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# Effect of Active Atmosphere Packaging using Natural Fungicide on Quality Characteristics and Shelf Life of Kinnow Fruit

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## Abstract

The present study was carried out to evaluate the influence of active packaging parameters i.e., dose of scavenger (3-5 g), fungicide concentration (5-7%), and polythene thickness (100-200 gauge) on the postharvest quality and shelf life of *Citrus reticulata* (kinnow) under cold storage. Response Surface Methodology (RSM) with Box Behnken Design (BBD) was employed to model and optimize the effects on key quality attributes, including O<sub>2</sub> and CO<sub>2</sub> gas concentration, physiological loss in weight, firmness, total soluble solids, titratable acidity, ascorbic acid, color difference, microbial load, and shelf life. Results indicated that higher doses of scavenger, increased fungicide concentration, and thicker films significantly ( $p < 0.05$ ) reduced O<sub>2</sub> and CO<sub>2</sub> levels, delayed ripening, retained firmness and nutritional quality, and minimized microbial growth, ultimately extending shelf life up to 65 days. The mathematical model suggested a high coefficient of determination with optimum conditions as 5 g dose of scavenger, 6.83% fungicide concentration and 192.12 gauge polythene thickness. A non-significant ( $p < 0.05$ ) difference was observed in the actual and predicted values of dependent variables. The findings suggest that active packaging incorporating optimized combinations of scavengers, natural fungicides, and polymer thickness can effectively preserve the quality of kinnow during cold storage. This approach offers a promising, low-residue alternative to conventional chemical treatments for enhancing citrus shelf life in postharvest handling systems.

**Keywords:** Active packaging; Coating; Fungicide; Kinnow; LDPE; Scavenger; Shelf life

## Introduction

'Kinnow' (*Citrus deliciosa* × *Citrus nobilis*) is the foremost citrus fruit of Punjab, and more than half of the total area covered with fruit is occupied by only this cultivar. In Punjab, January and February are the best times for its harvesting, but its demand remains constant throughout the year. At room temperature, it can be stored only for 7-8 days, so unable to fulfil its long-term demand.

Moreover, the lack of processing facilities and cold chain infrastructure during peak harvest season forces farmers to sell their produce at minimal prices in local markets (Randhawa et al., 2009). Being high in moisture, soft, and debilitative nature, the fruits undergo a significant postharvest loss, including microbial spoilage, weight loss, physiological damage, physical damage, and reduced



nutritional quality (Bhan et al., 2022). The reason behind its short shelf life is the evaporation of moisture at a higher rate when stored without cuticles (Dhall, 2013). Certainly, there is an urgent need to look for appropriate approaches that could maintain the quality of food for the long term and reduce postharvest losses.

During or after the harvesting of fruits, the balance of gases between oxygen consumption and carbon dioxide production has changed (Dhall, 2013). Several techniques, such as active packaging, surface coating, etc., are available to maintain the after-harvest life in fruits (Adhikary et al., 2022). Nowadays, consumers are aware of the cons of commonly used sanitizing techniques and are looking for safe, fresh, healthy, and easy-to-prepare food with high quality retention. To satisfy the requirements of consumers, active packaging (use of scavengers or certain agents) is used, which is a very innovative development in the area of packaging, based on deliberate interactions with the food itself or its environment (Ramos et al., 2013). Components included in active packaging are capable of scavenging O<sub>2</sub>, CO<sub>2</sub>, moisture, and ethylene and also remove undesirable flavours (De Kruijf et al., 2002). Various researchers have explored the use of scavengers such as Iron-based O<sub>2</sub> scavenger in oranges (Yadav and Ray, 2010), "Bartlett" pears (Kumar and Thakur, 2020), ethylene adsorber in Broccoli (Esturk et al., 2013), and economical adsorbents in sewage water treatment (Jha et al., 2024) etc. In another study on strawberries, O<sub>2</sub> and CO<sub>2</sub> scavengers were simultaneously used, which extended the shelf life up to 4 weeks at 4°C (Aday et al., 2011).

Apart from this, it is worth mentioning that, during washing/precleaning, the natural wax coating on fruit skin is removed, and it is essential to replace it with different synthetic and natural coating materials (Ali et al., 2015). Coatings on fresh produce can reduce quantity and quality losses by controlling and changing their inside atmosphere. Coatings can also reduce the migration of solute and moisture, rates of oxidative reactions, aerobic metabolism, and gas exchange, thereby extending the shelf life. Moreover, it is capable of carrying out some active ingredients like antimicrobials and anti-browning agents that can further enhance the shelf life of food and also reduce the chance of pathogen growth on its surface (Dhall, 2013). After harvesting, deterioration of produce due to fungus can be controlled by synthetic or chemical coatings. However, there are some hurdles in the use of chemical coatings, i.e., concerns of the public regarding their leftovers in produce and an increase in the resistance of pathogens. The use of coatings that are plant-based (natural) and biodegradable might be a good alternative to chemical coatings, which can also fulfil the requirements of consumers for healthy and natural foods (Mahajan et al., 2018).

Taking into consideration the aforesaid properties, neem leaf powder was chosen to evaluate its efficacy for the extension of Kinnow shelf life. Azadirachtin is an active substance present in neem, which is known for its insecticidal, growth-regulating, nematocidal, and fungicidal properties (Malik et al., 2016). In recent studies, neem oil on kinnow fruit, followed by HDPE packaging, has been explored (Randhawa et al., 2009). In another study, Gupta and Jain (2014) used neem leaf extract as a coating on Mango fruits cv. Dashehri.

However, to the best of our knowledge, the combined effect of various scavengers and neem leaf powder coating on postharvest quality attributes of kinnow fruit has not yet been studied. Therefore, the present study was designed to optimize the process parameters, namely the dose of scavenger, fungicide concentration, and polythene thickness and to evaluate their effect on preserving the kinnow quality and enhancing its shelf life.

## Materials and Methods

Freshly harvested uniform-sized mature kinnow fruits were procured from the local market, Ludhiana.

