AATCC Review

02 July 2025: Received
21 August 2025: Revised
29 August 2025: Accepted
27 September 2025: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Optimization of carboxymethyl cellulose and ethrel coatings using response surface methodology for management of post-harvest quality in mango (*Mangifera indica* L.)

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ABSTRACT

Background: *Mango (Mangifera indica L.) is an important tropical fruit and highly appreciated for its taste, flavour, colour and nutritional value. Its high respiration rate and climacteric nature, however, cause postharvest losses and rapid deterioration. Composite edible coatings are emerging as environmentally friendly solutions to maintain fruit quality, but studies on their optimisation for mango remain limited.*

Objective: *The study focused on developing and optimising a composite coating containing carboxymethyl cellulose (CMC), ethrel and glycerine to preserve the physical and biochemical quality of mango fruits during ambient storage.*

Methods: *A three-factor, three-level Box–Behnken design via Response Surface Methodology (RSM) was used with CMC (7.5–12.5 g L^{-1}), ethrel (500–1000 ppm) and glycerine (11.5–22.5 mL L^{-1}) as independent variables to study seventeen treatment combinations to measure the physical and biochemical parameters. Suitability of the models was evaluated using regression and ANOVA.*

Results: *All response models were highly significant ($p < 0.01$), with strong determination coefficients ($R^2 > 0.96$). Optimised coating with CMC (11 g L^{-1}) + ethrel (500 ppm) + glycerine (12 mL L^{-1}) reduced physical losses and maintained key biochemical parameters at ambient storage conditions with low residual error (<1%).*

Conclusions: *The optimised composite coating preserved the physical and biochemical quality of the mango cv. Langra during storage. Results confirmed that RSM is a useful technique for an optimised coating formulation for preservation of fruit quality.*

Keywords: *Mango, composite coating, climacteric fruit, carboxymethyl cellulose (CMC), ethrel, glycerine, response surface methodology (RSM)*

Introduction

Mango (*Mangifera indica L.*) is an important tropical fruit valued for its taste, flavour, aroma, succulent nature and high nutritional value (1). It is widely cultivated across Southeast Asia, Africa and Latin America (2). The major global producers are India, China and Thailand, with India alone contributing about 40% of the world's supply (3). There are approximately 1500 different mango cultivars in India and several commercial cultivars, including Alphonso, Chausa, Kesar, Dashehari, Neelum, Langra and Bombay Green, have gained popularity in the global market (4). It suffers post-harvest losses of 25-40% primarily because of rapid ripening, physiological disorders and post-harvest mismanagement, which affects quality, supply chain and global market (1, 5). It is a climacteric fruit and undergoes a sudden increase in ethylene production and respiration during ripening after harvest. This hastens the development of colour, tissue softening, sugar conversion and the release of aromatic compounds but it comes with less storage life and poor quality (1). However, the shelf life of mango under ambient conditions rarely exceeds one week, highlighting the importance and necessity of improved postharvest technologies to prevent quality degradation and loss.

Generally, the traditional methods of ripening, like natural ripened fruit from a tree, use of ripened fruit in storage, smoking exposure, have been used for centuries, but they cause uneven ripening, increase microbial infestation, fruit shrinkage and desiccation and affects the appearance (6). External ripening agents are mostly preferred by commercial growers and farmers, which include the use of ethylene gas (in chambers) and ethrel solution which causes rapid and uniform ripening by enhancing respiration and endogenous ethylene biosynthesis (7, 8). However, there may be a loss in flavour and storage interval after excessive exposure. In many developing nations, including India, calcium carbide (CaC_2) is still used due to its easy availability and affordability despite being banned. It acts as an ethylene substitute and releases acetylene after decomposition but it is hazardous to human health as it contains traces of compounds like arsenic and phosphorus (9). Fruits subjected to CaC_2 treatments are inferior in taste, texture, flavour, aroma and have low storage life as compared to natural or ethylene ripened fruit (10).

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DOI: https://doi.org/10.21276/AATCCReview.2025.13.04.572

Ethephon (ethrel) is another widely used ripening agent and produces ethylene gas after application and promotes faster and uniform ripening (7, 11). Nevertheless, some fruits are sensitive to ethylene and may experience premature decay if exposed to higher concentrations and for that there is a need for proper application, monitoring and precise control to prevent overripening and early decay (6). These limitations highlight the necessity for safe, sustainable and consumer acceptable postharvest methods for ripening while maintaining optimum fruit quality (6, 12).

Recently, edible coatings have gained popularity due to its safe, sustainable and consumer-friendly nature. They are thin layers of natural biopolymers either mixed with bioactive compounds or applied alone to maintain fruit quality and extend storage life (12). They create a barrier by forming a semipermeable layer on the fruit surface and prevent moisture loss and gaseous exchange, leading to slow respiration, prevent water loss and maintain firmness (13, 12). The most commonly used coating materials are polysaccharides, proteins and lipids and among them, polysaccharides are highly valued due to their easy availability, low cost and biodegradable nature (12). Carboxymethyl cellulose (CMC), a cellulose derivative, has the ability to form clear, transparent, flexible films with strong moisture and gas barrier properties (14). It has been reported that CMC results in a reduction of weight loss, maintains firmness and delay softening of tissues with optimum sensory quality (15, 16). Coating with a bioactive compound that enhances the efficacy is considered a composite, like CMC and Moringa leaf extract, combined results in reduced firmness loss, delayed colour changes in avocado (17). Similar results were reported by 18 in satsuma mandarin, (16) in aonla, (19) and (20) in strawberries. These results show the potential of polysaccharide-based composite coating to substitute artificial ripening agents, which are eco-friendly and extend the storage period.

CMC has proven effective in maintaining fruit quality and ethrel induces ripening. CMC does not regulate ripening and ethrel does not prevent water and firmness loss. A novel composite coating formulation with CMC and ethrel offers dual function approach which results in controlled release of ethylene for uniform and slow ripening, while CMC reduces water loss and maintains physical and biochemical attributes by forming semipermeable layer. Similar, glycerine used as a plasticiser improves flexibility, permeability and adhesion to the fruit surface (12). They (21) used 1-MCP and ethrel reporting that combining ripening agent and ripening inhibitor helps in uniform ripening and optimum quality with proper colour development, aroma and prevents quality loss in banana. So, CMC and ethrel composite coating may balance ripening and post-harvest quality for climacteric fruits like mango. However, there were no such studies in which ethrel-CMC composite coating was evaluated.

