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Effect of chlorine and silver grafted zeolite-ldpe composite bags packaging on the bio-chemical properties of sapota

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Abstract

In this study, zeolite-LDPE composite bags with or without infusion of antimicrobial compound (chlorine or silver) were employed as novel packaging materials. The influence of packaging innovation on bio-chemical parameters of sapota (*Manilkara achras*, Family: Sapotaceae) fruits under cold storage condition was investigated. Sapota fruits first packed in antimicrobial compound synergized zeolite-LDPE composite bags and then placed in corrugated fiber board (CFB) box showed significantly lower respiration rate, total soluble solids (TSS) and higher titratable acidity and more shelf-life than the sapota fruits packed in corrugated fiber board (CFB) box alone. Our results suggest that zeolite-LDPE composite bags synergized with antimicrobial compounds could maintained the postharvest quality and increase the shelf-life of sapota fruits.

Keywords: Zeolite, *Manilkara achras,* synergized, LDPE (Low density poly ethylene) and days after storage (DAS)

1. Introduction

Sapota fruits are classified under extremely high ethylene producers; they are highly perishable in nature, ripe quickly, lose moisture rapidly, spoiled faster and achieve senescence speedily after harvest (Singh *et al.,* 2017) [11]. The level of ethylene production in sapota fruits increases slowly from the beginning of ripening and reaches its peak at 144 hours, followed by a decline (Selvaraj and Pal, 1984) [10].

Ethylene is a gaseous plant hormone that plays a major role in the regulation of the metabolism of harvested horticultural crops at very low concentrations (Zhang *et al.,* 2012). The post-harvest life of both climacteric and non-climacteric fruits can be influenced by ethylene. This hormone affects their quality attributes, the development of physiological disorders and post-harvest diseases (Ernst, 2011) [3]. Effects of ethylene on quality attributes *viz*., external appearance, texture, flavour and nutritive value of fruits have been extensively reported (Saltveit, 1999; Ernst, 2011) [3]. Any closed environment such as truck trailer, shipping container, warehouses, cold rooms and consumer size package results in increase in concentration of ethylene. Therefore, the need to control ethylene activity to extend the post-harvest life of fruits through improvement in packaging, introducing anti-ethylene substances is greater than ever.

Zeolite is a large and diverse class of volcanic aluminosilicate crystalline material which has many useful applications (Khosravi *et al.*, 2015) [7]. The use of zeolite as an adsorbent has started in 1930s followed by Milton, who used zeolite for air purification (Kamarudin, 2006) [5]. Zeolite is a nanoporous crystalline alumina silicate having trihedral and tetrahedral structure. It contains large vacant spaces or cages in its structure that provide space for adsorption of cations or large molecules such as water and ethylene (Khosravi *et al.*, 2015) [7]. It has a rigid, three dimensional crystalline structure consisting of a network of interconnected channels and cages. Water moves freely in and out of these pores, but the zeolite framework remains rigid (Kamarudin, 2006) [5]. Moreover, the incorporation of antimicrobial compounds into zeolite-LDPE composite bags can further improve the physical, mechanical and biological properties of the bag (Lee *et al.*, 2017).

Among inorganic antimicrobial agents, chlorine and silver compounds could highly inhibit microbial growth and show strong biocidal effects on many species of bacteria including *Escherichia coli* (Kim *et al.,* 2007; Lee *et al.,* 2009 and Yang *et al.,* 2009) [12, 8]. The interaction of chlorine and silver ions with microbial cytoplasmic components and nucleic acids can inhibit the respiratory chain enzymes and interferes with the membrane permeability, limiting the development of bacteria, fungi and yeast (Russel and Hugo, 1994) [9].

In this study, the effect of antimicrobial compounds synergized zeolite-LDPE composite bags packaging on the postharvest quality of *M. achras* fruits was evaluated and recorded for the first time.

