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SYNERGISTIC EFFECTS OF CHITOSON AND MORINGA LEAF EXTRACT ENHANCED THE POST-HARVEST QUALITY OF SWEET ORANGE FRUITS (*CITRUS SINENSIS* L.)

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ABSTRACT

The experiment entitled "Effect of Chitosan and Moringa leaf extracts on the postharvest quality of sweet orange fruit" cv. Blood Red was conducted at the post-harvest laboratory, Department of Horticulture, The University of Agriculture, Peshawar, during 2021-2022. The experiment was design as completely randomized (CRD) replicated three times. Moringa leaf extract coating, Chitosan concentration and storage days were considered as three factors for conducting the experiments. Fruits were kept at room temperature (23ºC, RH =63 ±1). Results showed that in case of Moringa leaf extract coating, maximum score for appearance (7.50), taste (6.67), fruit firmness (2.84 kg cm^{-2}), total soluble solids (TSS) (14.41 $brix^{0}$), pH (4.76), titratable acidity (1.70%) and ascorbic acid was noted in fruits coated with 20% Moringa leaf extracts. while in case of chitosan coating favorable score for appearance (6.83), taste (6.69), fruit firmness (2.76 kg cm^{-2}), TSS (13.73 $brix^{0}$), pH (4.56), titratable acidity (1.61%), ascorbic acid (40.87 mg $100g^{-1}$), weight loss (9.97%) and decay incidence (2.24%) were noted on 4% coated fruit. In term of storage days, desirable mean values were detected on fresh day for all the studied traits. From the above finding it is concluded that 20% Moringa leaf extracts and 4% Chitosan coated fruits showed better results on shelf-life and post-harvest quality of sweet orange fruit.

Keywords: Orange, fruit quality, postharvest, tropical and subtropical fruit

INTRODUCTION

Sweet orange (*Citrus sinensis* L.) cv. Blood Red are classified as non-climacteric and belongs to family Rutaceae. Botanically, citrus fruit is a type of berry called hesperidium (Hussain et al., 2021). In Pakistan, the total area under citrus cultivation is 181.6 thousand hectares with average production of 2468.65 thousand tones. Brazil is the leading yielding country of oranges throughout the world, which produces 19,811,064 metric tons followed by United States (FAOSTAT, 2014). Pakistan ranks 11th in the oranges production in the world with a world share of 2.2 % (Siddique et al., 2018).Citruses constitute 85-90% water, 6-9% sugar, less than 2% acids, pectin, essential oils, protein, minerals and fiber (Sharma et al., 2017). Oranges contain huge amount of citrus limonoids, which fight against cancer (skin, lung, breast, stomach and colon). As they contain enough amount of soluble fiber which helps in lowering cholesterol, keep blood sugar level under control and provide protection against colon cancer. (Addi et al., 2021). To ensure maximum health benefits, edible coating is the prime aim to inhibit ripening mechanisms and preserve the fruit from water loss and spoiling and may be a viable approach to extend product shelf life (Suri et al., 2022). Therefore, coating fruits have become more resistant to pathogens and increasing their storage and marketing (Romero et al., 2022). Postharvest decay represents major losses of fruits. The application of chemicals creates imminent problems of residues to protect postharvest diseases; thus, there is a growing need to develop bioactive substances to control the decay. By toxicological studies it is indicated that Chitosan is a by-product from the seafood industry (Wang et al., 2022) and has been used widely in medicine, agricultural production and food industry (Strano et al., 2022). Based on the information collected from the previous studies the current research was conducted and designed to assess the effect of chitosan on sweet orange under different storage conditions. Moringa is one of the medicinal plant tree having numerous economic applications and utilizations in human consumption (Prajapati et al., 2022). The extract of the moringa leaves has a significant antimicrobial activity thus could be used as natural preservative because it is effective in preventing growth of fungi (Patil et al., 2022). Its leaves extract might be considered as a microbial inhibitor and it can increase the preservative activity of fortified juices. The phenolic hydrocarbon and alcoholic contents of Moringa extract increase the shelf-life of juices (Hashemi et al., 2021).

