



# Post-harvest Treatment of Different Concentrations of Gibberellic Acid on the Physiochemical Characteristics and Shelf Life of Mango (*Mangifera indica* L. cv. Malda)

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# Abstract

The study was undertaken to evaluate the effects of different concentrations of gibberellic acid on the physiochemical attributes and shelf life of mango (*Mangifera indica* cv. Malda) from  $17^{th}$  June 2022 to  $29^{th}$  June 2022. The main objective of this research was to assess the appropriate concentration of gibberellic acid to prolong the shelf life of mango and the physicochemical properties of mango. The study contained 5 different concentrations of gibberellic acid as 5 treatments (0ppm, 100ppm, 200ppm, 300ppm and 400ppm) with four replications of each on a Completely Randomized Design (CRD). For each treatment destructive and non-destructive samples were prepared, non-destructive samples were used for observation of pH, fruit firmness, Titratable acidity, TSS and TSS: TA ratio. The fruits were evaluated at the one-day interval after the initial reading taken on the day of storage and further data were recorded after 2, 4, 6, 8, 10 and 12 days of storage. Among all the GA<sub>3</sub> treatments, 400ppm GA<sub>3</sub> recorded the minimum physiological weight loss, the highest TSS (20.38<sup>0</sup>Brix), maximum fruit firmness (1.875 kg/cm<sup>2</sup>), maximum titratable acidity (0.1910%), maximum TSS: TA ratio (124.7) and minimum pH (6.725). The longest shelf life (12.75 days) was observed with fruit treated with a 400ppm concentration of gibberellic acid, similar to 300ppm of GA<sub>3</sub> (12 days). Maximum retardation of physicochemical changes and prolongation of mango fruit storage life was observed at 400 ppm Gibberellic acid.

Keywords: Malda, shelf life, physiochemical properties, gibberellic acid

# Introduction

In Nepal's tropical and subtropical climates, the mango plant which is a member of the Anacardiaceae family is commonly cultivated (Laohaprasit *et al.*, 2012). Because of its mouthwatering flavor, great palatability, eye-catching color and nutritional content, it is renowned as the "king of tropical fruits." The main tropical fruit growing in Nepal's terai, inner terai and slopes up to 1100 masl is the mango.

In terms of potential growth areas, productivity and consumption, the mango is among the highest-value crops in Nepal. Mangoes are nutrient-dense. A mango's ability to provide between 64 and 86 calories of energy per 100g is crucial for balancing the human diet (Rathore. *et al.*,2007). Insufficient arrangements for post-harvest management such as storage, processing, marketing, infrastructures and preservation lead to post-harvest losses (Bhande *et al.*, 2008). Therefore, appropriate measures should be adopted to prolong the shelf life of mangoes. The post-harvest life of mango is very less in Nepal because of the insufficiency of cold storage, transport facilities and a sound marketing system. **Error! Bookmark not defined.**(Hoa *et al.*, 2002).

The inherently present plant hormones gibberellin and gibberellic acid, which are pentacyclic diterpene acids, are utilized all over the world in agriculture to control the development of plants. The senescence-delaying compound gibberellins, an endogenous plant growth regulator, is accessible as a powder. When gibberellins are used, the production is improved since it corrects several physiological issues that lead to poor fruit quality and yield. Gibberellic acid is utilized to postpone the development of carotenoids and other ripening and softening changes in

produce. (Kader, 1986). Gibberellic acid was considered to be extremely important in preserving the quality and prolonging the shelf life of mango fruits.

# **Materials and Methods**

The cultivar used for this study was Malda and it was carried out in the horticulture lab of the CNRM Bardibas in Bardibas, Mahottari, Nepal. The field of study was situated between 26.8762°N and 85.8077°E in latitude and longitude respectively and at an altitude of 165 masl. The dates of this experiment were June 17, 2022, through June 29, 2022. Color and shape that have not been bruised or damaged and are free of disease and insect infestation are collected. The fruits were washed in water to remove any foreign objects, such as dust, grime and mud and then allowed to dry.

# Preparation of the gibberellic acid

With the use of a digital scale, gibberellic acid concentrations of 500 mg, 1000 mg, 1500 mg and 2000 mg were weighed. The varied amounts of gibberellic acid powder of 500 mg, 1000 mg, 1500 mg and 2000 mg correspondingly were dissolved in a little amount of ethanol at the mild warm state and produced up to 5 liters with distilled water to create gibberellic acid solutions of 0 ppm, 100 ppm, 200 ppm, 300 ppm and 400 ppm.

