus starch could maintain the overall quality of sapodilla fruit and extends the shelf life up to 12 days. GE in sesame protein and lotus starch-based coatings helps to retain the nutritional parameters by delaying respiration rate of fruit samples during storage.

Keywords: Sesame, protein, lotus starch, coatings, sapodilla

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INTRODUCTION

Sapodilla (*Manikara zapota* L.) from family *Sapotaceae*, is one of the extensively grown fruit crop in India, Mexico, Guatemala and Venezuela. Sapodilla is a source of sugars, antioxidants, total phenolics (gallic acid, catechin, chlorogenic acid, leucodephinidin, leucocyanidin and leucopelargonidin), ascorbic acid, carotenoids, minerals and proteins (Kulkarni et al., 2007; Bhutia et al., 2011). It is commonly known as sapota, *chiku, ciku* and *zapote*. Sapodilla is a climacteric fruit and due to high rate of ethylene production, leads to senescence followed by spoilage. By modifying the atmosphere around harvested sapodilla, the onset of ripening could be delayed. Edible coatings are effective as postharvest treatments to preserve food quality and provide additional benefit of reducing the volume of non-biodegradable packaging materials (Khaliq et al., 2019; Pavinatto et al., 2020; Salama et al., 2019; Robledo et al., 2018; Orsuwan and Sothornvit, 2018; Tosati et al., 2018; Li et al., 2012; Artés-Hernández et al., 2017). Proteins and carbohydrates are preferred for films and coatings especially for food applications because of their biodegradability, non-toxicity, excellent gas barrier properties.

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Sesame meal obtained from sesame (*Sesamum indicum*) is a by-product after oil extraction and is usually fed to animal as a protein source in India. The protein-based coatings impart good barrier properties and more strength as compared to polysaccharide-based coatings (Sharma et al., 2016; Sharma et al., 2018). Lotus seeds, from the lotus (*Nelumbo nucifera)* commonly known as "makhana seeds" are prominent agriculture product in India and have been widely consumed due to several health benefits. Lotus seeds provide bioactive ingredients such as flavonoids, antioxidants, antiviral substances and anti-steroids (Sridhar and Bhat, 2007). Lotus seeds have high potential of antioxidants and have high number of flavones. These seeds are reported to exhibit nutraceuticals properties. Starch is the major component of lotus seeds representing more than 50% of dry mater. Lotus seed starch may impart good film forming properties and may contribute to the formation of type III resistant starch due to its high amylose content (Man et al., 2012).

Plant extracts and essential oils in coating formulations are widely used nowadays to delay the deteriorative changes in fruits and vegetables. Moreover, pathogenic attacks on the plant tissues are another major reason for post-harvest spoilage. Application of plant extracts or essential oils such as *Fagonia indica* plant extract, pomegranate peel extract, moringa leaf extract, grape seed extract, cinnamon oil, clove oil, green tea extract, ginseng extract have demonstrated a delay in the respiration rate as well as in reduction of microbial spoilage in fresh produce (Khaliq et al., 2019; Nair et al., 2018; Tesfay and Magwaza, 2017; Aloui et al., 2014; Siripatrawan and Harte, 2010; Norajit et al., 2010).

Garcinia indica which is popularly known as *kokum* in India belongs to *Clusiaceae* (earlier *Guttiferae*) family. *Garcinia* is a large genus of polygamous trees or shrubs, dispersed in the Africa, tropical Asia, and Polynesia and contains bioactive substances including xanthones, flavonoids, benzophenones, lactones and phenolic acids, which exhibited antioxidant and antimicrobial properties (Selvi et al., 2003). Utilization of *Garcinia indica* extract in edible films and coatings could improve the shelf life of sapodilla.

Previous studies have reported that sapodilla coated with aloe vera gel and *Fagonia indica* plant extracts, stored under low temperature conditions delays the ripening and retained the overall quality till twelve days of storage (Khaliq et al., 2019). To the best of our knowledge, there is no report on the effect of sesame protein and lotus starch coatings enriched with *Garcinia indica* rind extract on the shelf-life extension of any horticulture produce. The objective of this work was to study the effects of sesame protein and lotus starch coatings enriched with *Garcinia indica* rind extract, on extension of shelf life with an emphasis on the nutritional status of sapodilla fruit during storage.