MATERIALS AND METHODS

The research was conducted at postharvest Horticulture lab, department of Horticulture, The University of Agriculture Peshawar Pakistan during the December 2021- January 2022 to assess the Effect of Chitosan and Moringa leaf extract on the postharvest quality of sweet orange fruit (cv. Blood Red). The experiment was laid out in completely randomized design (CRD) with three factors replicated three times. Factor A, Moringa leaf extract while factor B, Chitosan concentrations and Factor C, storage days were used in experiment (Table a). The data were taken at 7 days' interval up to 28 days.

Table a: detail oof three factors considered in the experiment.

Factor A (Moringa leaf extract)		
M_1=Control	M_2=10% moringa extract solution v/v	M_3=20% moringa solution v/v
Factor B (Chitosan concentrations)		
C_1=Control	C_2=2% chitosan powder solution (w/v)	C_3=4% chitosan solution w/v

Factor C (Storage days)				
S_1= Fresh	S_2= 7 days	S_3= 14 days	S_4= 21 days	S_5= 28 days

Ascorbic acid content (mg 100g^{-1})

Ascorbic acid was measured through titrimetric procedure as outlined in AOAC (2012). From each sample, 5 ml of juice was extracted. Then oxalic acid 0.4% was added and the volume was diluted to 50 ml. From this 50 ml, 10 ml were obtained as an ascorbic acid sample. A dye solution (50 mg 2-6 di-chloro phenol indo-phenol + 42 mg baking soda) from the burette was used to titrate the solution. The appearance of the colour pink served as evidence that the goal had been reached. After recording the burette reading, the following calculation or formula was used to determine the vitamin C levels:

$$Ascorbic\ acid\ \left(\frac{mg}{100g}\right) = \frac{L \times F \times 100 \times 100}{S \times P}$$

Where,

L = ml of dye used

F = Dye factor

P = Sample volume for titration.

S = ml of dilute solution taken for titration

Weight loss (%)

With a high-performance balance, it was possible to calculate the weight loss of the samples. Each treatment left a label on one fruit, indicating the amount of weight loss. The number of a particular period was multiply by 100 and removed from the sample's starting value. The below formula will be used to compute the % weight reduction.

$$Weight\ loss(\%) = \frac{Wi - Ws}{Wi} x100$$

Where;

Wi preliminary weight prior to storage

Ws last weight after storage

Decay incidence (%)

At the conclusion of the study, the number of rotten fruits in every treatment was recorded and computed using below formula.

$$Decay\ percentage = \frac{No.\ of\ rotten\ fruits}{Total\ no.\ of\ fruits} \times 100$$

Statistical analysis

The ANOVA (Analysis of Variance) method was used to conduct the statistical analysis of the data for the CRD with three factor research. In order to calculate the ANOVA and LSD value, statistical software Statistix (8.1) was used to evaluate the Means using Least Significant Differences (LSD) at the 5% level of significance (Jan *et al*., 2009).