Therefore, the development of such a coating with different variables requires precision and Response Surface Methodology (RSM) plays an important role (22). RSM is a multifunctional tool used to design experiments and determine the best formulation for coating from experimental runs and variables (23). Previous studies used RSM to optimise coating formulations (24) in dates, (25) in pear, (26) in papaya, (27) in guava and (28) in fresh cut apples. Focusing on the growing need for sustainable and cheap postharvest methods, this present study focuses on developing a novel and effective edible composite coating for mango.

2. Materials and Methods

2.1. Experimental site and plant material

The study was carried out in 2024 at the Horticulture Research Centre, Patharchatta, GBPUAT, Pantnagar, Uttarakhand, India (29.5°N, 79.3°E; 243.84 m altitude). Mango fruits (cv. Langra) were harvested at the physiologically mature stage and uniform and healthy fruits were selected. Precooling was done after harvest using cold water to remove field heat for 1 h. Fruits were subjected to washing, air drying to remove debris and wiped with a muslin cloth before treatment application.

2.2 Coating materials and chemicals

All chemicals were of commercial grade. Carboxymethyl cellulose (CMC) powder, ethrel (39% a.i.) and glycerine were purchased from commercial suppliers. Ethrel solutions were freshly prepared before use. Fruits were stored in cardboard boxes.

2.3 Coating preparation and application

CMC solution was prepared by dissolving of CMC (7.5–12.5 g) in 250 mL of distilled water with continuous stirring and heating (70–80 °C) until fully dissolved. The volume of the solution was made up to 1 L, resulting in a completely transparent solution. Glycerine (11.5–22.5 mL L^{-1}) was added to improve flexibility and ethrel (500–1000 ppm) was freshly mixed with the solution right before application. The composite solution was brushed uniformly on the surface of the mango fruits. The treated fruits were dried at room temperature and placed in cardboard boxes.

2.4 Experimental design and optimization

The coating formulation optimisation was performed using Response Surface Methodology (RSM). A three-factor, three-level Box–Behnken design (BBD) was used with the independent variables: carboxymethyl cellulose [CMC (A) 7.5, 10.0, 12.5 g L^{-1}), ethrel (B) 500, 750, 1000 ppm) and glycerine (C) 11.5, 17.0, 22.5 mL L^{-1}]. Overall, 17 runs of the experiment were generated including five replicates at the centre point (Table 1), using the Design-Expert software (v13.0.5.0). The experimental data were fitted to a quadratic polynomial model equation (1):

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ij} X_i X_j + \Sigma\beta_{ii} X_i^2$$

Where Y is the response, β_0 is the intercept, β_i are linear coefficients, β_{ij} are interaction coefficients and β_{ii} are quadratic coefficients.

Numerical optimisation was carried out to find the optimal combination of the independent variables to extend the shelf life of mango fruits while still retaining good quality. The independent variables (CMC, ethrel and glycerine) were allowed to be at their experimental levels, while the optimisation goal of the responses were identified and set as minimizing physiological loss in weight (PLW), fruit length reduction (FLR), fruit width reduction (FWR), titratable acidity and maximizing total soluble solids (TSS) and ascorbic acid content.

2.5 Response parameters

The procedure involved the optimisation of several response factors, including physiological loss in weight (PLW, %), fruit length reduction (FLR, %), fruit width reduction (FWR, %), titratable acidity (%), total soluble solids (°Brix) and ascorbic acid contents (mg 100 g^{-1}).

2.5.1 Fruit length and fruit width

Length and width were measured using a Vernier calliper and reduction was calculated as the percentage loss from the original length or width to the length/width measured on the evaluation day.

2.5.2 Physiological loss in weight

Each mango was weighed on a weighing balance and the weight loss was calculated as the percentage loss from the original weight to the weight measured on the evaluation day.

2.5.3 Total soluble solids

Fruits were crushed and juice drops were dispensed onto a digital refractometer and the results are presented in degrees Brix.

2.5.4 Titratable acidity

The titratable acidity was determined by titrating 10 ml aliquot with 0.1-N Sodium hydroxide solution using phenolphthalein as an indicator as per the method suggested by AOAC (**29**). It was expressed in terms of per cent.

2.5.5 Ascorbic acid

The ascorbic acid content was estimated by the use of 2, 6-Dichlorophenol indophenol visual titration method (**30**). The ascorbic acid was calculated on mg per 100 grams of pulp weight.

2.5.6 Total sugar

Total sugar was estimated by the standard method of AOAC (**29**). The sugar extract was hydrolysed with concentrated hydrochloric acid and titrated against 10 ml of mixed Fehlings solution (5 ml Fehling A + 5 ml Fehling solution B) using methylene blue as indicator. Results were expressed as per cent total sugar. The following formula was used to get the total sugar content:

$$\text{Total sugar (\%)} = \frac{\text{Fehling factor (0.05)} \times \text{Volume made up} \times \text{Final volume} \times 100}{\text{Titre value} \times \text{Weight of sample (g)}}$$

2.5.7 Total carotenoids

One gram of sample was weighed and grinded with acetone using acid and alkali washed sand in a pestle and mortar. The extract was decanted into a conical flask. The extraction was continued till the residue is colourless. The acetone extract was transferred to a separating funnel containing 10-15 ml of petroleum ether and mixed gently. After addition of 25 ml of 5 % sodium sulphate, the solution was shaked and kept for some time. The separated yellow colour pigment was transferred into the petroleum ether later. The layer was collected in a volumetric flask and acetone layer containing Na_2SO_4 was separated until the colour gets transferred into the petroleum ether. The colour intensity was measured at 452 nm by using spectrophotometer and the total carotenoid content was calculated by the following formula. The total carotenoids were calculated using the formula below and expressed as mg 100 g^{-1} fresh weight:

$$\text{Total carotenoids (mg 100 g}^{-1}) = \frac{O.D. \times 100 \times \text{Volume made up} \times 3.857}{\text{Weight of sample (g)} \times 100}$$

2.6 Statistical analysis

All statistical analyses were conducted using Design-Expert software version 13.0.5.0. Regression analysis and analysis of variance (ANOVA) were performed to assess both the significance of the models and the significance of the model terms. To determine the adequacy of the models, F-values, p-values, coefficient of determination (R^2), adjusted R^2, predicted R^2, coefficient of variation (CV%) and Adeq Precision were evaluated. Regression terms were deemed significant when their p-values were smaller than 0.05. Adequacy of the regression tests was further assessed by comparing predicted values to experimental values and checking residuals, along with using lack-of-fit tests to determine if the experimental data were well represented by the regression models.

