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Research Article

Effect of *Azadirachta indica* and *Tamarindus indica* leaf extract and evaporative cooling on the quality characteristics and shelf life of sapota (*Manilkara zapota*)

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Abstract
Tropical fruits such as Sapota (*Manilkara zapota*) are highly perishable. The main reasons for the quality deterioration of these fruits are field heat and microbial attack, which can be reduced by proper cooling techniques and by using antimicrobial agents. The present study was undertaken to extend the shelf life of Sapota using evaporative cooling and antimicrobial coating, namely, neem leaves (*Azadirachta indica*) and tamarind leaves (*Tamarindus indica*), at different concentrations (20, 50 and 100%). Coated Sapota fruits were stored for 24 days, whereas uncoated fruits were stored for only 7 days. Both the selected antimicrobial extracts showed antimicrobial activity, and the cooling efficiency of the evaporative cooling chamber was 81-85%. Fruits were cooled to 15-18°C (core temperature), which could enable retention of physio-chemical properties. The results indicated that fruits coated with the highest level (100%) of antimicrobial extract coating (neem leaves and tamarind leaves) and stored under evaporative cooling conditions had the lowest percentage loss in weight (5-7% after 24 days of storage) and good retention of total soluble solids (1-2%), pH (2-3%) and total sugars (3-5%). Thus, coating Sapota fruits with neem leaves and tamarind leaf extracts and storing them under evaporative cooling conditions can be a better way to enhance the shelf life of sapota.

Keywords: Antibacterial assay, Antifungal assay, Evaporative cooling, Loss in weight, Total soluble solids

INTRODUCTION

Tropical countries such as India are facing a major problem of storing horticultural produce and maintaining its postharvest quality. *Manilkara zapota* is a tropical and climacteric fruit that has a short shelf life due to its high respiration rate, transpiration and weight loss (Anu *et al*., 2015). Temperature, relative humidity, ambient condition variety and stage of ripening are some of the factors that influence the storage quality of produce (Babaremu *et al*., 2019; Azzolini, *et al*., 2004). Cooling is an appropriate technique to increase the storage quality of horticultural produce, and among different cooling techniques, the evaporative cooling technique is most suitable for tropical regions such as India. In this technique, cooling occurs by passing air that is not too humid on a wet surface. The efficiency of an evaporative cooler depends on the humidity of the surrounding air (Rajendra Kenghe *et al.,* 2015). Evaporative cooling not only lowers the surrounding temperature but also reduces drying of the produce by increasing the air moisture content. There are different methods to design evaporative coolers, but they depend upon the materials available and requirements to decide which design is more suitable for the particular horticultural produce (Amrat lal Basediya *et al*., 2013). The most common quality changes of horticultural produce during storage are because of microbial growth due to high temperature and loss of water (Ndukwu and Manuwa.,2015; Sandhya.,2010 and Tilahun.,2010). The development of coatings from polysaccharides has brought an increase in new types of coatings for extending the shelf life of fruit and vegetables.

Even though some edible coatings have been successfully applied to fresh products, other applications adversely affect quality (Riberio *et al*.,2007). Plant extracts have no negative impact on health and can be managed at low cost (Rajendra Kenghe *et al*., 2015 and Pervin, 2016). Neem leaves (*Azadirachta indica*) have been used as fungicides and insecticides for centuries (Chaturvedi *et al*.,2003). Additionally, studies have proven that tamarind leaves *(Tamarindus indica*) are a good antimicrobial agent (Julio *et al*.,2010; Abdallah and Muhammad,2018). Sapota or sapodilla is native to tropical America and originates in Mexico of Central America (Ajaykumar *et al*.,2012). The climacteric fruit sapota has a short shelf life due to its high respiration rate and ethylene production and leads to weight loss and quality deterioration (Anu *et al*.,2015). Hence, by considering factors such as temperature and microbial spoilage as the most critical factors for the storage quality of horticultural produce, especially fruits, the present study was carried out to analyse the antimicrobial activity of selected plant leaf extracts, i.e., neem leaf (*A. indica*) and tamarind leaf *(T. indica)* extracts, and the cooling effect of the designed evaporative cooler on the physiochemical properties during storage of sapota (*M. zapota*).