2. Materials and Methods
2.1 Materials and treatments
The present investigation was undertaken in the Department of Post Harvest Technology, University of Horticultural Sciences, Bagalkot, Karnataka, India during the year 2018-19. Sapota fruits (cv. Kalipatti) of uniform size and shape, free from any visible damage, scratches and decay were selectively harvested manually from a commercial orchard at proper maturity stage. The maturity stage was judged as right stage based on skin colour of the fruits which changed from light brown to dark brown (potato like colour) and brown scale like structure on the surface of fruit was disappeared. The fruits were brought to the laboratory in plastic crates. Soon, the plastic crates containing fruits were placed in the cold room for pre-cooling by room cooling method at 13ºC for 12 hours. Then fruits were packed in zeolite-LDPE composite bags with or without outer CFB box *viz.*, Silver-zeolite-LDPE composite bag (T_1), Zeolite-LDPE composite bag (T_2), Chlorine-zeolite-LDPE composite bag (T_3), Silver-zeolite-LDPE composite bag + CFB box (T_4), Zeolite-LDPE composite bag + CFB box (T_5), Chlorine-zeolite-LDPE composite bag + CFB box (T_6), Commercially used CFB (T_7) and Control (without any package) (T_8) @ 6 fruits/treatment (per bag) and stored under refrigerated (13±1°C, 85-90% RH) conditions. A thermostat of the walk-in cold room maintained the set temperature. Relative humidity in the storage chamber was maintained by with the help of a humidifier.

2.2 Respiration rate (ml CO_2 kg^{-1}h^{-1})
The rate of respiration was measured by static head space method using gas analyzer (Make: PBI, DANSENSOR, and CHECKMATE 2) and expressed as ml CO_2kg^{-1}h^{-1}. To carry out this method, four sapota fruits were trapped in 3000 ml airtight containers having twist-top lid fitted with a silicone rubber septum at the center of lid. The containers were kept for an hour for accumulation of respiratory gases at the head space. After specified time, the head space gas was sucked to the sensor of the analyzer through the hypodermic hollow needle and the displayed value of evolution of CO_2 concentration (%) was recorded. Further, the rate of respiration was calculated on the basis of amount of CO_2 evolved from the fruit per unit weight per unit time using the following formula.

$$\text{Rate of respiration } (CO_2/\text{kg/h}) = \frac{CO_2 \text{ concentration } (\%) \times \text{Head space}}{100 \times \text{weight of fruit (kg)} \times \text{Time (hr)}}$$

2.3 Total Soluble Solids (TSS) (°B)
The juice extracted by squeezing the homogenized fruit pulp through muslin cloth was used to measure the TSS. It was determined by using digital refractometer (Make: Hanna Instruments, Romania) replicated three times and the mean was expressed in °Brix.

2.4 Titratable acidity (%)
Five grams of homogenized pulp was made up to 100 ml and filtered through muslin cloth. Then, 10 ml of the filtrate was taken for titration against 0.1 N NaOH solution using phenolphthalein as an indicator. The appearance of light pink colour was considered as end point. The acidity was calculated and expressed as per cent malic acid.

$$\text{Acidity } (\%) = \frac{\text{Titre value} \times \text{N of NaOH} \times \text{Vol. made up} \times \text{Eq. weight of acid}}{\text{Vol. of aliquot} \times \text{Vol. of sample taken} \times 1000} \times 100$$

2.5 Shelf life (days)
The number of shelf life days was decided based on physiological loss in weight (PLW). A PLW of 10% was considered as the upper limit for determination of shelf life (Jaya, 2010).

2.6 Experimental design and data analysis
The first experiment was carried out with 3 replicate fruits and the experiment was repeated 3 times and pooled data was subjected to statistical analysis. Fruits were arranged in Complete Randomised Design. The second experiment was carried out for 3 replicate fruits and repeated 3 times. Randomly selected 8 fruits were taken to analyse respiration rate, Total Soluble Solids (TSS), titratable acidity and shelf life and all the experiments were repeated 3 times. The data of experiment was analyzed as applicable to completely randomized design (CRD). Statistical analyses of experiments were performed using Web Agri Stat Package (WASP) Version 2 (Jangan and Thali, 2010). The level of significance used in 'F' and 't' was p=0.01 and also p=0.05 for some parameters. Critical difference values were calculated whenever F-test was found significant.