MATERIALS AND METHODS

Raw material and film preparation

Sesame variety RT-127 and lotus seeds were obtained from Chandigarh, India. Fresh sapodilla (*Manikara zapota* L.) fruit having uniform size, color, maturity state without defects were procured from the farms of Haryana Agricultural University (HAU), Hisar Haryana. The fruits were sanitized with washing in chlorinated water for 5 min and allowed to dry naturally. Dehydrated *Garcinia indica* rind was obtained from Indo World Trading Co., New Delhi, India and used to develop *Garcinia* extract (GE). All other chemicals and solvents were of analytical grade and obtained from Merck.

Sesame protein isolate (SPI) was prepared by alkali method as described earlier by Sharma et al. (2016). Isolated sesame protein consists of 90.50% protein content, 2.08% ash content, 0.3% crude fiber, 6.86% moisture, 0.08% fat, and 0.81% carbohydrates as reported earlier (Sharma et al., 2016; Sharma et al., 2018).

Sesame protein (SP) solution was prepared by dissolving sesame protein isolate (5% (w/v) in distilled water, adjusted to pH 9 and denatured at 90 °C and cooled to 25 °C. 10 % glycerol (total solid basis) was added as a plasticizer and solution was magnetically stirred (Spinot, Tarsons, New Delhi, India) for 30 min at room temperature.

The lotus seed starch was isolated according to the method described Luo et al. (2016) with minor modification. 500 mL of distilled water was mixed with 50 g lotus seeds powder and filtered through 80-mesh stainless mesh. The starch filtrate was centrifuged at 4 °C and the precipitate was washed with distilled water. Obtained precipitate was dried to yield the crude starch. Crude starch is then defatted with diethyl ether in Soxhlet extractor and the residue was washed three times with 1M NaOH to de-proteinize. The residue was again washed with distilled water three times and vacuum dried to yield the starch.

Garcinia indica extract (GE) was prepared by grinding the dehydrated *G. indica* fruit rind with sterile pestle and mortar and the soluble components were extracted by soaking the ground material in water for 4 hrs. The extracts were filtered through Whatman filter paper no. 1 and filter sterilized with the help of Millipore syringe filters with the pore size of 0.2 µm and stored at 4 °C.

Four types of films were prepared by using combinations: (a) Sesame protein film (SP) with 5% sesame protein isolate (w/w, on total solid basis), 10% Glycerol (v/w, on total solid basis) ; (b) Lotus starch film (LS) with 5% lotus starch (w/w, on total solid basis), 10% Glycerol (v/w, on total solid basis); (c) Sesame protein film with 1 % *Garcinia indica* extract (v/w, on total solid basis) (SP-GE) and (d) Lotus starch film with 1 % *Garcinia indica* extract (v/w, on total solid basis) (LS-GE).

Coating of fruits

A total of 100 fruits were taken and divided into 5 groups. All fruits were dipped in following solutions for 2 min (a) 5% sesame protein solution (SP), (b) 5 % sesame protein solution + GE (SP-GE), (c) 5 % lotus seed starch solution (LS), (d) 5% lotus seed starch solution + GE (LS-GE) and (e) distilled water as control. The coating was accomplished on the same day, taken as day zero. Two coats were applied on the fruit with manual drying after an interval of 20 min. Fruits were stored at 70-75 % RH and 20 ±1 ºC temperature in plastic mesh trays in an environment chamber to store all coated and non-coated fruits. From each treatment, three samples were taken and analysed after the interval of 2 days till 12 days of storage.

Weight loss and Firmness

Weight loss of fruits was determined after the intervals of two days with a least count of 0.01 g. Three fruits per treatment were selected per treatment. Weight loss was expressed as percentage loss of the original fresh weight. The flesh firmness of the fruits was measured using a texture analyser (TAX T2i, Stable Microsystems, Surrey, UK) with a P/2 probe. Speed of test was kept at 2 mm/s and 5 mm penetration. Flesh firmness was considered by a mean force (N).

Respiration Rate

The method described by Nair et al., (2018) was adopted for determining respiration rate. Three fruits per treatment were selected. Sapodilla fruits with known weight were placed in airtight plastic container of 1000 mL capacity with rubber septum on lid. A headspace analyser (Dansensor, Checkmate 9900, Denmark) consisting of a syringe was inserted into the container through the rubber septum, to determine the respiration rate in terms of mg CO_2.