MATERIALS AND METHODS

Design of evaporative cooling chamber

The evaporative cooling chamber was designed for the storage of fresh fruits. This portable structure was made of fibre and consisted of two compartments with circular holes all around the periphery, which were kept offset by a 1-inch-thick pine grass mat. Water was dripped onto the mats continuously through a circular flexible pipe placed above the mats (dry straw). This decreased the temperature and maintained humidity. A thermocol sheet was placed in the evaporative cooling chamber as trays to place the fruits to avoid contact between the fruits. Fresh ripened Sapotas (*M. zapota*) were placed on a thermocol sheet. Cool water was poured into the circular pipe, and water was dripped continuously on the mat.

Preparation of antimicrobial extract

The antimicrobial extract treatment solutions were prepared on a percentage weight basis. Dried leaves were chopped and ground in a laboratory mortar to fine powder. The extracts were prepared by adding 100 ml of distilled water to 100 g of leaf powder separately and kept overnight. This resulted in a 100% concentration of every plant extract (1:1 w/v). For 20% and 50%, the same solution was diluted and made into two concentrations (Maragathavalli *et al*., 2012).

Determination of antibacterial and antifungal assay:

Neem leaf (*A. indica*) (E_1) and tamarind leaf *(T. indica)* (E_2) extracts were subjected to antimicrobial and antifungal activity against *Bacillus subtilis* (MTCC no. 441), *E. coli* (MTCC no. 42), *Pseudomonas aeruginosa* (MTCC no. 7952), *Aspergillus niger* (MTCC no. 281), *Rhizopus stolonifer* (MTCC no. 2198) and *Aspergillus flavus* (MTCC no. 873). The culture was purchased from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

This was done by an agar disc method with a 6 mm diameter paper disc for bacterial study and Sbouraud dextrose agar (SDA) plates for fungal study. Paper discs dipped with neem and tamarind leaf extracts were incubated for 24 hrs at 37°C and 72 h at 30°C for antibacterial and antifungal activities, respectively (Hudzicki and Kirby-bauer.,2009).

Cooling efficiency

Cooling efficiency is an index used to assess the performance of a direct evaporative cooler. Cooling efficiency in percentage can be defined as

$$h = \frac{Td - Tc}{Td - Tc} \; x\, 100 \qquad \text{….Eq. 1}$$

where *Td* and *Tw a*re the dry and wet bulb temperatures of the ambient air and *Tc* is the dry bulb temperature of the cooled air at ^{0}C (Mule.,2009; Tilahun.,2010; Zakari *et al*., 2016).

Cooling time of fruit and temperature relationship

Evaporative cooling decreased the chamber temperature and maintained relative humidity. The cabinet temperature and humidity were measured using a digital humidity-temperature meter, and the core temperature of the fruit was recorded throughout the cooling process.

Evaluation of *A. indica* and *T. indica* leaves extract coating and evaporative cooling on shelf life of Sapota:

Sapota fruits were washed, air-dried and subjected to coating at different concentrations. For treatment, 90 healthy uniform-sized Sapota fruits were selected and grouped into three groups with 30 fruits in three experimental groups treated with neem leaf (*A. indica*) and tamarind leaf *(T. indica*) extracts. The selected antimicrobial extract was prepared in three different concentrations of 20%, 50% and 100% and was used for coating. The chamber was tested for its suitability to reduce the temperature while maintaining increased relative humidity. The experiment was carried out using the developed evaporating cooling system. During the testing period, the humidity-temperature meter was suspended in the chamber, and a thermocouple was inserted at regular intervals to ascertain the variation in temperature in the chamber and in the fruit. Both coated and uncoated (Control) Sapota fruits were kept in the Evaporative Cooling Chamber.