RESULTS AND DISCUSSIONS

Appearance of sweet orange was significantly influenced by different concentration of Moringa leaf extracts coating and Chitosan coating while interaction between them was recorded significant (Table 1). Among the different moringa leaf extracts and chitosan coating maximum appearance score (7.50) and (6.83) was recorded in fruits coated with 20% coating of leaf extracts and 4% Chitosan, respectively. In storage days, maximum appearance score (8.07) was recorded in fruit at fresh day (Table 2). In term of appearance, the overall appearance of the skin improved. A group of an expert' judges assessed the appearance of sweet orange. The maximum appearance score was recorded in the coated fruits, which might be related to lowest respiration rate in these fruits, which decrease the loss of coloured pigmentation from the fruit surface, resultant in a higher appearance score. During prolonged storage the loss of colour is related to the evaporation of colour pigments from fruits surface (Gao et al., 2019). Fruits' appearance was enhanced by the formation of carotenoids and anthocyanins and the breakdown of chlorophyll during storage (Yao et al., 2020). Mean squares for taste of sweet orange was significantly affected by different concentration of Moringa leaf extracts coating and Chitosan coating while interaction between them was recorded non-significant Table 1. Desirable taste score was recorded in fruits coated with 20% Moringa leaf extract (6.67) and 4% Chitosan (6.69). Fresh fruit had maximum taste score (7.68) regarding storage Table 2. The coating reduces respiration, water loss, and suppresses the oxidation reaction by acting as a semi-permeable barrier to the movement of carbon dioxide, oxygen, moisture, and solutes. As a result, the consumer would be satisfied with the fruit's flavor (Ncama et al., 2019). The current findings are comparable to those of Saxena et al. (2020), who found that fruits of the Jincheng orange type had good eating quality without an unpleasant flavor because of the waxes. Kumar et al. (2023) published similar results, showing that the taste score in "Musambi" sweet orange (*Citrus sinensis*) fruits reached its peak after 30 days. Mean square for fruit firmness (kg cm^{-2}) of sweet orange was significantly affected by different concentration of Moringa Leaf Extract coating and Chitosan coating and their interaction Table 1. Maximum fruit firmness was recorded in fruits coated with 20% Moringa leaf extract and fruits coated with 4% Chitosan. Firmness gains high score for fresh fruits compare to stored Table 2. According to Rikhotso et al. (2022), oxygen stimulates the production of de-hydro ascorbic acid from ascorbic acid, which lowers fruit firmness. Similar to this, fruit firmness is impacted by transpiration from the fruit's surface. Fruits soften due to cellular disintegration, which increases membrane permeability, or the breakdown of insoluble proto-pectins into soluble pectins (Mattoo *et al.,* 1975). According to Firdaus et al. (2023), an edible coating of glycerin and gum arabic increased the shelf life of peach fruits by enhancing quality factors including firmness and colour, which support our findings. Significant mean squares were obtained for total soluble solid affected by different concentration of Moringa leaf extracts coating and Chitosan coating while the interaction between variety and coating was found non-significant Table 1. Maximum total soluble solids were recorded in fruit coated with 20% moringa leaf extract (14.41 ^{o}Brix) and fruit coated with 4% Chitosan (13.73 ^{o}Brix). In contrast of other parameter soluble solid was recorded (14.38 ^{o}Brix) maximum at 28 days compare to fresh fruit Table 2. Oranges are non-climacteric fruits; hence very little ethylene is produced. Fruits' storage time is extended by the conversion of starch to sugar and the hydrolysis of polysaccharides in the cell wall, which raises their TSS (Brizzolara et al., 2020). The elevated TSS at room temperature may be attributed to higher metabolic activity (Singh et al., 2017). On the other hand, increased TSS at 2oC occurred prior to the onset of chilling damage (Pott et al., 2020). According to a study, the rise in total soluble solids, weight loss, and loss of firmness were all significantly decreased in oranges coated with Aloe vera gel (Wu et al., 2020). Sweet orange was analyzed to have affected the pH significantly by different concentration of Moringa leaf extracts, Chitosan coating and their interaction Table 1. Highest pH (4.76) was recorded in fruits coated with 20% Moringa and 4% Chitosan. Desirable lowest pH (4.05) was recorded in fresh fruits compared to stored fruit Table 2. The pH level increased with longer storage times. The breakdown of acids during fruit storage due to respiration may be to blame for the rise in pH. A large increase in catabolic activities brought on by high respiration rates leads to the breakdown of organic acids and an increase in pH level (Sajid et al., 2019). Due to water vapors in the pack, pH rises throughout the last storage periods, which leads to saturation of the pack's environment (Nasir et al., 2016). Titratable acidity of sweet orange was influenced significantly by different concentration of Moringa leaf extract coating and Chitosan coating while interaction between them was found non-significant Table 1. Least desirable titrarable acidity was recorded in fruit coated with 20% Moringa leaf extracts and 4% Chitosan. In contrast of pH, the fruit showed minimum titrarable acidity (1.09%) after 28 days of storage Table 3. Our results support the claims presented by Bakhsh et al. (2020) who used coating that reduces water loss and the respiration process. Coatings prevent gas exchange, which causes fruit to accumulate CO2 and lose acidity while being stored. Fruits' reduced titratable acidity is caused by the Krebs cycle, which oxidises organic acids throughout the ripening process to create energy reserves for fruits (Sarvarian et al., 2022). According to some investigations, the higher concentration of Aloe vera gel coating suppressed the decline in titratable acidity, which may be related to a reduction in the respiration and catabolism of soluble solids like sugar and organic acid (Alsalhy et al., 2020). The analysis showed that the Ascorbic Acid (mg 100 g^{-1}) of sweet orange significantly affected by different concentration of Moringa leaf extract coating, Chitosan coating and their interaction Table 1. Moringa leaf extracts with 20% and chitosan 4% influenced ascorbic acid at highest in sweet orange. Maximum Ascorbic Acid (59.10 mg 100 g^{-1}) was recorded in fruit at fresh day compared to days Table 3. A less stable vitamin, ascorbic acid loses stability with prolonged storage (Baltazari et al., 2020). All of the orange samples revealed a decline in ascorbic acid level over the course of storage (Wibowo et al., 2015). Ascorbic acid decreases as moisture lost since it is water soluble. The advantage of coating is that it minimizes ascorbic acid oxidation by preventing oxygen from accessing the fruits (Arilla et al., 2024). Coating fruits delays its ripening which allows it to maintain its content of ascorbic acid for a longer period of time (Habibi et al., 2020). Significant variation sweet orange water loss (%) was detected from different concentration of Moringa leaf extract coating, Chitosan coating and their interaction Table 1. Minimum weight loss of 10.48% and 9.97% was recorded in fruit coated with 20 % Moringa leaf extract and 4% Chitosan coated fruits, respectively. In storage day, maximum weight loss (13.89%) was recorded on 28 days while no weight loss (0.00%)