### Experimental procedure

#### *Preparation of Neem leaf powder (Natural Fungicide)*

The neem leaves were collected and washed with tap water to remove adhering dirt and other impurities. Clean leaves were dried in a tray drier (Model 119, Creative Lab World, India) at 65°C until completely dried (Freitas et al., 2018), followed by grinding in a domestic grinder (Philips HL 7699/00) to make fine powder.

#### *Pre-treatment of kinnow with natural fungicide (Neem solution)*

Three different concentrations of fungicide, i.e., 3, 5, and 7%, were prepared by adding 30, 50, and 70 g of neem leaf powder in 1 L of water, respectively. The resulting solutions were filtered with a cotton cloth to obtain only an aqueous solution. Then, kinnow fruits were divided into three parts

and immersed separately in prepared neem leaf solutions for 1 min (Freitas et al., 2018). After treatment, the fruits were kept at room temperature for 5 h for surface drying (Richa et al., 2014).

#### **Preparation of the scavenger sachet and storage**

Iron powder, activated charcoal, and potassium permanganate were used as oxygen, carbon dioxide, and ethylene scavengers, respectively. Weighed quantities of these powders were packed separately in small pouches prepared from filter paper (inner layer) and high-density non-woven polypropylene (outer layer) has 0.11 mm thickness, 8-15 µm pore size, and 0.9±0.2 g/ml density (Shalini, 2017; Kumar and Thakur, 2020). Pre-treated kinnow (1kg ± 50g) was packed in LDPE pouches along with a scavenger sachet and stored in a cold chamber for 90 days at 6±1°C and 90-95% RH (Baswal et al., 2020). Observations were done at the interval of 5 days in terms of quality parameters viz. gas composition, physiological loss in weight, ascorbic acid, total soluble solids, titratable acidity, colour difference, firmness, microbial load, and shelf life.

#### **Experimental design and optimization**

The relationship between independent variables (dose of scavenger, fungicide concentration, and polythene thickness) and responses (O<sub>2</sub> and CO<sub>2</sub> gas concentration, physiological loss in weight, firmness, total soluble solids, titratable acidity, ascorbic acid, color difference, microbial load, and shelf life) was examined by using a three level and three factor Box–Behnken design (BBD). The corresponding levels of the three independent or process variables were selected based on previous literature and preliminary findings. These included: A, dose of scavenger (3-5 g), based on the effective range reported by Richa et al. (2014); B, fungicide concentration (5-7%), as recommended by Freitas et al. (2018) for effective microbial control while ensuring product safety; and C, polythene thickness (100-200 gauge), in accordance with the optimal packaging ranges suggested by Kumar and Thakur (2020) for maintaining moisture and gas barrier properties during storage. Table 1 shows the details of all 17 combinations of independent variables. All the experiments were done in triplicate. The relative effects of independent variables on the responses were evaluated by developing the second-order polynomial mathematical models.

#### **Quality parameters of kinnow**

The headspace gas composition (%), i.e., O<sub>2</sub> and CO<sub>2</sub>, was recorded at equal intervals of time using a portable Headspace Gas Analyzer (Model GS 3/P, Systech Instruments, Thames, UK). Headspace gas samples were taken by means of a hypodermic needle inserted through an adhesive septum (Mahajan et al., 2016). The firmness of fruits was measured using a handheld penetrometer (Tr di turonil Co. Snc. Italy) with 8 mm stainless steel probe, and results were expressed in terms of kg force (kgf) (Baswal et al., 2020). Yeast and mold counts were recorded using the method suggested by Farzana et al. (2017), and ascorbic acid (mg/100ml) using Indophenol dye was determined as described by Aggarwal et al. (2022).

Weight loss (%) and acidity (%) were determined according to the standard methods given in AOAC (2000). A hand-held refractometer (0-32°B) was used to record total soluble solids. The color was recorded as L, a, and b values using Color Reader CR-10 colorimeter (Konica Minolta Sensing Inc.) & color difference (ΔE) was calculated using following formula, where L, a, and b represent the color values on the first day of storage, and L<sub>s</sub>, a<sub>s</sub>, and b<sub>s</sub> correspond to the color values of the samples after storage.

$$\Delta E = \sqrt{(L_s - L)^2 + (a_s - a)^2 + (b_s - b)^2}$$

#### **Statistical analysis**

The final data was analyzed statistically using Design Expert (version 13.0.1.0) to determine the effects of different independent variables on dependent parameters. Least significant difference was calculated at 5% level of significance.