Percentage loss in weight:

Sapota fruits (two replicates) were labelled, and the initial weight and weight at regular intervals throughout the storage period were recorded. The average percent weight loss was calculated for each treatment according to the following equation:

weight loss (%) = $[(W_0-W_1)/W_0]$ X 100 …Eq. 2

where W_0 is the initial weight and W_1 is the weight measured on the sampling date (Elansari and Yehia., 2018).

Total soluble solids (0 Brix)

The total soluble solids (0 Brix) were recorded by using a hand held refractometer (Jadhav *et al*.,2018).

Determination of pH

The pH of the fruit pulp was measured using a digital pH meter. Initially, the pH meter was calibrated using the pH 7 buffer solution, and then the probe of the pH meter was placed into the fruit juice, and readings were taken directly. (Jadhav *et al*., 2018).

Total sugar

The total sugar content was calculated using the following formula: (Ranganna.,1977)

Total Sugar (%) = 0.05 x Volume make up X stock solution X 100 / Titre Value x weight of sample originally taken x 25 ….Eq.3

RESULTS AND DISCUSSION

Antibacterial and antifungal assay

It is evident from Table 1 that both extracts had antibacterial and antifungal activity. Higher concentration of extract had higher antimicrobial activity i.e Neem and Tamarind leaf extract had highest inhibition zone at 100 g/100 mL concentration against *B. subtilis* (MTCC no.441)*, E. coli* (MTCC no.42)*, P. aeruginosa* (MTCC no. 7952)*,* than 50 g/100 mL and 20 g/100 mL, respectively. Among two selected extracts, Neem. and tamarind; neem extract had higher inhibition than tamarind extract on *B. subtilis, E. coli* and *P. aeruginosa* with 14 mm, 12 mm and 7 mm zones of inhibition at 100 g/100 mL. Among the three microorganisms, *B. subtilis, E. coli* and *P. aeruginosa, B. subtilis* was inhibited the most at the highest concentration of extract (100 g/100 mL), while *P. aeruginosa* was the least inhibited, with a 0 mm value (no zone of inhibition) at the lowest concentration (20 g/100 mL) of tamarind extract. *E. coli* had a moderate zone of inhibition by both antimicrobial extracts at all concentrations. The antifungal activity results of the antimicrobial extracts are presented in Table 1. Higher concentration of extract had higher antifungal activity i.e Antifungal activity of Neem leaves was found to have higher inhibition than Tamarind leaves against the selected organisms *A. niger* (12 mm)*, R. stolonifer* (10 mm) and *A. flavus* (8 mm) at the

Table 1. Antibacterial activity of antimicrobial extract

Name of the Microorganism	Treatment	Zone of Inhibition (mm)	
		E1	E2
Bacillus Subtilis (MTCC no.441)	100%	14	10
	50%	9	6
	20%	3	5
Escherichia. coli (MTCC no.42)	100%	12	9
	50%	8	5
	20%	3	4
Pseudomonas aeruginosa (MTCC no. 7952)	100%	7	5
	50%	4	4
	20%	2	0
Aspergillus niger (MTCC no.281)	100%	12	9
	50%	8	6
	20%	3	5
Rhizopus stolonifer (MTCC no.2198)	100%	10	10
	50%	6	5
	20%	4	0
Aspergillus flavus (MTCC no.873)	100%	8	5
	50%	4	4
	20%	0	0