#### Design of experiment

Five treatments, four replications and a CRD were used in the experiment

#### Treatment details

- T<sub>1=</sub> (Control: distilled water) T<sub>2</sub>=100ppm of gibberellic acid
- $T_3$ = 200ppm of gibberellic acid
- $T_4=300$ ppm of gibberellic acid

T<sub>5</sub>=400ppm of *gibberellic acid* 

# An atmospheric condition of the laboratory

The temperature and relative humidity were recorded thrice a day i.e., 8:00 am, 1:00 pm and 5:00 pm daily with a thermo-hygrometer.

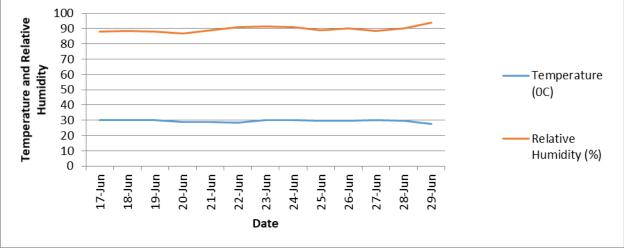


Figure 1. Geographical representation of Environmental conditions in the lab during the experiment period

The average maximum and minimum temperatures of 30.3°C and 30.25°C were recorded on 7<sup>th</sup> June 2022 and 8<sup>th</sup> June 2022 respectively.

The average maximum and minimum relative humidity of 88% and 94% were recorded on 7<sup>th</sup> June 2022 and 19<sup>th</sup> June 2022 respectively.

# Data collection

Two parameters viz. physical and chemical were recorded for observation at an interval of 1 day starting from the days of storage till the longevity of the fruits. In this study, the information below was gathered.

#### Physical observations

Three fruits of non-destructive sample from each treatment combination of every replication were kept recording the shelf life of the fruit and metabolic weight loss, fruit firmness.

#### Chemical observations

To measure the pulp pH, TSS, TA and TSS: TA ratio, 5 fruits of destructive samples from each treatment and replication were chemically tested.

# Observational Recording technique

# Physiological loss in weight (PLW)

Physical weight loss was determined at every 1-day interval from non-destructive sampling. An electronic digital balance was used to measure the fruit's weight. Initial weight was recorded at the 1<sup>st</sup> days of storage from the freshly harvested fruits. The physiological loss of weight was determined by using the following formulae (Moneruzzaman et al., 2009).

PLW (%) = (IW-FW) \*100/IW Where, PLW=Physiological loss in weight IW=Initial weight (g) FW=Final weight (g)

#### Total soluble solids (TSS)

A handheld refractometer was used to test the TSS of mango fruit. Using a clean pipette and fruit juice, the refractometer's prism is wetted for the measurement. TSS value is presented as %Brix. The reading was taken at room temperature. After every usage, the Refractometer was cleaned with water and a muslin cloth.

#### pН

An automated digital pH meter was used to measure the mango fruit juice's pH (Hanna instruments, pHep). Before determining the pH of the pulp juice, the pH meter was calibrated with a neutral buffer solution with a pH of 4.0 or 7.0.

#### TSS: TA ratio

TSS: TA ratio was evaluated by using the following formula. TSS: TA= Total soluble solid/Titratable acidity

# Titratable acidity (TA)

Titratable acidity was examined by the titration of diluted fruit juice (5ml) with 100 ml deionized water and 2 phenolphthalein droplets indicator (Ranganna, 1979) against base 0.1 N NaOH solutions. The following formula was used to compute the percent titratable acidity.

$$TA(\%) = \frac{[ml of NaOH used] \times [0.1N NaOH] \times [milliequivalent factor]}{grams of sample used} \times 100$$

Where, TA =Titratable acidity N NaOH =Normality of NaOH NaOH=Sodium hydroxide m.eq.wt. =Mill equivalent weight of predominant acid=0.067

# Fruit firmness

After treatment, fruit firmness measurements will be collected using a penetrometer device at 1-day intervals (Model GY-3, No. 400102024). Fruit firmness was evaluated using the peak pierce force (g) (Meng et al., 2014). With a penetrometer, the fruit's hardness will be evaluated at two sites and the average of these readings will be used as the real firmness.

# Shelf life

The storage time of a product is calculated by adding up the number of days between the first day of storage after treatment and the last day the fruit is edible. The shelf life was measured up until the mangoes in each treatment and replication had deteriorated by less than 50%.