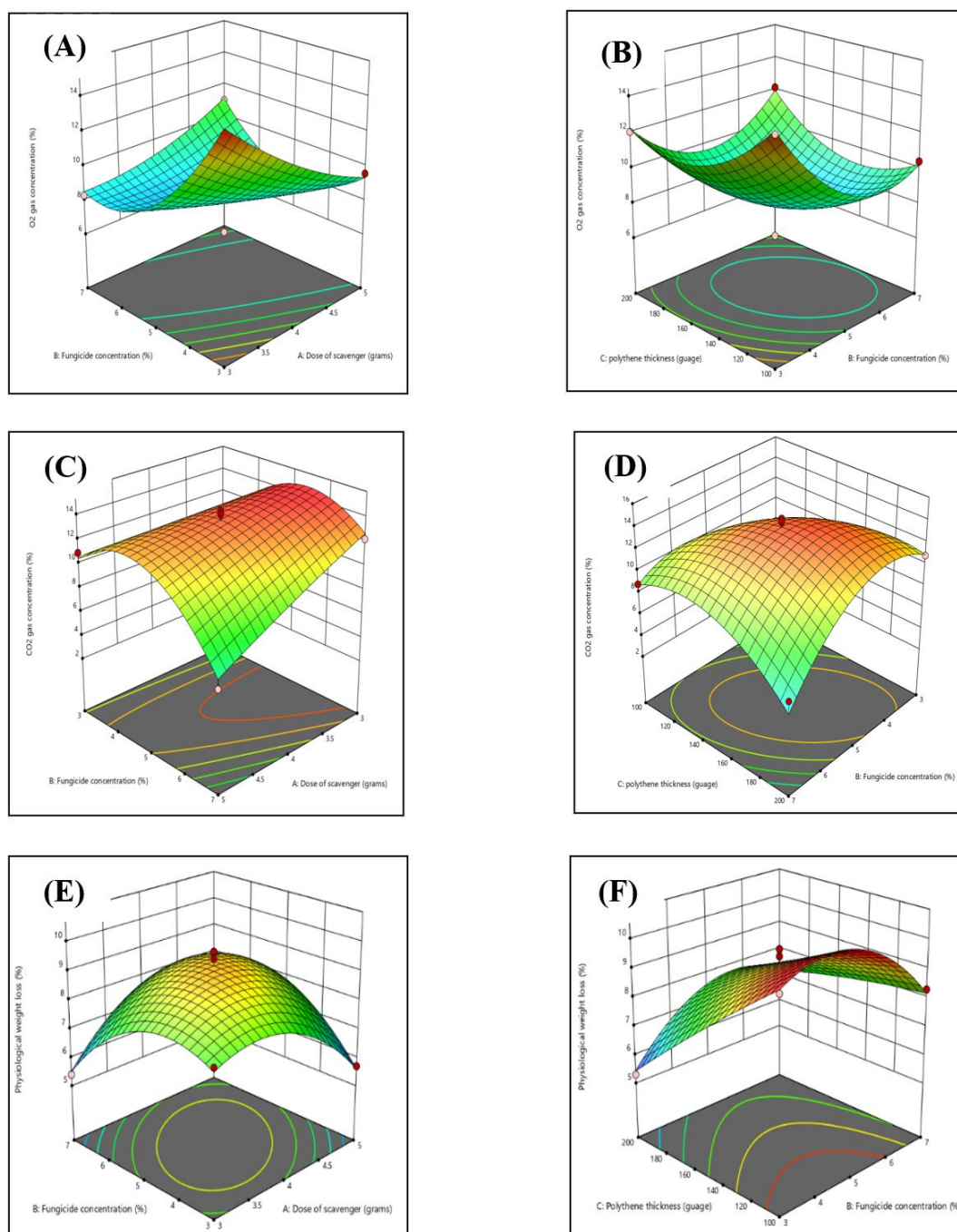
#### **Results and Discussion**

The effect of different independent variables, i.e., dose of scavenger, fungicide concentration, and polythene thickness, was investigated on the quality parameters and shelf life of kinnow fruit. It was observed that O<sub>2</sub> and CO<sub>2</sub> gas concentration, physiological loss in weight, firmness, total soluble solids, titratable acidity, ascorbic acid, color difference, microbial load, and shelf life varied from 6.10 to 15.36%, 2.10 to 14.43%, 5.30 to 10.05%, 2.11 to 4.44 kgf, 9.20 to 13.10°B, 0.23 to 1.71%, 17.11 to 35.71 mg/100ml, 0.22 to 7.99, 5.10 to 9.80×10<sup>3</sup> cfu/ml, and 15 to 65 days respectively (Table 1). The effect of independent parameters on the responses at linear, interaction, and quadratic levels has been studied (see Table 2); the significant (p<0.05) regression coefficient values were

highlighted with an asterisk (\*), where the quadratic response surface model was further used for the statistical analysis. The values of  $R^2$  for all the responses were more than 0.85 and the lack-of-fit test was found to be highly insignificant, indicating the selected model was sufficiently accurate for the prediction of responses.

**Table 1.** Different combinations of independent variables along with responses obtained during active packaging of kinnow

| Independent variables |                             |                             | Responses                            |                                       |                                  |                |                           |                         |                          |                  |                                          |                   |  |
|-----------------------|-----------------------------|-----------------------------|--------------------------------------|---------------------------------------|----------------------------------|----------------|---------------------------|-------------------------|--------------------------|------------------|------------------------------------------|-------------------|--|
| Dose of scavenger (g) | Fungicide concentration (%) | Polythene thickness (gauge) | O <sub>2</sub> gas concentration (%) | CO <sub>2</sub> gas concentration (%) | Physiological loss in weight (%) | Firmness (kgf) | Total Soluble Solids (°B) | Titrateable acidity (%) | Ascorbic acid (mg/100ml) | Color difference | Microbial load (×10 <sup>3</sup> cfu/ml) | Shelf life (days) |  |
| 4                     | 5                           | 150                         | 6.1                                  | 14.2                                  | 9.01                             | 2.58           | 11.3                      | 0.23                    | 20.81                    | 4.35             | 9.8                                      | 30                |  |
| 4                     | 3                           | 200                         | 12.07                                | 10.84                                 | 5.3                              | 3.11           | 12.3                      | 0.40                    | 23.97                    | 5.09             | 5.4                                      | 50                |  |
| 5                     | 5                           | 200                         | 9.63                                 | 9.9                                   | 6.61                             | 2.89           | 11                        | 1.09                    | 24.09                    | 0.22             | 5.3                                      | 65                |  |
| 4                     | 5                           | 150                         | 8.43                                 | 14.4                                  | 8.94                             | 2.56           | 11.4                      | 0.89                    | 17.11                    | 3.78             | 8.5                                      | 30                |  |
| 3                     | 5                           | 100                         | 11.78                                | 11.2                                  | 9.42                             | 2.59           | 12                        | 0.45                    | 21.65                    | 7.99             | 7.9                                      | 15                |  |
| 5                     | 5                           | 100                         | 9.65                                 | 9.04                                  | 9.11                             | 3.21           | 11.8                      | 0.4                     | 19.89                    | 4.51             | 7.1                                      | 35                |  |
| 4                     | 5                           | 150                         | 8.19                                 | 14.09                                 | 8.91                             | 2.41           | 11.6                      | 0.36                    | 17.56                    | 3.05             | 9.1                                      | 30                |  |
| 4                     | 5                           | 150                         | 8.22                                 | 11.3                                  | 9.67                             | 2.38           | 11.7                      | 0.41                    | 17.81                    | 2.7              | 8.8                                      | 30                |  |
| 4                     | 7                           | 100                         | 10.4                                 | 8.85                                  | 8.3                              | 3.37           | 12.2                      | 0.58                    | 24.79                    | 6.2              | 6.2                                      | 35                |  |
| 4                     | 3                           | 100                         | 14.67                                | 2.1                                   | 10.05                            | 3.69           | 10.9                      | 0.84                    | 27.13                    | 6.62             | 8.1                                      | 20                |  |
| 4                     | 5                           | 150                         | 8.77                                 | 14.43                                 | 9.43                             | 2.11           | 11.1                      | 0.43                    | 17.36                    | 2.12             | 9.5                                      | 25                |  |
| 3                     | 5                           | 200                         | 10.92                                | 11.97                                 | 6.24                             | 2.9            | 12                        | 0.51                    | 20.76                    | 3.11             | 8.7                                      | 45                |  |
| 5                     | 3                           | 150                         | 9.59                                 | 10.93                                 | 5.7                              | 3.55           | 13.1                      | 0.51                    | 21.82                    | 5.35             | 5.5                                      | 40                |  |
| 3                     | 7                           | 150                         | 8.3                                  | 12.2                                  | 5.4                              | 3.89           | 12.5                      | 0.43                    | 21.47                    | 5.83             | 6.4                                      | 40                |  |
| 4                     | 7                           | 200                         | 12.15                                | 6.05                                  | 7.3                              | 4.44           | 9.2                       | 1.71                    | 35.71                    | 2.84             | 7.5                                      | 60                |  |
| 5                     | 7                           | 150                         | 11.01                                | 5.67                                  | 7.48                             | 4.34           | 10.6                      | 0.96                    | 29.17                    | 1.81             | 5.1                                      | 55                |  |
| 3                     | 3                           | 150                         | 15.36                                | 9.85                                  | 7.9                              | 3.27           | 12.1                      | 0.48                    | 23.26                    | 7.47             | 6.3                                      | 20                |  |