Ascorbic acid content

Ascorbic acid content (mg/100g) in the juice was determined with 2-6 Dichlorophenolindophenol (DCPIP) visual titration method as described by AOAC (1990). DCPIP dye, which is blue in alkaline solution, is reduced by ascorbic acid to a colorless form. 5 mL of 3% metaphosphoric acid extract of sapodilla pulp was titrated with DCPIP dye to a pink color end point. The ascorbic acid content was calculated using the dye factor, determined by the titration of the standard ascorbic acid solution with DCPIP dye.

Total carotenoids (TC)

Carotenoids were extracted using method described by Bushway and Wilson (1982) using about 10 g of fruit. Total carotenoid (TC) content (mg/100g) was measured by spectrophotometric method. About 10 g of sapodilla pulp was blended with 100 ml cold acetone in a pestle and mortar, and filtered. The extraction was repeated until the residue was colorless. Acetone extract was placed in the separating funnel and mixed with 25 ml petroleum ether and 5 ml of water. The mixture was left to stand for 30 min. The yellow petroleum ether extract was collected and filtered over anhydrous sodium sulphate on a Whatman filter paper no. 1 (Whatman International Ltd., Maidstone, England). The extract was made up to 25 ml and the color intensity of carotenoid extract was measured at 450 nm in a UV-Visible Spectrophotometer (UV-160A, Shimadzu Co., Kyoto, Japan). The total carotenoid content was calculated based on the calibration curve of β -carotene and expressed as β-carotene equivalents.

Total phenolic content (TP)

Total phenols were measured spectrophotometrically using method standardized by Gao et al. (2014) using Folin–Ciocalteu reagent with gallic acid as a standard. 50 µL of sapodilla extract were added to 3 mL of deionized water plus 250 µL of Folin–Ciocalteu reagent (1 N). After a 5 min reaction time, 750 µL of 20 % Na_2CO_3 solution was added. The mixture was increased to 5 mL by adding deionized water. The phenols were measured at 760 nm after a 30 min incubation time. The results were expressed as mg GAE/100g.

Antioxidant activity

The antioxidant activity in samples were determined by 2,2- Diphenyl-1-picryl-hidrazil (DPPH) radical scavenging method as well as Ferric Reducing Ability of Plasma (FRAP) method. DPPH solution was prepared by dissolving 0.025g DPPH in 100 mL of 70% methanol. Then, to 0.1 mL of sample extract, 3.9 mL DPPH was added and mixed well using vortex. The mixture was incubated for 30 min in a dark room. The absorbance was taken at 517 nm using a UV–visible spectrophotometer and 70% methanol as blank. Radical scavenging activity (RSA) was expressed as the percentage inhibition of DPPH radical. FRAP reagent mixture was prepared in the ratio of 1:1:10 consisting of 20 mM $FeCl_3$, 10 mM TPTZ (2,4,6-Tripyridyl-s-triazine) in 40 mM dilute HCl with an acetate buffer (300 mM) of 3.6 pH (Benzie and Strain, 1996). To 0.1 mL of the sample, 3 mL FRAP reagent was added and absorbance was recorded in a UV–visible spectrophotometer at 593 nm after an incubation period of 4 min at 37 °C. Results were expressed in mg TE/g.

Statistical analysis

Triplicate runs were carried out for each experiment and the data were subjected to statistical analysis. StatSoft (Statistica 12.0) was used to evaluate data by analysis of variance (ANOVA) and Duncan's multiple range test was employed to evaluate significant ($p \leq 0.05$) differences.

RESULTS AND DISCUSSION

Weight loss and firmness

Weight loss and reducing firmness are the main drawbacks of sapodilla fruit during storage and ripening process which leads to decrease in shelf life. All the fruits exhibited weight loss which ultimately escalated with storage. Maximum weight loss was observed by control samples and minimum weight loss was observed for SP-GE coated samples (Fig.1). This might be due to excellent water barrier properties of sesame proteins and GE. Transpiration process, which is analysed by the gradient of water vapour pressure between the fruit and the surrounding air, is responsible for the weight loss of fruits. Both epidermal cell layer and cuticle are helpful in the reduction of transpiration. Edible coatings decreased the rate of transpiration by acting as an extra layer which coats the stomata and leads to reduction in weight loss. All coated samples showed reduced weight loss as compared to non-coated fruit. Similar trend was observed in case of other coated fruits like red guavas (Formiga et al., 2019), tomatoes (Davila-Avina et al., 2014) and eggplant fruits (Singh et al., 2016).