Table 1: Experimental design matrix of Box–Behnken design with coded and actual values of independent variables

Test number	Point type	Coded value of variables			Actual values of variables		
		A	B	C	CMC (g L^{-1})	Ethrel (ppm)	Glycerine (mL L^{-1})
1	Factorial	-1	0	+1	7.50	750	22.5
2	Factorial	+1	-1	0	12.5	500	17.0
3	Factorial	+1	+1	0	12.5	1000	17.0
4	Factorial	0	-1	-1	10.0	500	11.5
5	Factorial	-1	-1	0	7.50	500	17.0
6	Centre	0	0	0	10.0	750	17.0
7	Factorial	0	+1	+1	10.0	1000	22.5
8	Centre	0	0	0	10.0	750	17.0
9	Centre	0	0	0	10.0	750	17.0
10	Centre	0	0	0	10.0	750	17.0
11	Factorial	+1	0	-1	12.5	750	11.5
12	Factorial	+1	0	+1	12.5	750	22.5
13	Factorial	-1	0	-1	7.50	750	11.5
14	Factorial	-1	+1	0	7.50	1000	17.0
15	Centre	0	0	0	10.0	750	17.0
16	Factorial	0	+1	-1	10.0	1000	11.5
17	Factorial	0	-1	+1	10	500	22.5

3. Results

The optimization of mango pretreatments using CMC, ethrel and glycerine was performed to improve fruit post-harvest quality under ambient conditions, using eight response variables related to fruit physiology and quality. Regression models for each response showed high coefficients of determination ($R^2 \geq 0.964$), confirming excellent model fits to the experimental data. The fruit quality and physicochemical parameters were analysed at 3-day intervals from the day of harvest (0 day) up to 15 days during ambient storage, continuing until the fruits were no longer fit for consumption. The mangoes displayed acceptable quality and appearance for up to 12 days with little or no deterioration. Hence, all results presented here are based on the 12-day evaluation, the final point that the mangoes were acceptable for analysis and consumption without major quality loss.

This study aims to establish optimized conditions for prolonging the postharvest quality of mangoes under ambient storage conditions, through a set of experimental conditions including CMC, ethrel and glycerine concentrations. The optimized condition could be single point or a range of points in which all the possible combinations result in optimal quality and the constraints decided (Table 2), to guide the optimization and ensure that used solution yields good results and effective in real world limits.

Table 2: Constraints for optimization of pretreatments of mango under ambient storage conditions

Name	Goal	Lower Limit	Upper Limit
A: CMC	In range	7.5	12.5
B: Ethrel	In range	500	1000
C: Glycerine	In range	11.5	22.5
Physiological loss in weight	Minimize	8.9523	12.2585
Per cent reduction in fruit length	Minimize	1.11623	6.1082
Per cent reduction in fruit width	Minimize	1.60085	8.6067
Total soluble solids (^{o}Brix)	Minimize	16.42	19.8
Titratable acidity (%)	Minimize	0.165	0.343
Ascorbic acid (mg 100 g^{-1})	Maximize	42.51	50.98
Total Sugars (%)	Minimize	12.32	16.63
Total Carotenoids (mg 100 g^{-1})	In Range	2.37	4.04

3.1 Physical response

3.1.1 Physiological loss in weight (PLW, %)

The PLW of stored mangoes ranged between 8.95 to 12.26% (Table 4). The regression model for PLW was highly significant, suggesting that the fitted quadratic model is adequately explaining the variation in PLW (F-value = 44.26, $p < 0.01$, R^2 = 0.9827) (Table 3). The R^2 (0.9827) and adjusted R^2 (0.9605) values were consistent with the predicted and experimental data and the lack-of-fit F value (2.62) was not significant confirming excellent model adequacy and predictive reliability. The concentration of the CMC (A) had a negative coefficient (-0.7756), suggesting that CMC assisted in preventing water loss due to the formation of a semi-permeable barrier on the surface of the fruit. The ethrel (B) had a positive coefficient (+0.9729), due to the ripening process which increases the water loss. The glycerine (C) had a small positive effect (+0.1377), which aligns with its hygroscopic properties. The quadratic terms (A^2, B^2 and C^2) indicate that excessive concentrations could result in increased water loss. The 3D plot (Fig. 1) in the RSM illustrated the aforementioned finding that PLW decreased with CMC concentrations, while with concentrations of ethrel PLW increased. Overall results show the optimum concentration of CMC that could help decrease PLW, in conjunction with lower concentrations of ethrel.

The regression equation (2) for PLW is:

$$PLW = 11.94 - 0.7756A + 0.9729B + 0.1377C - 0.5344AB - 0.1653AC - 0.1787BC - 1.26A^2 - 0.5918B^2 - 0.7280C^2.$$

3.1.2 Fruit length reduction (FLR, %)

The FLR of stored mangoes ranged between 1.12 to 6.11% (Table 4). The regression model for FLR was highly significant, suggesting that fitted quadratic model is adequately explaining the variation in FLR (F-value = 51.91, $p < 0.01$, R^2 = 0.9855) (Table 3). The R^2 (0.9855) and adjusted R^2 (0.9663) values were consistent with the predicted and experimental data and the lack-of-fit F value (5.29) was not significant, confirming that this model was suitable. The concentration of CMC (A) showed negative effect (–0.6520), indicating its role in length reducing by forming barrier on the fruit surface that reduces moisture loss and enzymatic cell wall degradation. However, ethrel (B) had a strong positive effect (+1.43), suggesting its role in softening tissue through increase in ripening which increases the cellular breakdown, causing reduction in length. Glycerine (C) had a minor effect (–0.0662) whereas the AB interaction (-0.9450) showed that combining CMC and ethrel helps in minimising the shrinkage. The quadratic term A^2 and B^2 shows nonlinear effects on reduction of length. The 3D plot (Fig. 2) showed that reduction in length was minimised with high CMC and low ethrel concentration.