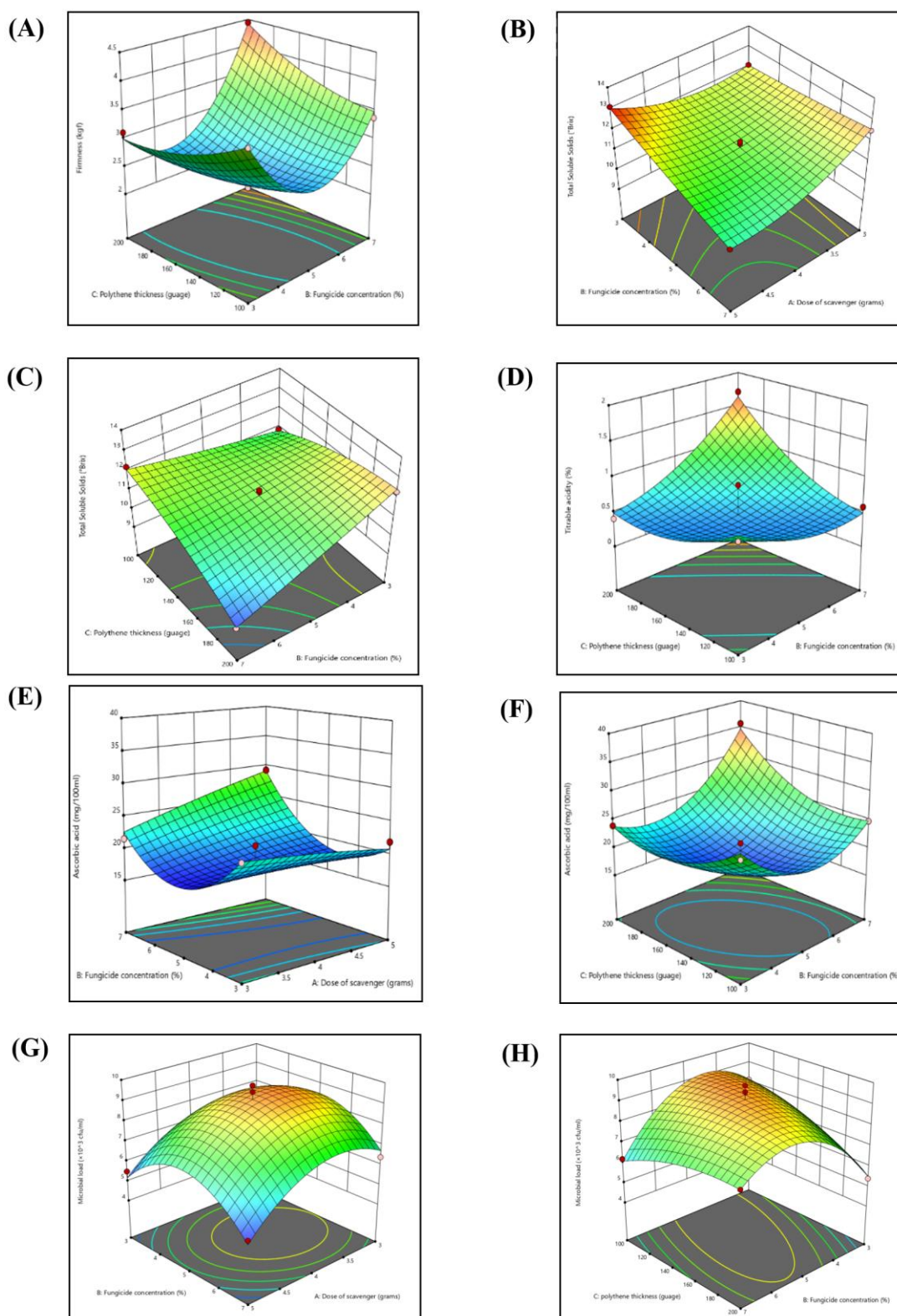


**Fig. S1.** Response surface 3-D plot showing the effect of independent variables on (A-B) O<sub>2</sub> gas concentration, (C-D) CO<sub>2</sub> gas concentration and (E-F) physiological loss in weight