E1: Neem leaf (*A. indica*) extract and **E2**: Tamarind leaf *(T. indica)* extract highest concentration 100 g/100 mL, respectively. Among the three microorganisms *A. niger* (MTCC no.281), *R. stolonifer* (MTCC no.2198) and *A. flavus* (MTCC no.873); *Aspergillus flavus* had the least inhibition zone with a value of 8 mm and 5 mm inhibition zone for the highest antimicrobial extract concentration 100 g/100 mL and no zone of inhibition (0 mm) at least antimicrobial extract concentration 20 g/100 mL. At 50 g/100 mL, a moderate zone of inhibition was observed for all three microorganisms in both antimicrobial extracts. The results of the antibacterial and antifungal studies indicate that both antimicrobial extracts have antibacterial and antifungal activity; neem has a better inhibition zone than tamarind at a 100 g/100 mL concentration. Two seaweeds, *Kappaphycus alvarezii* (red algae) and *Sargassum tenerrimum* (brown algae), were selected as antibacterial and antifungal extracts against *B. subtilis*, *Staphylococcus aureus, Lactobacillus acidophilius, Escherichia coli, Pseudomonas aeruginosa*, and *Proteus mirabilis* and against *Apergillus flavus, Aspergillus niger, Aspergillus furigate, Candida albicans and Candida tropicalis* (at 40, 60, 80 and 100 mg/mL) by Thahira *et al*. (2020) to increase the shelf life of tomato, and it was found that the highest concentration, i.e., 100 mg/mL, had the highest inhibition value.

Cooling efficiency

The highest cooling efficiency of 81-85% was recorded in the developed evaporative cooling system. It was observed during the study that the evaporative cooling process cooling rate completely depends on the cooling temperature and humidity within the chamber, which has to be maintained throughout the storage period. An average cooling efficiency of evaporative cooler was found to be 83.0% by Zakari *et al*.,2016 by evaluating the cooling efficiency of the cooler stored with tomatoes.

Cooling time and temperature relationship

The time-temperature relationship is important to determine the time required to reduce the core temperature of Sapota fruits, as it is the deciding factor for the effectiveness of the cooling process. Fig. 1 demonstrates the relationship between time and temperature of coated and control fruit. A linear trend was observed for all treatments, and the R^2 values were observed to be close in all cases. It was observed that there was little effect of the coating on the cooling pattern. With evaporative cooling, the core temperature of Sapota fruit could be reduced to 15-18°C, which is the desirable temperature for safe storage of Sapota fruits.

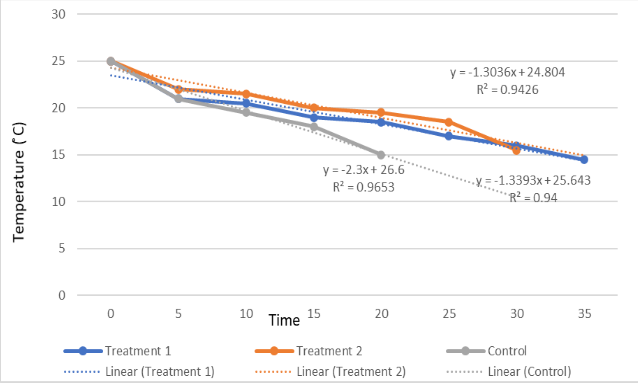

Fig. 1. *Cooling time and temperature relationship of coated and control fruits*

Weight loss of the uncoated and coated Sapota during storage

Table 2 represents the weight loss values of both controls, neem- and tamarind leaf-coated sapota, which were recorded for a total storage period of 24 days. Weight loss recorded for Sapota fruits coated with the highest concentration of Neem was lowest compared to Sapota fruits coated with Tamarind leaf extract coating. Sapota coated with 100% of both extracts contained the lowest level of weight loss, followed by 50% and then 20% of extract coating. Antimicrobial coating was found to provide a protective barrier against gases such as oxygen, carbon dioxide, and water vapour transmission, hence reducing the problem of transpiration. Uncoated Sapota fruits showed the highest weight loss of 10.12% within 6 days of storage, whereas for

Table 2. Weight loss of the uncoated and coated Sapota during storage

Treatment	Storage period (Days)				
	0th day	6th day	12th day	18th day	24th day
E1:100%	0	0.62 ±0.12	2.31 ±0.56	3.66 ±0.33	5.19±0.21
E1: 50%	0	1.44 ±0.16	2.22 ±0.43	3.11 ±0.22	6.50 ±0.12
E1:20%	0	1.56 ±0.12	3.29±0.16	4.51±0.17	7.11±0.56
E2: 100%	0	0.98 ±0.12	3.11 ±0.44	4.15 ±0.23	6.31±0.41
E2:50%	0	0.91 ±0.50	3.23 ±0.25	4.33 ±0.12	6.13 ±0.12
E2:20%	0	1.51 ±0.18	4.11 ±0.12	5.12 ±0.33	6.69±0.47
Control	0	10.12 ±0.12	-	-	-