3. Results and discussion
3.1 Respiration rate (ml CO_2 kg^{-1}h^{-1})
Fresh sapota fruits used in the study had respiration rate of 78.09 ml CO_2 /Kg/hr. With storage, mean of respiration rate increased in all the treatments up to 15 days and a decrease at 18 and 21 DAS (Table 1). The data revealed significant differences with respect to respiration rate of sapota during 21 DAS. The rate was found to be minimum in the treatment T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) throughout the storage (3 DAS-81.4; 6 DAS-92.2; 9 DAS-108.5; 12 DAS-128.9; 15 DAS- 195.6; 18 DAS-192.3; 21 DAS-164.1 ml CO_2 /Kg/hr) in comparison to all other treatments. In the present study, hurdle to respiration process was created by low temperature as well as zeolite composed package. Porous zeolite is effective in adsorbing gases such as oxygen, carbon dioxide and ethylene and water vapour, and thereby reducing respiration (Khosravi *et al.*, 2015) [7]. This is possibly due to biocidal effect of chlorine (Kim *et al.*, 2007; Lee *et al.*, 2009 and Yang *et al.*, 2009) [8, 12] leading to delay in fruit senescence. However, T_6 treatment had non-significant difference with T_3 (Chlorine-zeolite-LDPE composite bag), T_4 (Silver-zeolite-LDPE composite bag + CFB box) and T_5 (Zeolite-LDPE composite bag + CFB box) throughout the storage except at 12 DAS when T_6 differed significantly over all other treatments. Reduced respiration of fruits in zeolite-LDPE variants (with or without chlorine or silver) in CFB boxes (T_4, T_5, T_6) than those without CFB (T_1, T_2, T_3) could be due to beneficial synergistic effect of combined packages. Sapota fruits kept open condition (T_8) had significantly higher respiration rate followed closely by T_7 (Commercially used CFB) throughout the storage period. This defends the beneficial effect of zeolite-LDPE bags.

3.2 Total Soluble Solids (TSS) (°B)
TSS was found to increase gradually along the 21 days period of storage (Table 2). But this parameter TSS did not present statistical difference, Minimum TSS was found in T_6

(Chlorine-zeolite-LDPE composite bag + CFB box) at all the days of observation (3 DAS-16.41 °B; 6 DAS-17.72°B; 9 DAS-18.90°B; 12 DAS-19.30°B; 15 DAS-20.26°B; 18 DAS-22.80°B and 21 DAS-24.71°B). Zomoroid (2005) recorded minimum TSS in cut apples stored along with a pouch of zeolite (1 gm) for a period of 6 days. Zeolite has adsorbing property of oxygen, carbon dioxide and ethylene gases from storage environment thus checking the biochemical differentiation and metabolic reorganization leading to reduction in TSS (Khosravi *et al.*, 2015) [7]. Maximum TSS was observed in T_8 (Control) at all the 7 observations taken during the study (3 DAS-18.09 °B; 6 DAS-21.78°B; 9 DAS-22.97°B; 12 DAS-23.74°B; 15 DAS-24.92°B; 18 DAS-24.31°B and 21 DAS-28.82°B). However, a difference of 3-4^{o}B between the treatments practically has tremendous impact on taste, flavour and ripening of fruit. In climacteric fruits like sapota, the conversion of starch to sugars in the fruit is an important component of the ripening process, giving the fruit its distinctive sweet flavour as well as precursor for many of the aromatic flavour compounds (Kays, 1998) [6].