### ***Oxygen gas concentration***

It can be observed from Table 2 that A and B were the significant ( $p < 0.05$ ) parameters that affected the concentration of oxygen at the linear level; AB and BC at the interaction level, whereas  $B^2$  and  $C^2$  at the quadratic level. It was noted that the O<sub>2</sub> concentration decreased by increasing the dose of scavenger and fungicide concentration, as illustrated in Fig. S1 (A-B). These findings align well with recent research showing how effective active oxygen scavengers are in reducing headspace O<sub>2</sub> and preventing oxidative spoilage. For instance, iron-based sachets and scavengers embedded in packaging materials have been shown to bring O<sub>2</sub> levels down to nearly undetectable amounts (below 0.01%) when used with low oxygen-permeable films (Cichello, 2015). In another study, a gallic acid-based scavenger film cut dissolved O<sub>2</sub> levels by half within just 24 hours under refrigeration (Coray and Yildirim, 2024). It might be due to higher scavenging of O<sub>2</sub> by 5g scavenger than other scavengers and thus, preventing deterioration due to oxidation and growth of microorganism (Aday et al., 2011). Fungicide treatments, especially those using neem extract also played a key role in reducing O<sub>2</sub> levels. When used at higher concentrations, these treatments appear to support the action of O<sub>2</sub> scavengers by slowing down microbial respiration, helping to keep internal oxygen levels low. This combined effect is similar to what was observed in studies on

orange juice packaging, where oxygen-scavenging films helped block O<sub>2</sub> entry and preserved both vitamin C and flavor quality (Xu et al., 2012). In fact, higher doses of neem extract can suppress the growth of aerobic microbes and fungal pathogens, leading to less overall O<sub>2</sub> consumption inside the packaging (Strano et al., 2022).



**Fig. S2.** Response surface 3-D plot showing the effect of independent variables on (A) firmness, (B-C) total soluble solids, (D) titratable acidity, (E-F) ascorbic acid, and (G-H) microbial load

### Carbon dioxide gas concentration

In the present study, AB and BC had a significant ( $p < 0.05$ ) effect on CO<sub>2</sub> concentration at the interaction level; B<sup>2</sup> and C<sup>2</sup> at the quadratic level, whereas only A was found significant at the linear level (Table 2). The CO<sub>2</sub> concentration decreased with an increase in the dose of scavenger, i.e., packaging with higher scavenger dose had lower CO<sub>2</sub> concentration as compared to that contained

lower dosages of scavenger (Fig. S1 C-D). This might be due to the scavenging activity of scavengers, which can reduce the increase in gas pressure inside the package by absorbing carbon dioxide produced through respiration (Aday et al., 2011).

### **Physiological loss in weight**

Among all the variables under this research, C was the only parameter that significantly ( $p < 0.05$ ) affected the loss in physiological weight at the linear level,  $A^2$  and  $B^2$  at the quadratic level, whereas AB and BC at the interaction level (Table 2). It was noticed from Fig S1 (E-F), that the physiological loss in weight decreased with an increase in the polythene thickness indicating thicker LDPE bags retained better physiological quality & moisture in fruits as compared to thinner LDPE. This can be correlated to higher relative humidity maintenance inside the thick package, thus, reducing transpiration losses and ultimately physiological loss in weight of packaged commodity (Kumar and Thakur, 2020). According to Kaur et al. (2013) thicker polythene helps to maintain the moisture of packed commodity by creating a saturated micro-atmosphere around it and preventing dehydration. Moreover, it slowed down the rate of transpiration by restricting the diffusion of gases thus, reduced physiological loss in weight. Similar results had been reported by Buthelezi and Mafeo (2024) where the authors found comparatively less weight loss of avocado in thick polythene (40  $\mu\text{m}$ ) than 20  $\mu\text{m}$  thickness polythene.

Kumar and Thakur (2020) concluded that during cold storage of 60 days, 50.8  $\mu\text{m}$  LDPE packaging effectively lessen the weight loss of pear fruit with 7.4% loss whereas 8% loss was reported in fruits that packed in bags with 25.4  $\mu\text{m}$  thickness. However, 3D graph shows that at low fungicide and scavenger levels, microbial control is weak, but limited respiration may cause only moderate weight loss. At moderate levels, respiration and transpiration are more active due to partial microbial suppression and sufficient oxygen, leading to peak weight loss. At high levels, strong scavenger action limits oxygen, and high fungicide doses suppress microbial and enzymatic activity together reducing respiration, transpiration, and thereby minimizing physiological weight loss.

### **Firmness**

Firmness of kinnow fruits was significantly ( $p < 0.05$ ) affected by  $A^2$  and  $B^2$  at quadratic level; BC at interaction level, whereas A and B at linear level as shown in Table 2. It is revealed from Fig S2 (A), that the higher firmness had been recorded for fruits packed with higher scavenger dose as well as fungicide concentration. Similar results for dose of scavenger have been reported by Niazmand and Yeganehzad (2020) for packed barberries using oxygen-scavenger sachets under refrigerated conditions. The authors concluded that packages with effective  $\text{O}_2$  scavenging maintained higher fruit firmness, attributed to the suppression of microbial and enzymatic degradation pathways that typically soften tissues. Arowora et al. (2013) revealed that the retention of firmness in fruits treated with higher fungicide concentration may be due to decline in breakdown of insoluble protopectins into soluble pectin and pectic acid. It was revealed that during the ripening of fruits, shortening or depolymerization of chain length of pectin substances occurs with an increase in polygalacturonase and pectinesterase activities. Hence low  $\text{O}_2$  and high  $\text{CO}_2$  concentrations reduce the activities of these enzymes and allows retention of the firmness during storage. Similar results had been reported by Wijewardane and Guleria (2009) where researchers found that the treatment with 2% neem oil was the most effective for retaining firmness (i.e., 66.4-67.3 N) of apple fruit than 1% and 1.5% concentration.