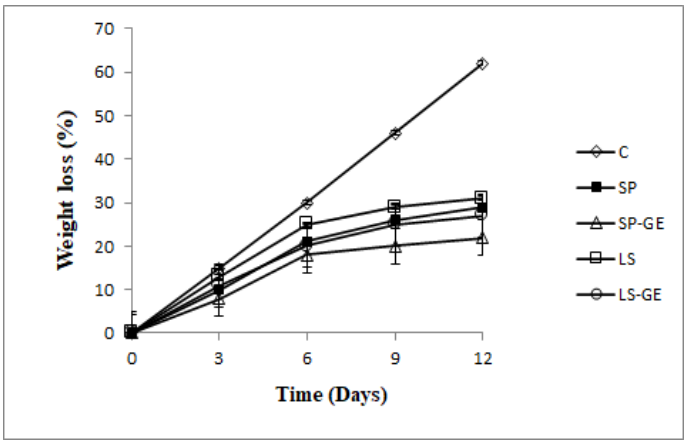

Fig. 1. Effect of edible coatings on weight loss (%) of both coated and non-coated sapota fruit under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations. C: Control sample; SP: Sesame protein film; SP-GE: Sesame protein + *Garcinia indica* film; LS: Lotus starch film; LS-GE: Lotus starch + *Garcinia indica* extract film

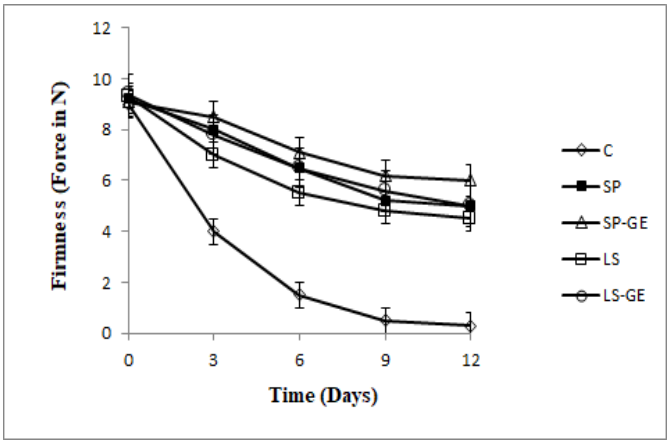

Fig. 2. Effect of edible coatings on firmness (force in N) of both coated and non-coated sapota under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations.

Firmness is considered as an important factor that influences consumer acceptability. Weight loss and moisture loss are responsible for the reduction of firmness of fruits. Pectins and hemicelluloses undergo solubilisation and depolymerisation during fruit ripening, which causes cell wall loosening and disintegration (Wakabayashi, 2000). Overripe and mealy fruits have poor juice release and a dry mouth feel due to high level of cell separation (Waldron et al., 2003). Edible coatings maintain the firmness by declining the rate of respiration and transpiration, slowing ripening, delaying senescence and retarding degradation of cell wall (Baldwin et al., 2011). A common reduction in firmness (Fig. 2) with increase in storage period was observed in all coated and non-coated fruit samples, which can be mainly due to loss in cell turgidity pressure, cell wall degradation, cell breakdown and loss of extracellular and vascular air (Del-Valle et al., 2005). All coated samples succeeded to maintain the fruit firmness as compared to control sample, but maximum firmness was retained by SP-GE and LS-GE coated fruits. Sesame protein and lotus starch alone and with the incorporation of GE significantly ($p \leq 0.05$) maintained the firmness up to 12th day of storage. It was observed that incorporation of GE in sesame protein and lotus starch was further responsible for the reduced respiration rate and helps to maintain the firmness of fruit samples. Formiga et al. (2019) reported that edible coatings based on hydroxylpropyl methylcellulose and beeswax was effective in the retention of firmness of red guava. These observations are

also in agreement with Pullulanase - Octenyl Succinic Anhydride (OSA) coated sapota fruits stored for nine days (Shah et al., 2016).