3.3 Titratable acidity (%)

Titratable acidity (%) was found to increase from the initial value (0.14%) up to 3 DAS and later declined in all the storage treatments of sapota (Table 3). At all the days of observation, maximum acidity was associated with T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) (3 DAS-0.60%; 6 DAS-0.58%; 9 DAS-0.55%; 12 DAS-0.51%; 15 DAS-0.49%; 18 DAS-0.46% and 21 DAS-0.42%). The most important feature of zeolite is effective adsorption of gases such as oxygen, carbon dioxide and ethylene and water vapour leading to reduced respiration and metabolism of fruits (Khosravi *et al.*, 2015) [7]. Reduction in metabolism could reduce the use of organic acids as substrates in respiration (Islam *et al.*, 1996) [4], thus resulting higher acidity in fruits. The T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) recorded higher acidity than the fruits without any package (T_8). Further, higher titratable acidity in antimicrobial compounds synergized zeolite-LDPE variants in CFB boxes (T_4, T_5, T_6) than those without CFB (T_1, T_2, T_3) could be due to beneficial synergistic effect of combined packages. Lower titratable acidity observed in fruits kept in CFB alone (T_7) strongly justifies the effect of zeolite-LDPE bags with or without antimicrobial compounds.The treatment T_8 (Control) showed significantly minimum titratable acidity (3 DAS-0.28%; 6 DAS-0.26%; 9 DAS-0.23%; 12 DAS-0.20%; 15 DAS-0.18%; 18 DAS-0.18% and 21 DAS-0.13%) closely followed by T_7 (Commercially used CFB) throughout the study duration. Dhua *et al.* (2006) [2] and Bhutia *et al.* (2011) [1] revealed that sapota fruits packed along with ethylene absorbers showed marked retardation of ripening and also maintenance of higher acidity in comparison to control fruits.

3.4 Shelf –life (days)

Sapota fruits recorded significantly maximum shelf life (days) in T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) (17.66 days) (Table 9). It was however non-significant with T_1 (Silver-zeolite-LDPE composite bag), T_2 (Zeolite-LDPE composite bag), T_3 (Chlorine-zeolite-LDPE composite bag) and T_4 (Silver-zeolite-LDPE composite bag + CFB box). Significantly minimum shelf life (days) was recorded in T_8 (Control) (12.00 days) and this treatment was on par with T_7 (Commercially used CFB) (9.66 days).

The packaging yielded an extra shelf-life of 8 days over control (T_8) and 6.33 days over T_7 (Commercially used CFB). Slow rate of ripening of sapota fruits in T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) is documented in terms of slower decrease in fruit firmness, reduced respiration rate, physiological loss in weight and disease score. Realization of more shelf life in T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) was mainly due to retarding ethylene effect on ripening process and due to effect of chlorine on post harvest decay. Altered micro-environment and cushioning benefit provided by CFB box in T_6 (Chlorine-zeolite-LDPE composite bag + CFB box) was supportive to the health and wholesomeness of sapota fruits.

Table 1: Effect of antimicrobial compounds synergized zeolite-LDPE composite bags on respiration rate (ml 0f CO_2 /Kg/hr) of sapota fruits under refrigerated condition (13 °C)

Treatments	Respiration rate (ml 0f CO_2 /Kg/hr)							
	3 DAS	6 DAS	9 DAS	12 DAS	15 DAS	18 DAS	21 DAS	Mean
Initial	78.09							
T_1	89.1^{bcd}	98.7^{abc}	122.2^{cd}	153.8^{cd}	208.9^{cd}	199.2^{b}	183.9^{bc}	150.82
T_2	91.5^{abc}	100.8^{ab}	125.3^{bc}	160.6^{bc}	213.8^{bc}	201.5^{ab}	185.6^{abc}	154.15
T_3	87.8^{bcde}	96.0^{abc}	118.6^{de}	149.0^{de}	206.8^{cd}	198.8^{b}	180.3^{c}	148.18
T_4	83.4^{de}	92.4^{c}	111.8^{fg}	137.6^{f}	200.6^{de}	196.4^{bc}	168.3^{de}	141.50
T_5	85.7^{cde}	94.3^{bc}	115.1^{ef}	144.5^{ef}	204.8^{cde}	196.6^{bc}	171.7^{d}	144.67
T_6	81.4^{e}	92.2^{c}	108.5^{g}	128.9^{g}	195.6^{e}	192.3^{c}	164.1^{e}	137.57
T_7	93.9^{ab}	102.7^{a}	128.4^{ab}	163.6^{ab}	220.4^{ab}	202.8^{ab}	189.8^{ab}	157.37
T_8	96.2^{a}	104.1^{a}	131.0^{a}	169.6^{a}	224.7^{a}	206.0^{a}	192.7^{a}	160.61
Mean	88.62	97.65	120.11	150.95	209.45	199.20	179.55	
S.Em±	2.33	2.74	1.53	2.77	3.45	2.14	2.47	
CD@1%	9.65	8.23	6.36	11.46	14.26	6.41	10.22	