The regression equation (3) for FLR is:

$$FLR = 3.05 - 0.6520A + 1.43B - 0.0662C - 0.9450AB - 0.6771AC + 0.0106BC - 0.5693A^2 + 0.4826B^2 - 0.0506C^2$$

3.1.3 Fruit width reduction (FWR, %)

The FWR of stored mangoes ranged between 1.60 to 8.61% (Table 4). The regression model for FWR was highly significant suggesting that fitted quadratic model is adequately explaining the variation in FWR (F-value = 39.62, $p < 0.01$, R^2 = 0.9807) (Table 3). The R^2 (0.9807) and adjusted R^2 (0.9560) values were consistent with the predicted and experimental data and the lack-of-fit F value (2.45) was not significant confirming that this model was suitable. The concentration of CMC (A) showed a negative effect (–1.03) indicating its role in width reducing by forming barrier on the fruit surface that reduces moisture loss and cellular dehydration. However, ethrel (B) had a negative effect (-0.2324) suggesting its role in softening tissue through increase in ripening which increases the cellular breakdown. Glycerine (C) had a positive effect (+0.5865) because it contributes in coating flexibility and maintains cellular turgidity. Whereas the interaction AB and BC highlight complex interactions among coating components and ethrel. (-0.9450) showed that combining CMC and ethrel helps in minimising the shrinkage. Significant quadratic terms indicate that higher concentrations of coating components or ethrel may adversely affect fruit dimensional stability. The 3D plot (Fig. 3) showed that the reduction in width was minimised with high CMC and low ethrel concentration.

The regression equation (4) for FWR is:

$$FWR = 4.41 - 1.03A - 0.2324B + 0.5865C + 1.11AB - 0.5155AC - 0.7960BC + 0.8981A^2 + 0.9078B^2 - 2.73C^2$$

3.2 Biochemical responses

3.2.1 Total soluble solids (TSS ^{o}B)

The total soluble solids content of mangoes during storage ranged from 16.42 to 19.80 ^{o}Brix (Table 4). The regression analysis revealed that the quadratic model was statistically significant (F-value=34.09, $p<0.01$, R^2=0.9777) (Table 3).

The adjusted R^2 (0.9490) is in agreement with R^2 and the lack-of-fit F value of (1.74) is not statistically significant, indicating the model reliability. The concentration of CMC (A) displayed a negative effect (= –0.7988), which slow down the accumulation of sugar by reducing respiration and delaying ripening. Ethrel (B) exhibited a significant positive effect (+0.5300), which is consistent with its role as a ripening agent that increases the rate of starch to sugar conversion. Glycerine (C) also had a positive effect (+0.5487), which also affected sugar accumulation by contributing to the metabolic processes at an acceptable moderate level due to its plasticizing nature. The quadratic term (A^2, B^2, C^2) shows nonlinear effects. The 3D plot (Fig. 4) showed that TSS was maximum when the application levels of ethrel and glycerine were higher, while the CMC application reduced the total sugar solids accumulation.

The regression equation (5) for TSS is:

$$TSS = 18.71 - 0.7988A + 0.5300B + 0.5487C + 0.3350AB + 0.0025AC - 0.6050BC - 0.4542A^2 - 0.2717B^2 + 0.2007C^2$$

3.2.2 Titratable acidity (%)

The titratable acidity (TA) content of mangoes during storage ranged from 0.165 to 0.343% (Table 4). The regression analysis revealed that the quadratic model was statistically significant (F-value= 20.85, $p<0.01$, R^2=0.9640) (Table 3). The adjusted R^2 (0.9178) is in agreement with R^2 and the lack-of-fit F value of (1.74) is not statistically significant, indicating the model reliability. The concentration of CMC (A) showed slight positive effect (= +0.0243), which aided in retention of acid during storage. Ethrel (B) and Glycerine (C) exhibited negative effect (–0.0289 and –0.0131, respectively), suggesting their role in decreasing acid content during storage due to the increase in rate of ripening due to ethrel. The quadratic term (A^2, B^2, C^2) were significant and their excess concentration led to alteration in acidic content. The 3D plot (Fig. 5) showed that higher concentration of ethrel lead to decrease in acidity while CMC coating preserved it.

The regression equation (6) for TA is:

$$TA = 0.1880 + 0.0243A - 0.0289B - 0.0131C - 0.0268AB - 0.0073AC + 0.0150BC + 0.0337A^2 + 0.0415B^2 + 0.0275C^2$$

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

The ascorbic acid (AA) content of mangoes during storage ranged from 42.51 to 50.98 mg 100 g^{-1} (Table 4). The regression analysis revealed that the quadratic model was statistically significant (F-value= 48.46, $p<0.01$, R^2=0.9842) (Table 3). The adjusted R^2 (0.9639) is in agreement with R^2 and the lack-of-fit F value of (3.97) is not statistically significant, indicating the model reliability. The concentration of CMC (A) showed positive effect (= +1.10), which aided in retention of ascorbic acid with ethrel and glycerine interaction. Ethrel (B) and Glycerine (C) exhibited negative effect (–0.4487 and –0.6688, respectively), suggesting their role in increasing ascorbic acid degradation due to accelerated ripening and associated oxidative process along with glycerine.

The quadratic term (A^2, B^2, C^2) were significant, showing nonlinear response at higher concentration. The 3D plot (Fig. 6) showed that higher concentration of CMC plays protective role with higher ascorbic acid retention with lower ethrel concentration showing the protective role of hydrocolloid coating in reducing vitamin c loss.