Respiration Rate

Respiration rate (RR) was observed to be similar for all coated and control samples and did not exhibit much difference till the 3rd day of storage (Fig. 3). Slight increase was observed in RR after the 4th day of storage in case of control samples, which increased sharply by 12th day. All samples showed significantly ($p \leq 0.05$) lower level of respiration rate as compared to control samples. SP-GE and LS-GE coated sapodilla fruits evinced a significant ($p \leq 0.05$) decrease in RR as compared to SP and LS coated samples up to 12 days of storage. Highest respiration rate was exhibited by control sample (61.5 mg CO_2 $kg^{-1}hr^{-1}$) at the end of 12th day of storage, whereas all coated samples demonstrated RR to level of 41.2, 43.1, 44.5 and 48.2 mg CO_2 $kg^{-1}hr^{-1}$in case of SP-GE, LS-GE, SP and LS coated samples, respectively. Results depict that the applications of different coating treatments could decelerate the respiration of sapodilla fruits samples. Coatings could alter the internal atmosphere by enhancing CO_2 to significant levels. Addition of GE could also be responsible for the utilization of O_2 and production of CO_2. As GE have antimicrobial properties and this could be responsible for enhanced barrier properties of the coatings and reduced gas diffusion (Nair et al., 2018). Similar trends were observed for the tomatoes coated with composite edible coatings based on pectin, corn flour and beetroot powder. Coated tomatoes exhibited significant reduction in ripening index over control samples during the shelf-life study of 30 days (Sucheta et al., 2019).

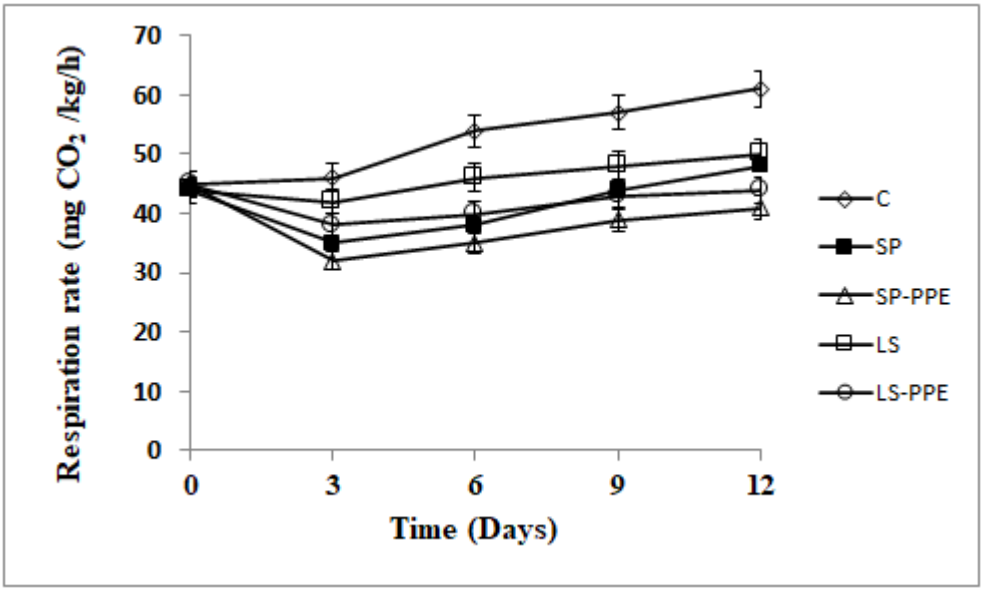

Fig. 3 Respiration rate of sapota under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations.

Ascorbic acid

Reduction in ascorbic acid (AA) in sapodilla fruit during ripening is due to oxidative destruction of ascorbic acid by enzymes, mainly ascorbic acid oxidase (Pawar et al., 2011). Ascorbic acid content of both non-coated and coated fruits reduced during storage (Fig. 4). All coated fruits exhibited significantly ($p \leq 0.05$) less reduction in ascorbic acid as compared to control sapodilla

fruit. On 6th day of storage, AA content of SP-GE coated samples was observed to be significantly ($p \leq 0.05$) different as compared to other coated samples. AA content of SP-GE and LS-GE coated fruits was significantly (($p \leq 0.05$) higher as compared to SP and LS coated fruits. In autoxidation, ascorbic acid combines with oxygen, which occurs during respiration of fruits and this might be responsible for the reduction of ascorbic acid in fruits during ripening. Edible coatings control the permeability of oxygen and carbon dioxide by acting as protective layer and reducing the autoxidation of ascorbic acid followed by the reduction of enzyme activity. Sesame protein and lotus starch-based coatings enriched with GE could decelerate the oxidation process in sapodilla by maintaining higher levels of AA as compared to control during storage. Reduction in ascorbic acid loss was observed in case of guavas coated with chitosan and alginate-based coatings enriched with pomegranate peel extract stored for 20 days (Nair et al., 2018).