was found in fresh fruits Table 3. Losses in weight are important parameter and significantly impact the appearance (Shiekh et al., 2021). Fruits with coatings may have served as a barrier, preventing moisture from fruit surfaces from evaporating. transpiration, respiration, and other metabolic processes are the key factors that lead to weight loss in fruits and other horticulture products (Maringgal et al, 2020). Additionally, the process of transpiration in which water vapors is moved from inside cells to the outside atmosphere. Our results support Bajaj et al. (2023) assertion that edible coatings can easily preserve fruit weight and minimize water loss (Alvarez et al., 2022). Mean square showed that decay incidence (%) of sweet orange significantly affected by different concentration of Moringa leaf extract coating and Chitosan and their interaction Table 1. Minimum decay incidence (2.16%) was recorded in fruits coated with 20% Moringa leaf extracts. Similarly, minimum score was recorded on 4% coated fruit (2.24%) with chitosan. Maximum decay incidence (3.95%) score was recorded with highest storage days compared to fresh fruits Table 3.Decay is one of the most important postharvest factors in reduction of quality horticultural Crops. Yeshiwas et al., (2021) studied how pathogens infected strawberry fruits were affected by the coating of chitosan. Chitosan was fatal to moulds cells, stopped the polygalacturonases from secreting, and stimulated enzymes involved in defensive mechanisms. Fruits damage less quickly during storage time in the early days of fresh commodities, when respiration is higher and sugar loss is greater, antifungal appearance was less (Ahmad et al., 2021) and antimicrobial activities was increase (Lufu et al., 2020).

Table 1: Mean square of Appearance, taste, Fruit firmness, TSS, PH, Tiratable acidity, Ascorbic acid, Weight loss and Decy incidence of Sweet orange as effected by different concentration of Moringa and Chitosan coating during storage.

SOV	Appearance	Taste	Fruit Firmness	TSS	PH	Tiratable acidity	Ascorbic acid	weight loss	Decy Incidence
Moringa	35.17**	35.17**	0.70**	26.60**	3.69**	1.16**	4.07 ns	3.58 ns	0.58*
Chitosan	4.98**	4.98**	0.32 ns	1.71**	0.58*	0.31**	272.20**	38.93**	0.09 ns
Storage	32.69**	32.69**	33.13**	10.45**	1.99**	4.28**	7301.59**	857.52**	91.89**
M x C	0.52*	0.52 ns	0.37*	0.50 ns	0.74**	0.04 ns	308.00**	93.17**	0.11 ns
M x S	0.10ns	0.10*	2.46**	0.23 ns	0.30*	0.07 ns	355.19**	8.78**	0.47*
C x S	0.04 ns	0.04 ns	3.50**	0.35 ns	0.03 ns	0.05 ns	205.31**	21.71**	0.12 ns
M x C x S	0.04 ns	0.04 ns	1.98**	0.16 ns	0.12 ns	0.04 ns	136.88**	22.88**	0.17 ns
Error	0.16	0.16	0.14	0.35	0.12	0.03	29.38	2.24	0.18
CV%	6.13	9.96	14.08	4.38	7.88	10.71	14.22	15.48	18.64

**= significant at 1% , * =significant at 5% ns=non significant, TSS= Total soluble solid, M=moringa, C=chitosan, S=storage and CV= coefficient of variation.