Data are expressed as the mean±SD, n=3.

fruits stored in Evaporative Cooler even after 24 days, the percentage of weight loss was approximately 5-7%. This was a similar finding as that studied by Balogun *et al*., 2020 who reported that weight loss was highest for mangoes stored at ambient conditions (25%) than the fruits stored in two evaporative coolers, a non-cladded burnt-clay-brick (NBBEC)(13.4%) and an aluminum-cladded burnt-clay brick evaporative coolers (ABBEC) (6.7%) to reduce postharvest loss.

Total soluble solids (TSS °Brix) of the uncoated and coated Sapota during storage

It is evident from Table 3 that 20% of E_2 extract, i.e., Tamarind extract-coated Sapota had the highest and 100% Neem-coated Sapota had the least increase in Total Soluble Solids content during the 24 days of storage period compared to other treatments. The total soluble solids of the control (uncoated Sapota) group were high within 7 days of storage, similar to the highest value of coated fruit after 24 days. This shows that there was control on the ripening process due to the temperature and humidity maintenance in the evaporative cooler. The total soluble solids value was only increased which may be because it was still in its ripening stage. Total soluble solids generally increase during the ripening process due to the polysaccharide hydrolysis to maintain the respiration rate, as stated by Azzolini *et al*. (2004) for the physiochemical properties of guavas harvested at three different stages of maturation on the basis of skin color. Thahira *et al*. (2020) stated that the higher level of total soluble solids in tomato coated with *K. alvarezii* (red algae) and *S. tenerrimum* (brown algae) extract may be due to a protective O_2 barrier reduction of oxygen supply on the fruit surface, which inhibited respiration.

pH of the uncoated and coated Sapota during storage

The increase in pH from 5.19 to 6.36 (as per Table 4) during ripening of sapota fruit may be attributed to the decrease in acidity during ripening. The results of the present study were supported by Paralkar (1985) and Pawar *et al*., (2011) studied the physicochemical properties of Sapota at different ripening stages. There was significant increase in pH value in control sample within 6 days whereas in coated samples pH was increased only 2-3% within 24 days similar difference was observed by Padmaja *et al*. (2015) who studied physical and chemical properties of Sapota coated with edible aloe vera gel in 1:2 ratio for 7 minutes.

Table 3. Total soluble solids (TSS °Brix) of the uncoated and coated Sapota during storage

Treatment	Storage Period (Days)				
	0th day	**6th day**	**12th day**	**18th day**	**24th day**
E1:100%	19.31±0.33	19.33±0.33	20.01 ±0.52	20.36 ±0.33	20.40±0.12
E1: 50%	19.31±0.33	19.63±0.18	20.23±0.36	20.51 ±0.25	20.58±0.16
E1:20%	19.31±0.33	19.99 ±0.19	20.11 ±0.15	21.16 ±0.19	22.31 ±0.11
E2: 100%	19.31±0.33	19.36±0.13	20.36 ±0.25	22.11 ±0.23	22.29 ±0.10
E2:50%	19.31±0.33	19.61±0.56	20.51±0.23	21.36 ±0.18	21.19 ±0.25
E2:20%	19.31±0.33	20.10 ±0.22	20.63 ±0.12	20.18 ±0.39	23.16 ±0.75
Control	19.31±0.33	22.31 ±0.88	-	-	-

Data are expressed as the mean±SD, n=3.