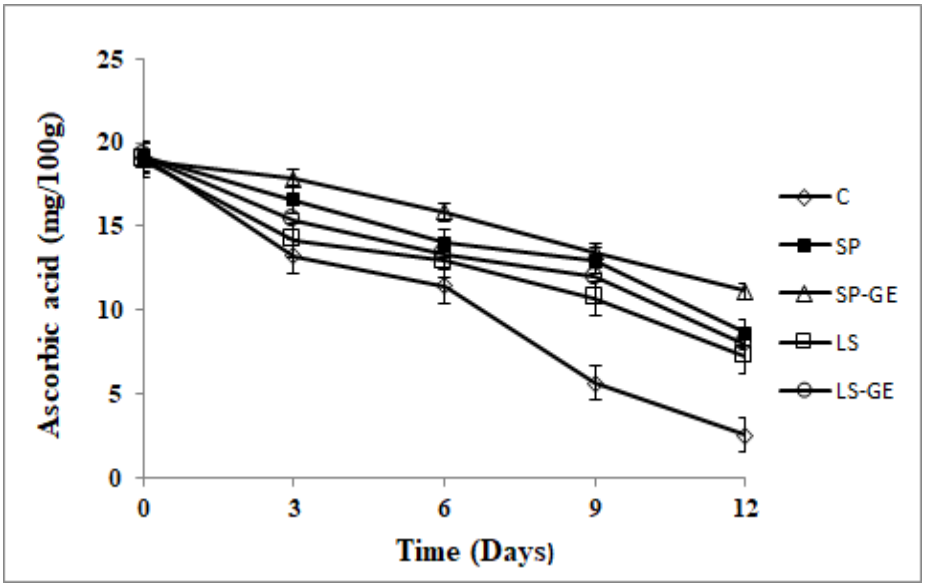

Fig. 4. Effect of edible coatings on ascorbic acid (mg/100g) of both coated and non-coated sapota under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations.

Total carotenoids

Carotenoids are present in chromoplast with β-carotene in major proportion. On the beginning of ripening, the chloroplasts differentiate into chromoplast and *de novo* synthesis of carotenoids takes place, which inevitably increased the content of carotenoids during ripening to senescence of fruits (Bramley, 2013). Less accumulation of carotenoids and total phenols was also related to slow ripening process. It was observed that total carotenoids increased during the storage period (Fig. 5) up to 6th day for all coated and control sample After 6th day, gradual decrease in carotenoid content was observed in control sample. Minimum decrease in carotenoid content after 6th day was observed in SP-GE and LS-GE coated samples. GE was observed to be efficiently maintaining the carotenoid content of coated fruits till the storage of 12 days. Coatings act as semipermeable barrier; decelerate the respiration rate, chlorophyll degradation and biochemical metabolism within the fruits. Similar trend was reported by Saha et al. (2015) for persimmon fruit coated by chitosan and guar gum based edible coatings. Increase in total carotenoids was also observed in plums of both purple and yellow cultivars, which was reported to be delayed by alginate coatings (Valero et al., 2013).

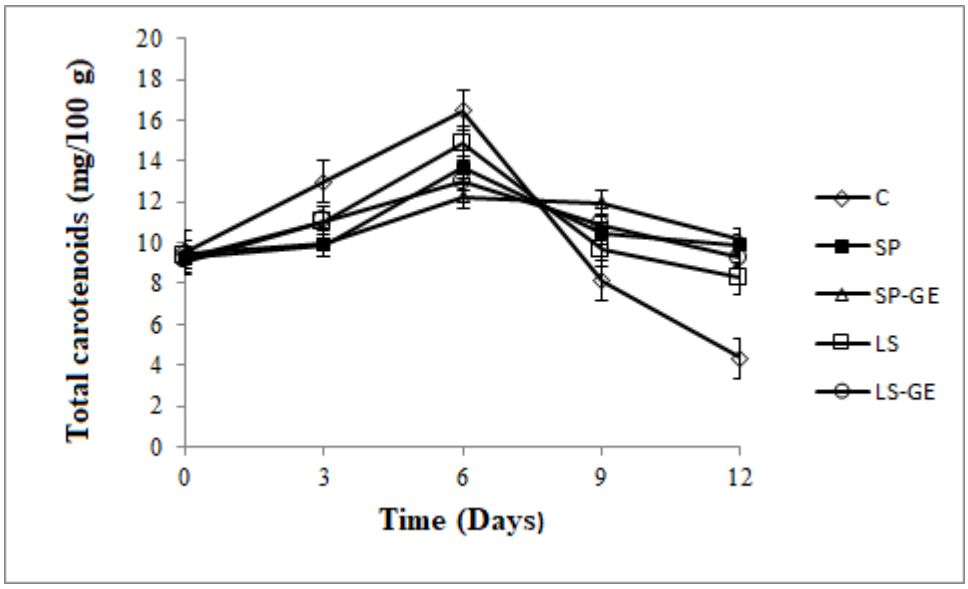

Fig. 5. Effect of edible coatings on carotenoids (mg/100g) of both coated and non-coated sapota under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations.