Table 2: Effect of moringa leaf extract coating, chitosan coating and storage duration on Appearance, Taste, Fruit Firmness (kg.cm^{-2}), TSS (^{o}brix) and pH of Sweet orange

Moringa Leaf Extract	**Parameters**				
Coating (%) (MC)	**Appearance**	**Taste**	**Fruit Firmness (kg cm^{-2})**	**TSS (brix^o)**	**pH**
0	5.79 C	6.13 B	2.60 B	13.02 B	4.22 B
10	6.23 B	6.47 A	2.64 B	13.15 B	4.32 B
20	7.50 A	6.67 A	2.84 A	14.41 A	4.76 A
LSD (P≤0.01)	0.16	0.26	0.15	0.24	0.14
Chitosan Coating (C)					
0	6.17 C	6.27 B	2.60 B	13.35 B	4.34 B
2	6.51 B	6.31 B	2.72 AB	13.49 AB	4.40 B
4	6.83 A	6.69 A	2.76 A	13.73 A	4.56 A
LSD (P≤0.01 OR 0.05)	0.16	0.26	0.15	0.24	0.14
Storage Days (SD)					
Fresh	8.07 A	7.68 A	4.43 A	12.73 D	4.05 D
7	7.14 B	6.57 B	3.10 B	13.24 C	4.32 C
14	6.23 C	6.14 C	2.35 C	13.45 C	4.45 BC
21	5.76 D	5.96 CD	1.86 D	13.84 B	4.56 B
28	5.33 E	5.78 D	1.73 D	14.38 A	4.78 A
LSD (P≤0.05)	0.21	0.34	0.20	0.32	0.18

Mean in their respective columns followed by the same letters are non-significant 1% level of probability

Table 3: Effect of moringa leaf extract coating, chitosan coating and storage duration on Titratable Acidity (%), Ascorbic Acid (mg. $100g^{-1}$), weight loss (%) and Decay incidence (%) of Sweet orange.

Moringa Leaf Extract Coating (%) (MLE)	Parameters			
	Titratable Acidity (%)	Ascorbic Acid (mg. $100g^{-1}$)	Weight loss (%)	Decay incidence (%)
0	1.70 A	37.78 A	11.00 A	2.38 A
10	1.54 B	38.19 A	10.82 A	2.33 AB
20	1.38 C	38.36 A	10.48 A	2.16 B
LSD (P≤0.01)	0.06	2.27	0.81	0.17
Chitosan Coating (C)				
0	1.61 A	36.13 B	11.82 A	2.32 A
2	1.56 AB	37.33 B	10.50 B	2.31 A
4	1.44 B	40.87 A	9.97 B	2.24 A
LSD (P≤0.01)	0.06	2.27	0.81	0.17
Storage Days (SD)				
Fresh	2.08 A	59.10 A	0.00 D	0.00 E
7	1.73 B	51.66 B	9.67 C	0.59 D
14	1.56 C	32.72 C	11.58 B	3.27 C
21	1.23 D	24.89 D	13.13 A	3.63 B
28	1.09 E	22.18 D	13.89A	3.95 A
LSD (P≤0.01)	0.08	2.93	0.80	0.23
Interactions (LSD at P≤0.05)				

Conclusions and Recommendations

It was concluded from the current research that sweet orange coated with 20% moringa leaf extract were found for retaining post-harvest quality of sweet orange. Similarly, the traits that influenced the quality of sweet orange after harvesting were best enhanced from 4% chitosan coating with 28 days storage limit. Sweet orange at fresh days were found with no weight loss, no decay incidence, minimum total soluble solids (Brix°), maximum fruit firmness (kg cm^{-2}), maximum ascorbic acid (mg 100^{-1}), minimum titratable acidity (%), good taste and appearance of sweet oranges at fresh days. It is recommended, based on the obtained conclusions that sweet orange should be coated with 20% moringa leaf extract and 4% chitosan for better postharvest quality and shelf life of sweet orange up to 28 days of storage.

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