Table 4. pH of the uncoated and coated Sapota during storage

Treatment	Storage Period (Days)				
	0th day	**6th day**	**12th day**	**18th day**	**24th day**
E1:100%	5.19±0.14	5.19±0.30	5.30 ±0.52	5.60 ±0.33	6.30±0.15
E1: 50%	5.19±0.14	5.11±0.26	5.15±0.29	5.94 ±0.25	6.33±0.25
E1:20%	5.19±0.14	5.12 ±0.36	6.01 ±0.15	6.52 ±0.82	6.98 ±0.11
E2: 100%	5.19±0.14	5.36±0.88	5.36 ±0.31	5.63 ±0.23	6.56 ±0.77
E2:50%	5.19±0.14	5.46±0.53	5.87±0.17	5.96 ±0.51	6.31 ±0.42
E2:20%	5.19±0.14	5.22±0.22	6.30 ±0.12	6.35 ±0.39	6.36 ±0.12
Control	5.19±0.14	22.31 ±0.88	-	-	-

Data are expressed as the mean±SD, n=3.

Table 5. Change in total sugar content during storage

Treatment	Storage Period (Days)				
	0th day	**6th day**	**12th day**	**18th day**	**24th day**
E1:100%	14.4±0.12	14.56±0.30	15.69 ±0.61	17.22 ±0.33	18.20±0.15
E1: 50%	14.4±0.12	14.63±0.24	16.32±0.32	17.88 ±0.54	18.36±0.24
E1:20%	14.4±0.12	15.12±0.36	15.62 ±0.15	17.86 ±0.65	19.10 ±0.15
E2: 100%	14.4±0.12	14.53±0.81	15.12 ±0.31	16.93 ±0.61	18.66 ±0.77
E2:50%	14.4±0.12	14.63±0.55	15.29±0.19	17.99 ±0.51	18.52 ±0.42
E2:20%	14.4±0.12	15.63±0.22	16.82 ±0.33	18.88 ±0.39	19.00 ±0.12
Control	14.4±0.12	19.77 ±0.88	-	-	-

Data are expressed as the mean±SD, n=3.

Total sugars of the uncoated and coated Sapota during storage

The total sugar content of sapota fruit at different stages of ripening increased significantly from the mature stage (14.40%) to the ripe stage (19%), as shown in Table 5. An increase in sugars during the ripening process in sapota fruit may probably be due to the accumulation of more sugars in the fruit due to hydrolysis of starch, and a slight decline at the overripe stage was due to utilization of sugars during the respiration process. The results of this investigation are in agreement with the results obtained by Raut (1999) when effect of postharvest processing and handling on maturity indices of Sapota were studied at different ripening stages. Also, similar results were observed by Bindu *et al*.,2013 while investigating the effect of packaging of sapota fruits in polythene bags with different gauges (100, 200 and 300 gauge) and with different levels of ventilations (0.8, 1.2 and 1.6%) on the postharvest shelf life and quality of Sapota under low temperature 12±1°C and RH 85-90%.

Conclusion

In the present study, it was observed that coating Sapota fruit with antimicrobial agents did not affect the efficiency of the evaporative cooling technique and in turn provided good storage conditions. From the mentioned results, it was concluded that the 100% *A. indica* (Neem) extract coating and low-temperature storage using the evaporative cooling technique were the most effective treatments in maintaining the quality characteristics of Sapota fruits during the storage period of 24 days when compared to the control treatment (uncoated) at low temperatures stored for 7 days. Treated fruit with 100% Neem extract had the highest antimicrobial activity, the lowest percentage of weight loss, the lowest increase in total soluble solids and less change in pH compared with the control. Of course, the second coating with *T. indica* (Tamarind) leaf extract was on par with the first coating in all the results. Neem and tamarind leaf extracts are effective antimicrobial agents that could be used to improve the shelf life of sapota, and evaporative cooling will help to maintain fruit quality by reducing the fruit core temperature to 15°C; both factors together enhance the shelf life of the produce. Thus, the shelf life of Sapota can be increased by evaporative cooling and coating with antimicrobial extracts.

Conflict of interest

The authors declare that they have no conflicts of interest.

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