The regression equation (7) for AA is:

$$AA = 48.49 + 1.10A - 0.4487B - 0.6688C - 0.0250AB + 0.1400AC + 0.9525BC - 2.18A^2 + 2.64B^2 - 2.22C^2$$

3.2.4 Total sugar (%)

Total sugar content in mangoes varied from 12.32 to 16.63% (Table 4). The regression model was significant ($F = 40.14$, $p < 0.01$) with an observed $R^2 = 0.9810$ and adjusted $R^2 = 0.9566$, indicating adequate fit (Table 3) with a non-significant lack-of-fit value (2.03) to support model adequacy. CMC (A) showed a negative coefficient (–0.8250), indicating a reduction of sugar accumulation at higher concentration as a result of lower respiration and starch hydrolysis. Ethrel (B) and glycerine (C) had positive effects (+0.5050 and +0.5475, respectively) indicating their support in completing the sugar conversion during ripening and the coating effect in promoting stability of the film, respectively. The AB interaction had a positive effect, suggesting that moderate levels of ethrel with sufficient coating permeability increased ripening to an optimal level. Additionally, the 3D response plot (Fig. 7) indicated that total sugar content generally increased with ethrel and glycerine concentrations to a middle level before declining.

The regression equation (8) for TS is:

$$TS = 16.34 - 0.8250A + 0.5050B + 0.5475C + 0.3350AB + 0.0550AC - 0.6550BC - 1.32A^2 - 1.14B^2 - 0.6130C^2$$

3.2.5 Total carotenoids (mg 100 g^{-1})

The overall carotenoid content was between 2.37 to 4.04 mg 100 g^{-1} (Table 4). The regression model was statistically significant ($F = 29.54$, $p < 0.01$) and had $R^2 = 0.9743$ and adjusted $R^2 = 0.9414$ verifying the adequacy of fit described by the model (Table 3). The lack-of-fit value (1.35) was not significant verifying its adequacy. Ethrel (B) had a strong positive coefficient (+0.1588), indicating it occurred increased carotenoid biosynthesis due to ethylene-mediated degradation of chlorophyll. CMC (A) had a slight negative effect (–0.0688), which we think could be attributed to it being semi-permeable and restrictive to gas exchange, thus prolonging pigment formation. The combination of CMC with ethrel (AB) was also statistically significant, indicating the moderate ethrel level that utilized the barrier properties of CMC to enhance colour development. Figure 8 illustrates the 3D response surface plot and reflects our findings that carotenoid content was positively related to the applied ethrel levels (up to moderate levels), but slightly declined as the ethrel concentration increased (after moderate levels).

The regression equation (9) for TC is:

$$TC = 3.51 - 0.0688A + 0.1588B - 0.0475C - 0.0975AB - 0.0650AC - 0.1350BC - 0.5498A^2 - 0.3998B^2 + 0.5428C^2$$

Table 3: Regression coefficients, R^2 and probability values for eight response variables

Regression Coefficient	PLW (%)	FLR (%)	FWR (%)	TSS (°B)	TA (%)	AA (mg 100 g^{-1})	TS (%)	TC (mg 100 g^{-1})
Intercept	11.94	3.05	4.41	18.71	0.1880	48.49	16.34	3.51
A-CMC	-0.7756	-0.6520	-1.03	-0.7988	0.0243	1.10	-0.8250	-0.0688
B-Ethrel	0.9729	1.43	-0.2324	0.5300	-0.0289	-0.4487	0.5050	0.1588
C-Glycerine	0.1377	-0.0662	0.5865	0.5487	-0.0131	-0.6688	0.5475	-0.0475
AB	-0.5344	-0.9450	1.11	0.3350	-0.0268	-0.0250	0.3350	-0.0975
AC	-0.1653	-0.6771	-0.5155	0.0025	-0.0073	0.1400	0.0550	-0.0650
BC	-0.1787	0.0106	-0.7960	-0.6050	0.0150	0.9525	-0.6550	-0.1350
A^2	-1.26	-0.5693	0.8981	-0.4542	0.0337	-2.18	-1.32	-0.5498
B^2	-0.5918	0.4826	0.9078	-0.2717	0.0415	2.64	-1.14	-0.3998
C^2	-0.7280	-0.0506	-2.73	0.2007	0.0275	-2.22	-0.6130	0.5428
Mean	10.73	2.98	3.97	18.46	0.2364	47.66	14.89	3.32
R^2	0.9827	0.9855	0.9807	0.9777	0.9640	0.9842	0.9810	0.9743
Adjusted R^2	0.9605	0.9663	0.9560	0.9490	0.9178	0.9639	0.9566	0.9414
Model F-value	44.26	51.91	39.62	34.09	20.85	48.46	40.14	29.54
Lack of fit	2.62	5.29	2.45	1.74	0.59	3.97	2.03	1.35

**Significant at $p < 0.05$.*

Table 4: Experimental results for treated mango fruits stored under ambient conditions on the 12th day of storage

Test number	Response							
	PLW (%)	FLR (%)	FWR (%)	TSS (°B)	TA (%)	AA (mg 100 g^{-1})	TS (%)	TC (mg 100 g^{-1})
1	11.30	3.43	4.45	19.8	0.227	42.51	15.70	3.59
2	9.00	1.71	3.89	16.42	0.343	50.98	12.32	2.37
3	9.96	2.57	6.04	18.15	0.240	49.49	14.05	2.51
4	9.47	1.98	1.60	16.85	0.322	50.85	12.75	3.47
5	9.16	1.47	8.61	18.48	0.233	48.36	14.38	2.42
6	12.05	3.07	4.89	18.55	0.194	48.67	16.14	3.58
7	11.42	5.01	1.98	19.21	0.222	48.86	15.11	3.57
8	11.96	3.17	4.46	18.84	0.165	48.02	16.63	3.38
9	12.00	2.99	4.13	18.95	0.185	48.40	16.54	3.50
10	11.61	3.18	4.12	18.63	0.191	48.60	16.22	3.45
11	8.95	2.78	1.74	17.10	0.286	45.39	13.00	3.55
12	9.10	1.12	1.81	18.00	0.253	44.63	13.90	3.43
13	10.49	2.39	2.32	18.91	0.231	43.83	15.02	3.45
14	12.26	6.11	6.32	18.87	0.237	46.97	14.77	2.95
15	12.10	2.83	4.44	18.56	0.205	48.76	16.15	3.65
16	11.70	4.94	2.33	19.12	0.226	48.59	15.02	4.04
17	9.91	2.01	4.44	19.36	0.258	47.31	15.46	3.54