*DAS- Days After Storage

Table 2: Effect of antimicrobial compounds synergized zeolite-LDPE composite bags on TSS (°B) of sapota fruits under refrigerated condition (13°C) *DAS-Days After Storage

Treatments	TSS (°B)							
	3 DAS	6 DAS	9 DAS	12 DAS	15 DAS	18 DAS	21 DAS	Mean
Initial	16.36							
T_1	17.21	19.62	20.93	22.32	22.84	23.83	25.91	21.80
T_2	17.48	19.98	21.56	22.87	23.09	23.98	26.36	22.18
T_3	17.02	19.07	20.22	21.98	22.61	23.55	25.74	21.45
T_4	16.69	18.79	19.19	20.02	21.48	22.97	24.95	20.58
T_5	16.85	18.91	19.85	20.88	21.84	23.16	25.30	20.97
T_6	16.41	17.72	18.90	19.30	20.26	22.80	24.71	20.01
T_7	17.82	20.02	22.19	23.07	24.26	24.00	27.28	22.66
T_8	18.09	21.78	22.97	23.74	24.92	24.31	28.82	23.51
Mean	17.19	19.48	20.72	21.77	22.66	23.57	26.13	
S.Em±	0.44	2.14	2.10	2.90	2.22	1.22	0.40	
CD@5%	NS	NS	NS	NS	NS	NS	NS	

Table 3: Effect of zeolite-LDPE composite bags on titratable acidity (%) of sapota fruits under refrigerated condition (13°C)

Treatments	Titratable acidity (%)								Shelf life (days)
	3 DAS		9 DAS	12 DAS	15 DAS	18 DAS	21 DAS	Mean	
Initial	0.14								
T_1	0.42^{e}	13.33^{d}	0.36^{de}	0.33^{cde}	0.30^{d}	0.28^{c}	0.26^{bcd}	0.33	13.33^{d}
T_2	0.38^{f}	11.66^{e}	0.32^{ef}	0.30^{def}	0.28^{de}	0.26^{cd}	0.24^{cde}	0.30	11.66^{e}
T_3	0.47^{d}	13.66^{d}	0.41^{cd}	0.38^{bcd}	0.35^{cd}	0.33^{bc}	0.30^{abc}	0.38	13.66^{d}
T_4	0.56^{b}	16.00^{b}	0.51^{ab}	0.47^{ab}	0.44^{ab}	0.40^{ab}	0.38^{ab}	0.47	16.00^{b}
T_5	0.51^{c}	14.66^{c}	0.46^{bc}	0.43^{abc}	0.41^{bc}	0.38^{ab}	0.36^{abc}	0.43	14.66^{c}
T_6	0.60^{a}	17.66^{a}	0.55^{a}	0.51^{a}	0.49^{a}	0.46^{a}	0.42^{a}	0.51	17.66^{a}
T_7	0.31^{g}	11.33^{e}	0.26^{fg}	0.23^{ef}	0.21^{ef}	0.18^{de}	0.16^{de}	0.23	11.33^{e}
T_8	0.28^{g}	9.66^{f}	0.23^{g}	0.20^{f}	0.18^{f}	0.15^{e}	0.13^{e}	0.20	9.66^{f}
Mean	0.44	13.49	0.38	0.35	0.33	0.30	0.28		13.49
S.Em±	0.02	0.30	0.05	0.03	0.05	0.05	0.03		0.30
CD@1%	0.03	1.28	0.10	0.17	0.10	0.12	0.17		1.28

4. Conclusion

Our results have shown that antimicrobial compounds synergized zeolite-LDPE composite bags packaging had a positive effect on the postharvest quality of *M. achras* fruits. The preservation effect of antimicrobial compounds synergized zeolite-LDPE composite bags could be attributed to its intrinsic ethylene adsorbing property as well as antimicrobial property. Thus sapota fruits packaged with antimicrobial compounds synergized zeolite-LDPE composite bags showed lower respiration rate, total soluble solids (TSS), titratable acidity and more shelf-life. These indicated that antimicrobial compounds synergized zeolite-LDPE composite bags could be explored as a novel active packaging material for the postharvest storage of sapota fruits.

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