### **Total soluble solids**

All three parameters i.e., A, B, and C at linear level;  $A^2$  and  $C^2$  at quadratic level; whereas AB and BC at interaction level were the most significant ( $p < 0.05$ ) parameters that affects the total soluble solids of kinnow fruit (Table 2). As shown in Fig S2 (B-C), total soluble solids decreased with an increase in the dose of scavenger, fungicide concentration, and polythene thickness. In our work, higher scavenger dose can delay in ripening process of fruit due to less availability of oxygen inside the package thus slower the conversion of starches into sugars. As a result, scavenger dose had negative effect on total soluble solids. Fruits treated with higher fungicide concentration had lower total soluble solids and vice versa. Similar results have been reported by Freitas et al. (2018). The authors reported that papaya fruits treated with 10% neem leaf extract substantially inhibited increase in total soluble solids during storage than lower levels. The reduction in metabolic process, rate of respiration, and slower conversion of starch to sugars might have delayed the ripening process (Liaquat et al., 2019). Fruits packed in thicker LDPE bags had lower total soluble solids as compared to those packed in thinner packaging. These findings were in agreement with the work carried out by Baswal (2019), who reported that the kinnow fruits packed in 150 gauge of polythene thickness had lower total soluble solids i.e., 9.93 °B than fruits packed in 100 gauge. Adhikaria et al.

(2020) correlated this with retardation of ripening process due to slower respiration rate and metabolic activities.

**Table 2.** ANOVA and regression coefficient of second-order polynomial model for variables

| Source                    | p-values (Responses) | O <sub>2</sub> gas concentration (%) | CO <sub>2</sub> gas concentration (%) | Physiological loss in weight (%) | Firmness (kgf)            | Total Soluble Solids (°B) | Titrateable acidity (%)   | Ascorbic acid (mg/100ml)  | Color difference          | Microbial load (×10 <sup>3</sup> cfu/ml) | Shelf life (days)         |
|---------------------------|----------------------|--------------------------------------|---------------------------------------|----------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|------------------------------------------|---------------------------|
| Model                     | 7.94*                | -0.81*                               | -1.23*                                | -0.1187                          | 0.9463                    | 1.43*                     | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                                  | 0.3025*                   |
| A-Dose of scavenger       | 7.94*                | -0.81*                               | -1.23*                                | -0.1187                          | 0.9463                    | 1.43*                     | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                                  | 0.3025*                   |
| B-Fungicide concentration | -1.23*               | -0.1187                              | 0.9463                                | 1.43*                            | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                                  | 0.3025*                   |
| C-Polythene thickness     | -0.2163              | 0.9463                               | 1.43*                                 | 0.3025*                          | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                   | 0.3025*                                  | 0.3025*                   |
| AB                        | 2.12*                | -1.90*                               | 0.0225                                | -2.88*                           | -0.2270                   | -3.79*                    | -2.93*                    | 0.4736                    | 0.9308                    | 12.91                                    | 0.8418                    |
| AC                        | 0.21                 | 1.09*                                | 0.6478                                | 2.48*                            | 1.91*                     | 0.9655                    | 0.9485                    | 7.98                      | 0.8823                    | 0.8758                                   | * is significant (p<0.05) |
| BC                        | 1.09*                | 0.6478                               | 2.48*                                 | 1.91*                            | 0.9655                    | 0.9485                    | 7.98                      | 0.8823                    | 0.8758                    | * is significant (p<0.05)                |                           |
| A <sup>2</sup>            | 0.6478               | 2.48*                                | 1.91*                                 | 0.9655                           | 0.9485                    | 7.98                      | 0.8823                    | 0.8758                    | * is significant (p<0.05) |                                          |                           |
| B <sup>2</sup>            | 2.48*                | 1.91*                                | 0.9655                                | 0.9485                           | 7.98                      | 0.8823                    | 0.8758                    | * is significant (p<0.05) |                           |                                          |                           |
| C <sup>2</sup>            | 1.91*                | 0.9655                               | 0.9485                                | 7.98                             | 0.8823                    | 0.8758                    | * is significant (p<0.05) |                           |                           |                                          |                           |
| Lack of Fit               | 0.9655               | 0.9485                               | 7.98                                  | 0.8823                           | 0.8758                    | * is significant (p<0.05) |                           |                           |                           |                                          |                           |
| R <sup>2</sup>            | 0.9485               | 7.98                                 | 0.8823                                | 0.8758                           | * is significant (p<0.05) |                           |                           |                           |                           |                                          |                           |
| C.V. (%)                  | 7.98                 | 0.8823                               | 0.8758                                | * is significant (p<0.05)        |                           |                           |                           |                           |                           |                                          |                           |
| Adj. R <sup>2</sup>       | 0.8823               | 0.8758                               | * is significant (p<0.05)             |                                  |                           |                           |                           |                           |                           |                                          |                           |
| Predicted R <sup>2</sup>  | 0.8758               | * is significant (p<0.05)            |                                       |                                  |                           |                           |                           |                           |                           |                                          |                           |

### Titrateable acidity

The BC showed significant ( $p<0.05$ ) results for the titrateable acidity at the interaction level; B and C at the linear level; however, none of the independent variables were found to have a significant effect at the quadratic level (Table 2). Fig S2 (D) shows that the titrateable acidity increased with an increase in the fungicide concentration and polythene thickness. Zewdie et al. (2022) studied tomatoes treated with nectar extract and edible coatings and found that those coated with a combination of 9% beeswax and 25% neem leaf extract maintained their titrateable acidity (TA)

much better over a 36-day storage period compared to untreated fruits. This slower drop in acidity was likely due to reduced respiration and slower breakdown of organic acids, thanks to the improved gas barrier provided by the coating. It might have happened due to the decrease in metabolic activity of organic acids. Similarly, Ahlawat et al. (2018) reported that the coating delayed the respiration rate and conversion of organic acids into sugars. Fruits packed in thicker polythene had higher titratable acidity as compared to fruits packed in thinner LDPE. It might be due to decrease in respiration rate followed by delay in ripening might have prevented the acidity reduction (Khankeshi et al., 2018). These results are supported by Ediriweera et al. (2016), who found that avocados stored in thicker LDPE films (40  $\mu\text{m}$ ) held on to significantly more titratable acidity over 12 days compared to those packed in thinner films (20  $\mu\text{m}$ ). The better performance was credited to the thicker film's stronger barrier against oxygen, which helped slow down respiration and the breakdown of acids. Richa et al. (2014) revealed that maximum titratable acidity was retained in 125 gauge polythene thickness than 75 and 100 gauge.