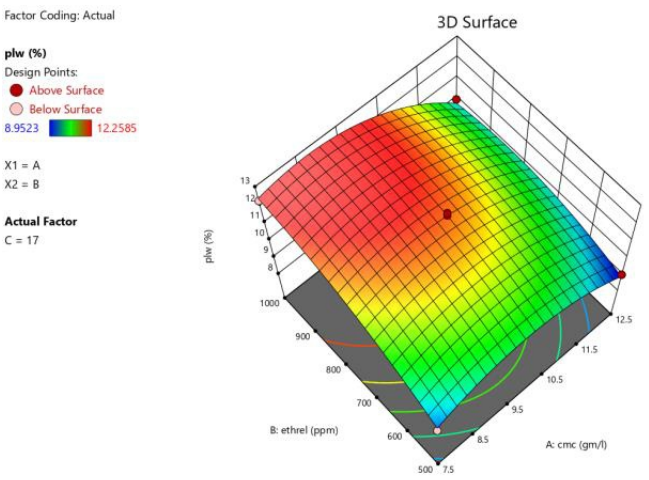

Figure 1. 3D Response surface plots showing the interactive effects of CMC and ethrel on physiological loss in weight

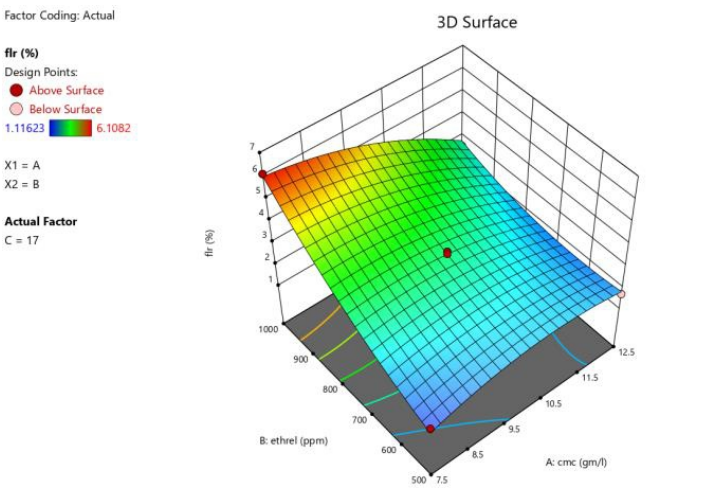

Figure 2. 3D Response surface plots showing the interactive effects of CMC and ethrel on reduction in fruit length

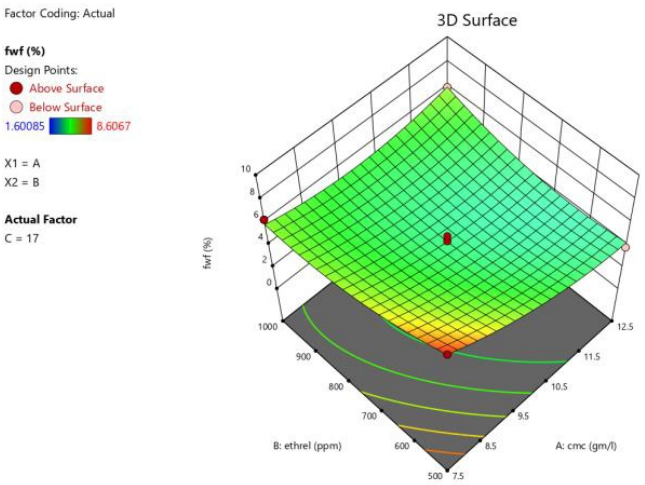

Figure 3. 3D Response surface plots showing the interactive effects of CMC and ethrel on reduction in fruit width

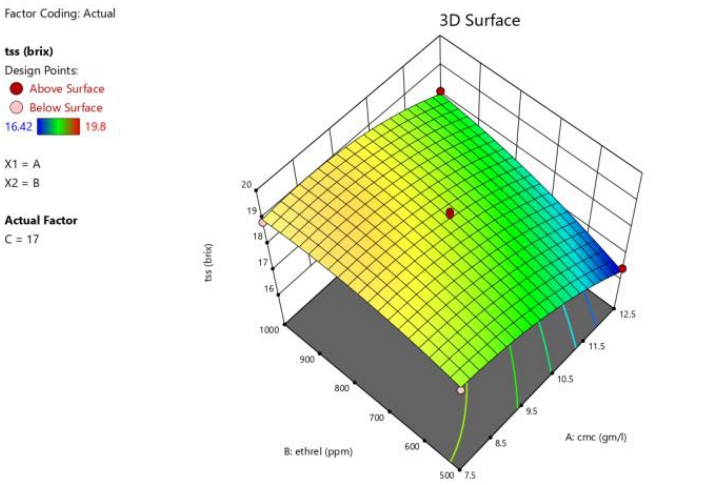

Figure 4. 3D Response surface plots showing the interactive effects of CMC and ethrel on total soluble solids

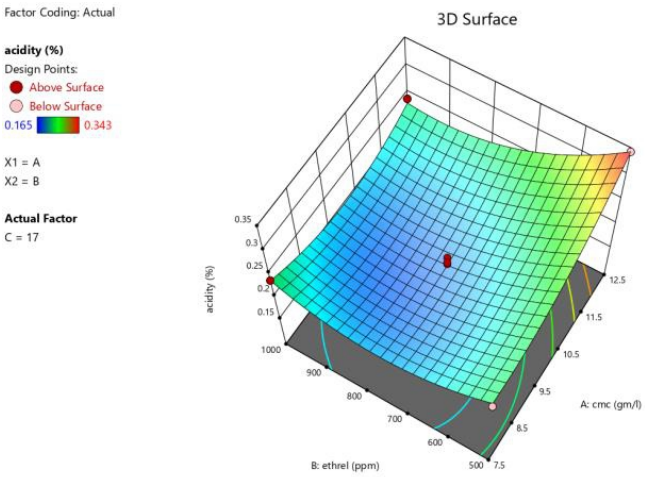

Figure 5. 3D Response surface plots showing the interactive effects of CMC and ethrel on titratable acidity