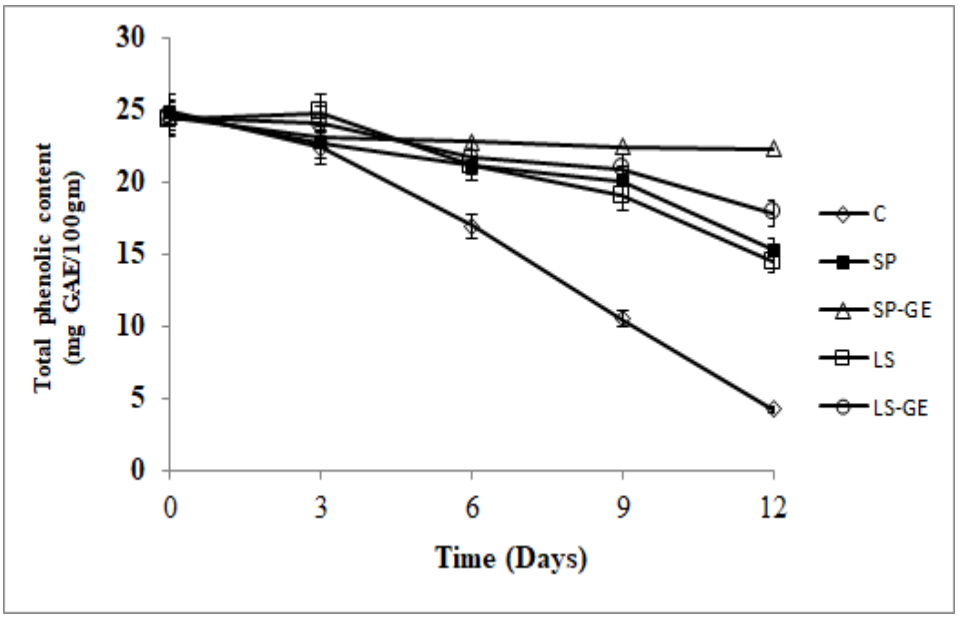

Fig. 6. Effect of edible coatings on total phenolic content (mg GAE/100 g) of both coated and non-coated sapota fruit under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations.

Total phenolic content (TP)

Phenolic content for sapodilla was observed to be 19.81-19.86 mg GAE/100 g on fresh weight basis in non-coated fruit and decreased rapidly during storage in both non-coated and coated fruits (Fig. 6). The decrease in total phenolic content for coated fruits was observed to be significantly ($p \leq 0.05$) less as compared to control fruits. In control sample, TP content of fruits exhibits

sharp decline after 6th day of storage. Minimum TP retention was observed for control sample (16.89%) after the 12th day of storage. Maximum TP content was retained by SP-GE (91.20%) followed by LS-GE (72.82%) coated fruits. TP content of SP and LS coated samples was observed to be significantly ($p \leq 0.05$) different from SP-GE and LS-GE coated samples. SP and LS exhibited retention of 61% and 72.82%, respectively on the 12th day of storage.

Phenolic and flavonoid compounds are secondary metabolites in plants with capability to protect human body tissue against oxidative attacks (Davila-Avina et al., 2014). The production of TP of fruit during storage could be varied depending on the species, cultivar, temperature and environmental conditions during the growth period (Kalt, 2005). Abiotic stress can be produced by edible coatings on fruits, altering its metabolism and affecting the production of secondary metabolites such as phenolic compounds (Gonzalez-Aguilar et al., 2010). This might be the reason for retention of total phenolics in coated fruits compared to non-coated fruits. Sapodilla fruit treated with coatings based on aloe vera gel and *Fagonia indica* plant extract reportedly exhibited higher total phenolic content as compared to non-coated fruits during storage (Khaliq et al., 2019). Ali et al. (2010) observed the positive effect of aloe vera gel coatings on the total phenolic content of litchi fruit.