**Table 3.** Selection criteria for the numerical optimization for active packaging of kinnow

| Name                                   | Goal        | Lower limit | Upper limit |
|----------------------------------------|-------------|-------------|-------------|
| Dose of scavenger (g)                  | is in range | 3           | 5           |
| Fungicide concentration (%)            | is in range | 3           | 7           |
| Polythene thickness (gauge)            | is in range | 100         | 200         |
| O <sub>2</sub> gas concentration (%)   | minimize    | 6.1         | 15.36       |
| CO <sub>2</sub> gas concentration (%)  | minimize    | 2.1         | 14.43       |
| Physiological loss in weight (%)       | minimize    | 5.3         | 10.05       |
| Firmness (kgf)                         | maximize    | 2.11        | 4.44        |
| Total Soluble Solids (°B)              | minimize    | 9.2         | 13.1        |
| Titratable acidity (%)                 | maximize    | 0.23        | 1.71        |
| Ascorbic acid (mg/100ml)               | maximize    | 17.11       | 35.71       |
| Color difference                       | minimize    | 0.22        | 7.99        |
| Microbial load ( $\times 10^3$ cfu/ml) | minimize    | 5.1         | 9.8         |
| Shelf life (days)                      | maximize    | 15          | 65          |

**Table 4.** The predicted optimized conditions for the active packaging of kinnow and their comparison with the experimental values of responses

| Parameter                              | Predicted values | Actual values    | Percentage deviation (%) |
|----------------------------------------|------------------|------------------|--------------------------|
| Dose of scavenger (g)                  | 5                | 5                |                          |
| Fungicide concentration (%)            | 6.83             | 7                |                          |
| Polythene thickness (gauge)            | 192.12           | 200              |                          |
| <b>Desirability</b>                    |                  |                  | <b>0.812</b>             |
| O <sub>2</sub> gas concentration (%)   | 12.84            | 12.45 $\pm$ 0.60 | 3.04                     |
| CO <sub>2</sub> gas concentration (%)  | 3.75             | 3.66 $\pm$ 0.12  | 2.4                      |
| Physiological loss in weight (%)       | 7.34             | 7.19 $\pm$ 0.27  | 2.04                     |
| Firmness (kgf)                         | 4.44             | 4.3 $\pm$ 0.02   | 3.15                     |
| Total Soluble Solids (°B)              | 9.2              | 9.4 $\pm$ 0.43   | 2.17                     |
| Titratable acidity (%)                 | 1.72             | 1.68 $\pm$ 0.12  | 2.32                     |
| Ascorbic acid (mg/100ml)               | 35.28            | 34.09 $\pm$ 1.51 | 3.37                     |
| Color difference                       | 0.55             | 0.38 $\pm$ 0.01  | 3.1                      |
| Microbial load ( $\times 10^3$ cfu/ml) | 4.87             | 5.0 $\pm$ 0.03   | 2.67                     |
| Shelf life (days)                      | 69               | 66 $\pm$ 3.01    | 4.35                     |

### Ascorbic acid

Ascorbic acid of kinnow retained significantly ( $p < 0.05$ ) with increasing B<sup>2</sup> and C<sup>2</sup> at quadratic level; AB and BC at interaction level; whereas B and C at linear level (Table 2). Fig S2 (E-F) shows that the fruits treated with higher neem leaf solution had higher ascorbic acid content. This can be related to impaired O<sub>2</sub> uptake by fruits due to coating with fungicide thereby slowing down their metabolism and consequently resulting in reduction in the rate of degradation/oxidation of ascorbic acid (Kumar, 2018). Similar results were reported by Palei et al. (2024), who studied mangoes treated with different concentrations of neem leaf extract (5%, 10%, 15%, and 20%). They found that mangoes treated with 20% neem extract and kept in cold storage retained the highest levels of ascorbic acid (28.5 mg/100 g). These fruits also showed less weight loss and lower spoilage rates compared to those treated with lower concentrations. Likewise, Bhuvaneswari et al. (2015) looked at onion slices treated with calcium lactate and packed in polypropylene films of different thicknesses (25  $\mu\text{m}$  and 50  $\mu\text{m}$ ). The thicker 50  $\mu\text{m}$  films helped reduce respiration, preserved more nutrients including vitamin C and kept the slices firmer after 12 days at 10 °C, compared to the thinner films. This might be due to low availability of O<sub>2</sub> for respiration owing to high barrier properties of the thicker film and degradation of ascorbic acid. In contrast, beyond an optimal point, very high fungicide levels or excessively thick packaging can restrict gas exchange, causing anaerobic conditions, moisture buildup, or stress-induced degradation. Therefore, moderate levels

of both treatments provide an optimal balance-sufficiently reducing oxygen exposure and microbial growth without disrupting the fruit's natural respiration or storage environment.

### **Color difference**

There was a significant ( $p < 0.05$ ) effect of all independent variables, i.e., A, B, and C, on color difference of kinnow fruit at linear level;  $B^2$  at quadratic level; whereas no significant effect of parameters at interaction level was observed in the present study (Table 2). Fruits packed with a higher dose of scavengers had lower color difference value as compared to those packed with a lower dosage. This negative effect can be due to lower oxygen and higher carbon dioxide concentration inside the package, which delays oxidation, respiration as well as enzyme reaction. Hence, color value was maintained by higher scavenger dosage (Custode, 2017). Fruits treated with higher fungicide concentration had lower color difference and vice versa. Similar results have been reported by Malik (2016) where kinnow fruits treated with a higher concentration of chemical fungicide (Azoxystrobin) showed lower color difference during storage. Munoz et al. (2008) concluded that the control of moisture loss by coatings might have contributed to minimize external color changes. Also, kinnow fruits packed in thicker LDPE bags had lower color difference. The results of the present study are in agreement with the findings of Zhai et al. (2020), who reported that packaging Cuixiang kiwifruit in thicker modified-atmosphere films helped maintain lower oxygen and higher carbon dioxide concentrations. This modified internal atmosphere significantly delayed respiration, oxidation, and enzymatic browning, ultimately reducing chilling injury and preserving fruit color during cold storage.