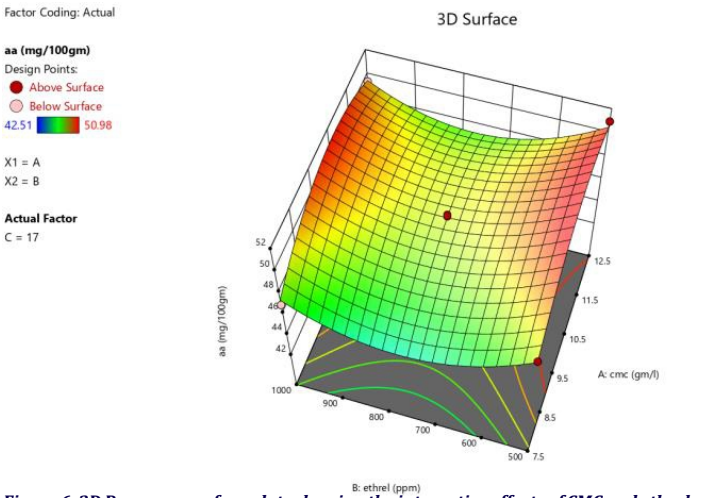

Figure 6. 3D Response surface plots showing the interactive effects of CMC and ethrel on ascorbic acid

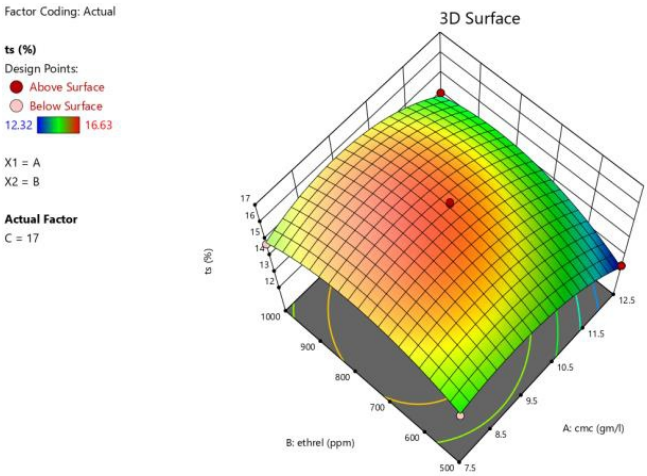

Figure 7. 3D Response surface plots showing the interactive effects of CMC and ethrel on total sugar

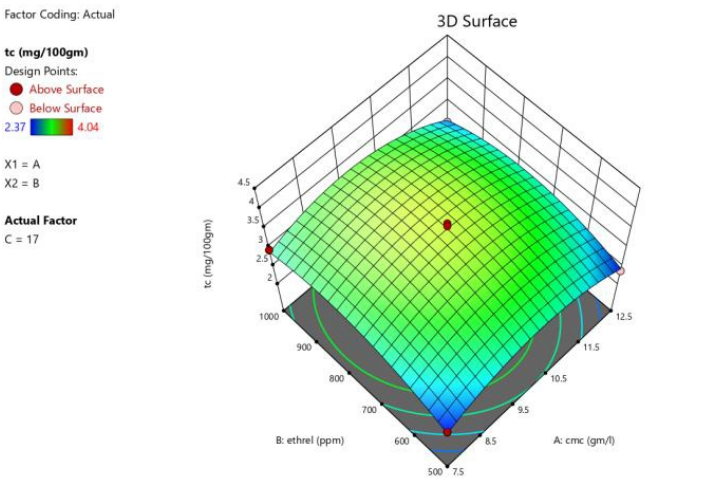

Figure 8. 3D Response surface plots showing the interactive effects of CMC and ethrel on total carotenoids

4. Discussion

4.1 Physical Responses

Physiological weight loss (PLW) is a significant parameter indicating fruit quality after harvest, primarily due to the effects of transpiration and respiration. The main contributor to weight loss, shrivelling, loss of texture and poor market appeal, is transpiration, or water vapour loss from the fruit epidermis (31). Respiration contributes to moisture loss by consuming substrates and releasing CO_2 and water, which negatively affect fruit quality (32). PLW will differ based on fruit morphology, epidermal skin properties, environmental influences of temperature and humidity and metabolic activity (31, 33). Edible coatings, especially polysaccharide-based films, such as CMC, are considered to be useful in reducing the potential for PLW (34). Edible coatings provide a semi-permeable barrier to water vapour diffusion but allow necessary gas exchange (13). The coatings help retain moisture, turgor pressure and delay degradation of texture. Research on mangoes supports the use of CMC coatings to reduce weight loss associated with controlling transpiration and respiration (56, 16). In addition, coatings alter the internal atmosphere of fruit, by reducing available oxygen, slowing respiration and minimising the metabolic contributions to PLW (36, 37). Glycerine's role as a plasticiser increases flexibility and integrity of the coating, preventing cracks otherwise result in loss of barrier function (38). On the other hand, ethrel increases ripening by hastening respiration and increasing metabolically related water loss (39). Ethrel is useful for enhancing quality attributes related to ripening but increases PLW. When used together, CMC coatings will help to counter-balance ethrel effects by reducing excessive water loss while not inhibiting ripening like when ethrel and 1-MCP are used together (21).