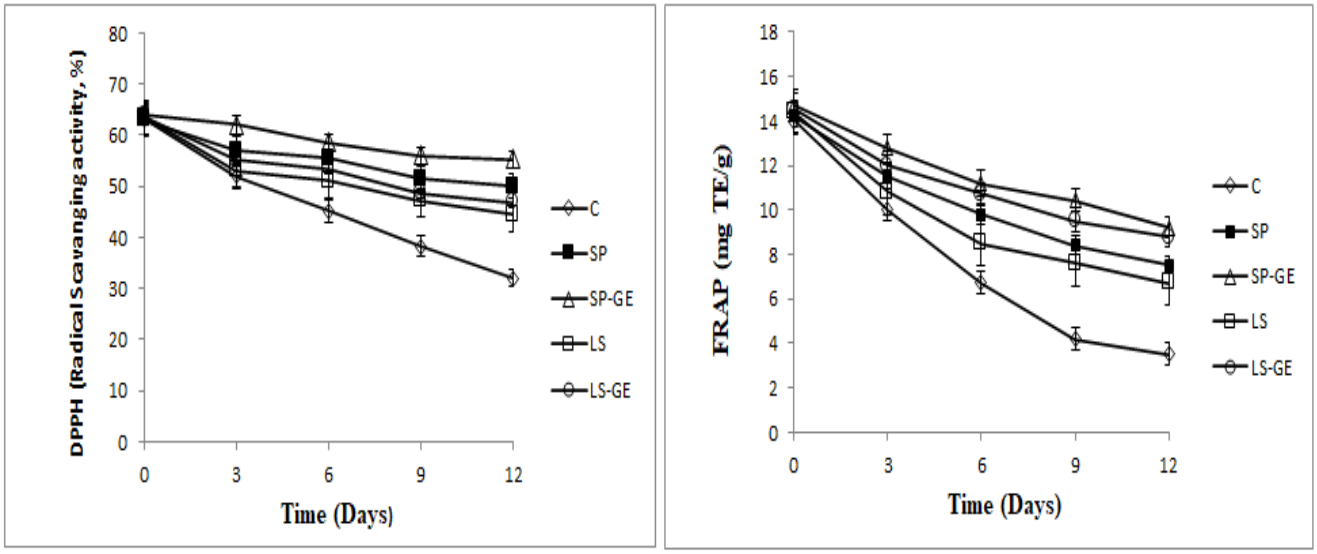

Fig. 7. Variation in antioxidant capacity expressed in terms of (a) DPPH and (b) FRAP in sapota under storage at 20℃. Error bars represent the standard deviations. Results are means of triplicate determinations.

Antioxidant activity (AOA)

Antioxidant potential of all coated samples was observed to be higher as compared to control samples at the end of storage period (Fig. 7). The maximum AOA in terms of DPPH (Fig. 7a) and FRAP (Fig. 7b) was observed in SP-GE coated samples (55.2% RSA and 9.2 mg TE/g, respectively) followed by LS-GE coated samples (46.8% and 8.8 mg TE/g, respectively), whereas minimum AOA was observed for control samples (32 % and 2.5 mg TE/g, respectively) at the end of storage period. Edible coatings are responsible for slowing down the metabolism of fresh produce due to the modification of internal atmosphere, which reduces the synthesis of phenolics, flavonoids and antioxidants (Nair et al., 2018). These secondary metabolites accumulate during storage and responsible for increase in the antioxidant levels. Khaliq et al. (2019) reported the increased levels of antioxidants of coated fruit samples till 6th day and then sudden decline up to 12th day of storage. Antioxidant retention of aloe vera coated fruits was reported to be significantly higher as compared to control samples during storage.

CONCLUSION

Results suggested that utilization of plant extracts such as *Garcinia indica* extract with the combination of sesame protein and lotus starch could maintain the overall quality of sapodilla fruit and extends the shelf life up to 12 days. GE in sesame protein and lotus starch-based coatings helps to retain the nutritional parameters by delaying respiration rate of fruit samples during storage. Also, phytochemicals such as ascorbic acid, carotenoids and total phenolic was found to be retained with the application of coatings. Sesame protein-based coatings were observed to be more efficient as compared to lotus starch which might be due to better barrier properties of proteins then polysaccharide-based coatings. Further, research should be focused to scrutinize the effect of *Garcinia indica* in edible films and coatings developed from different polysaccharides and proteins on other fruits and vegetables.

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