### **Microbial load**

As evident from Table 2, only one parameter i.e., A at linear level had significant effect while AC and BC at interaction level whereas  $A^2$  and  $B^2$  were found significant ( $p < 0.05$ ) at quadratic level. The microbial load decreased with an increase in the dose of scavenger (Fig S2 G-H). However, beyond an optimal point, further increase appears to reduce efficacy might be due to the saturation of active antimicrobial agents, development of microbial resistance, and chemical antagonism or instability at higher doses. Similar findings were reported by Lee et al. (2018), where the application of a sulfite-based oxygen scavenger in kimchi packaging significantly reduced headspace oxygen and microbial load, without affecting the natural fermentation process. The treated samples showed a significant reduction in total aerobic bacteria (up to 2.97 log CFU/g) and yeasts/molds (up to 3.04 log CFU/g) during 12 weeks of storage. The reduction in microbial load with increasing scavenger dose is primarily due to the decreased availability of oxygen in the packaging environment. Many spoilage microorganisms and aerobic bacteria require oxygen for growth and metabolic activity. By absorbing oxygen, scavengers create a low-oxygen or anaerobic environment, which inhibits the growth of these microbes (Cichello, 2015).

### **Shelf life**

Shelf life of kinnow was affected significantly by all the process parameters at both linear and quadratic levels; whereas no parameter was found to be significant at interaction level (Table 2). It was seen that the shelf life was positively affected with the dose of scavenger, fungicide concentration, and polythene thickness. The use of all three independent parameters at higher level was effective in enhancing the quality and shelf life of kinnow fruit during cold storage. Hence, active packaging might be an effective way to maintain or delay in the changes in all the dependent parameters and could be considered as a suitable non-chemical treatment for the shelf-life extension and conservation of quality parameters without any chemical hazards for human consumption. Khan et al. (2021) concluded that 30% neem leaf extract could be considered a suitable coating for shelf-life enhancement of grapefruit than lower (10% and 20%) coating concentrations. The positive findings of polythene thickness and dose of scavenger were in line with the study carried out by Kumar and Thakur (2020) who observed that fruit could be safely stored at cold conditions up to 4 months when packed in LDPE bags (50.8  $\mu\text{m}$ ) in the presence of 15% oxygen scavenger i.e., iron powder.

### **Numerical Optimization**

To find out the optimum conditions for storage of kinnow fruit, intensity and goals was allocated to each independent variable and responses. The main criteria for optimization of constraints were to minimize  $\text{O}_2$  and  $\text{CO}_2$  gas concentration, physiological loss in weight, total soluble solids, color difference, microbial load, and maximize firmness, titratable acidity, ascorbic acid, shelf life while the process parameters should be within range (Table 3). The optimized conditions for active packaging of kinnow fruit were 5 g dose of scavenger, 6.83% fungicide concentration, and 192.12 gauge of polythene thickness with 0.812 overall desirability. Corresponding to these variables, the

predicted value of O<sub>2</sub> gas concentration, CO<sub>2</sub> gas concentration, physiological loss in weight, firmness, total soluble solids, titratable acidity, ascorbic acid, color difference, microbial load, and shelf life were 12.84%, 3.75%, 7.34%, 4.44 kgf, 9.2°B, 1.72%, 35.28 mg/100ml, 0.55,  $4.87 \times 10^3$  cfu/ml, and 69 days respectively (Table 4). For the model validation of optimized value, percentage deviation between the predicted and actual values were calculated for various parameters as shown in Table 4. Percent deviation was less than 5% for all the quality parameters.

### Conclusion

The present study elucidates the significant effect of three independent variables i.e., dose of scavenger, fungicide concentration, and polythene thickness on the postharvest physiology, physicochemical quality, and shelf life of kinnow fruits under cold storage. Statistical analysis confirmed that these variables exhibited notable effects ( $p < 0.05$ ) on key quality attributes, including gas concentration (O<sub>2</sub> and CO<sub>2</sub>), physiological loss in weight, firmness, total soluble solids, titratable acidity, ascorbic acid, color difference, microbial load, and shelf life. Higher doses of scavenger and fungicide, in combination with increased polythene thickness, was associated with reduced oxidative degradation and respiration rates, thereby delaying senescence and microbial spoilage. The fitted second-order polynomial regression models demonstrated strong predictive capacity ( $R^2 > 0.85$ ) and non-significant lack-of-fit, validating their applicability for process optimization. Overall, the results underscore the potential of active packaging strategies, incorporating tailored scavenger systems and bio-based fungicidal treatments within optimized polymeric films as an effective, non-chemical approach to extend the storage life and preserve the nutritional and sensory quality of citrus fruits. These findings hold substantial implications for sustainable postharvest management and value retention in commercial citrus supply chains.

### Future Scope

Future studies should focus on evaluating the commercial scalability and economic feasibility of the proposed active packaging system. The use of biodegradable films in place of LDPE offers a promising direction for enhancing environmental sustainability. Further research on combining oxygen scavengers with other active agents (e.g., ethylene absorbers or natural antimicrobials) may yield synergistic effects on quality retention. Integrating smart indicators for real-time monitoring and exploring fruit physiological responses at the molecular level could deepen mechanistic understanding. Validation across different citrus varieties and under dynamic cold-chain conditions would also improve the applicability of this technology.

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**Author Contributions**

PKP, VK, NK, GKS, AJ and SB conceived the concept, wrote and approved the manuscript.

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