Postharvest fruit storage can lead to reductions in both fruit length and width, which shows tissue shrinkage and moisture loss. Such dimensional changes negatively affect fruit appearance, texture, consumer preference and economic value (40). The primary cause is dehydration of the cellular tissue due to a loss of water through the epidermis as a result of transpiration (41). After fruit was detached from the plant, they will no longer have a source of water, which reduces the turgor pressure of the cells and leads to shrinkage and loss of firmness (42). The environment, including temperature, relative humidity and airflow, affects the rate of dimensional reduction and high temperatures and low humidity will hasten this process (43). CMC coatings can help minimise these losses by forming semi-permeable barriers that restrict diffusion of water vapour and improve the cellular hydration of the fruit (13,57). Coatings can lower respiration rates by modifying the atmosphere around the fruit thus reducing the rate of shrinkage as well (44). Plasticisers like glycerine will increase the mechanical strength and flexibility of a coating, prevent cracking of the coating and enhance its protective capacity (45). Ethrel promotes ripening of fruit by accelerating the enzymatically-driven degradation of the cell wall thus weakening the tissues of the fruit, making them more prone to shrinkage (39). Therefore, balancing ethrel application with coatings and environmental controls (temperature, humidity) is important for preserving fruit quality (46, 47).

4.2 Biochemical Responses

Total soluble solids (TSS) is an important quality parameter that describes the ripening flavour and sweetness of a fruit. Their content is influenced by the enzymatic conversions of starch to simple sugars during the ripening process (48). TSS content usually increases until it reaches its climacteric peak, then declines due to the expense of sugars during respiration (56, 48). Temperature during storage has a significant influence on TSS content, with ambient conditions being more favourable for starch hydrolysis than cooler storage (49). CMC coatings moderate TSS content by slowing down respiration and also extending the period of enzymatic breakdown of starch (13, 57). However, ethrel also induces starch degradation and ripening which promotes TSS (39). Therefore, CMC coatings and ethrel can be used in combination to regulate TSS content and prevent premature overripening while also maintaining sweetness. Plasticisers such as glycerine aid in improving coating functionality further in regulating biochemical changes (45).

Titratable acidity, mainly from citric and malic acids, is important in maintaining balanced fruit flavour. Naturally, TA declines during ripening because organic acids are converted into sugars, as well as through respiratory losses (48). A moderate decrease in acidity promotes flavour, but excessive losses may adversely affect consumer acceptability. CMC coatings reduce the rate of decline in TA by reducing diffusion of oxygen and thus respiratory metabolism (16, 13). Ethrel, on the other hand, markedly contributes to the degradation of acids due to its ability to activate metabolism (39). However, as observed in the prior treatment, achieving a balance of sugars and acids is key to an acceptable flavour and avoiding rapid senescence in the fruit. The use of plasticisers like glycerine also reduces the possibility that coatings become unstable while also reducing any environmental stress that may accelerate the loss of acids (45).

Ascorbic acid (AA) or vitamin C has prominent antioxidative and nutritional properties but it is quite sensitive to oxidative degradation during storage. During the ripening process, the ascorbic acid is diminished via oxidative stress and degradation due to enzymatic activity (39). The ascorbic acid degradation during the storage also fluctuates with temperature, water activity, oxygen concentration and humidity during the storage (13). The CMC coating reduces oxidation and overall oxidative stress. CMC coating creates a barrier between the fruit surface and the surrounding atmosphere by achieving mechanical and chemical stability and helps retain ascorbic acid content with slowing down its degradation (45). However, with exposure to an ethrel treatment ripening was accelerated which induces additional metabolic activity and an ample deflation of ascorbic acid (39). Therefore, when improved mechanical stability is paired with better CMC coating using plasticizers and combined with ethrel, there is less oxidation and oxidative stress and there is greater chance of ascorbic acid retention as well as the nutritional values of the fruit are retained (56, 13).

The increase in total sugar in the mangoes during ripening can be attributed to enzymatic hydrolysis of starch that yields glucose, fructose and sucrose, which is enhanced by ethylene action (50). Uncontrolled respiration requires increased demand of sugar and affects fruit quality (51). The CMC coating reduces gas exchange and slows respiration rates of the mango, preventing rapid utilisation of sugars and allowing a slow accumulation of soluble sugars (56). Ethrel at desired concentration improve starch conversion at a desirable pace and ensure uniformity of ripening (52).

Glycerine maintains the flexibility of the coating and reduces moisture loss when used as a plasticiser (53). This balance of reduced respiration along with controlled ethylene release will maintain desirable sweetness and textures, which is desirable to the consumer. Overall, it can be determined that a composite coating is moderating biological processes, allowing for shelf-life extension of fruit and sustaining the retention of naturally occurring sugars and sensory quality (52,57).

Carotenoids are significant bioactive pigments that give mango fruit its characteristic yellow-orange hue and support its antioxidant qualities (54). Ethylene-mediated chlorophyll breakdown and the activation of carotenogenic enzymes like phytoene synthase and lycopene β-cyclase during ripening promote carotenoid accumulation (55). On the other hand, excessive ethylene or unchecked respiration can hasten pigment breakdown and oxidation. A semi-permeable layer is created by applying a CMC-based coating, which maintains pigment formation while decreasing oxygen diffusion and slowing chlorophyll loss (16). Glycerine reduced desiccation stress and increased coating flexibility. Extended shelf life, carotenoid stability and visual appeal were all preserved by the gradual ethylene diffusion made possible by the optimised CMC–Ethrel–Glycerine coating.

Conclusion

Under ambient storage, a composite edible coating consisting of CMC, ethrel and glycerine effectively maintained the postharvest quality of Langra mangoes, minimising physiological weight loss and fruit shrinkage while preserving sugars, acidity, vitamin C and colour development up to 12 days. Effective levels of CMC, ethrel and glycerine that balanced moisture retention with controlled ripening were identified by the RSM-based models, which also correctly predicted quality responses. According to these results, CMC-ethrel-glycerine coatings are a safe, economical and user-friendly way to control ripening and prolong the shelf life of mangos and possibly other climacteric fruits.

Future Work

The optimised CMC–ethrel–glycerine coating should be validated across different mango cultivars and other climacteric fruits under both ambient and cold storage conditions. Future studies may also integrate molecular, enzymatic and metabolomic analyses to better understand the mechanisms of ripening regulation and quality retention.

Acknowledgements: The authors gratefully acknowledge the Department of Horticulture, College of Agriculture and the Department of Chemistry, College of Basic Sciences and Humanities, GBPUAT, Pantnagar, for their support and facilities provided for this study.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Approval: Not applicable.

Funding: Not applicable.

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