#### Statistical method

All collected data were entered into MS-Excel for statistical analysis and subjected to Analysis of variance (ANOVA) using the Gen Stat 15<sup>th</sup> edition. Duncan's Multiple Range Test at a 5% level of significance (DMRT) was used to separate the means of treatments that were significantly different.

#### **Results and Discussion**

#### Physiological weight loss (PLW)

Table 1 and Figure 2 exhibit the finding related to physiological weight reduction. There was a consistent increase in physiological weight loss across all treatments when the storage duration was extended. Gibberellic acid 400 ppm treatment resulted in the fruits with the lowest physiological weight of all the treatments, followed by those treated with 300 ppm, 200 ppm and 100 ppm of GA<sub>3</sub>, with control fruits losing the most weight on each sampling at 8 days following storage.

**Table 1.** Post-harvest treatments of gibberellic acid on physiological loss in weight of mango (*Mangifera indica* L.cv. Malda) in Bardibas, Mahottari, 2022

Turnet	Physiological loss in weight									
Treatments	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS	12 DAS				
Control	4.126°	8.280 <sup>c</sup>	12.24°	15.98 <sup>b</sup>	-	-				
100 ppm GA <sub>3</sub>	4.001 <sup>bc</sup>	8.006 <sup>bc</sup>	11.96 <sup>bc</sup>	15.74 <sup>b</sup>	19.83 <sup>b</sup>	-				
200 ppm GA <sub>3</sub>	3.672 <sup>ab</sup>	7.929 <sup>bc</sup>	11.49 <sup>bc</sup>	15.40 <sup>b</sup>	17.83ª	47.59 <sup>b</sup>				
300 ppm GA <sub>3</sub>	3.538ª	7.472 <sup>ab</sup>	11.03 <sup>ab</sup>	14.34 <sup>ab</sup>	17.73ª	46.58 <sup>b</sup>				
400 ppm GA <sub>3</sub>	3.380ª	6.907ª	10.34ª	13.44ª	17.27ª	20.85ª				
Grand Mean	3.743	7.72	11.41	14.98	18.17	38.34				
CV%	6.1	6.4	6.3	7	6.2	2.5				
SEm(±)	0.1619	0.349	0.511	0.742	0.799	0.688				
F-test	**	*	*	*	*	**				

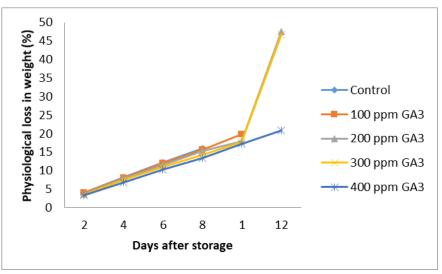


Figure 2. Gibberellic acid effect on physiological weight loss

#### Fruit firmness

Table 2 and Figure 3 illustrate the results of treating fruits with gibberellic acid. Untreated fruits had the least stiffness whereas fruits with 400 ppm of gibberellic acid had the most firmness ( $1.875 \text{ kg/cm}^2$ ), followed by 300 ( $1.865 \text{ kg/cm}^2$ ) and 200 ( $1.633 \text{ kg/cm}^2$ ) after 12 days of storage.

Transformer	Fruit firmness								
Treatments	Initial	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS	12 DAS		
Control	8.3	3.200ª	2.800ª	2.425ª	-	-	-		
100 ppm GA3	8.3	3.763ª	2.825 <sup>ab</sup>	2.725 <sup>b</sup>	2.5ª	-	-		
200 ppm GA3	8.3	5.065 <sup>b</sup>	2.925 <sup>bc</sup>	2.750 <sup>b</sup>	2.675 <sup>b</sup>	2.175 <sup>a</sup>	1.633ª		
300 ppm GA <sub>3</sub>	8.3	5.900 <sup>b</sup>	2.975°	2.775 <sup>b</sup>	2.775 <sup>bc</sup>	2.287 <sup>b</sup>	1.865 <sup>b</sup>		
400 ppm GA <sub>3</sub>	8.3	5.933 <sup>b</sup>	3°	2.800 <sup>b</sup>	2.8°	2.362°	1.875 <sup>b</sup>		
Grand Mean	8.3	4.77	2.905	2.695	2.687	2.275	1.791		
CV%	-	14.9	2.6	4.4	2.5	1.9	4.6		
SEm(±)	-	0.503	0.0524	0.0842	0.0479	0.03	0.0582		
F-test	-	***	**	**	***	***	**		

 Table 2. Post-harvest treatments of different concentrations of gibberellic acid on fruit firmness of mango

 (Mangifera indica L. cv. Malda) in Bardibas, Mahottari, 2022

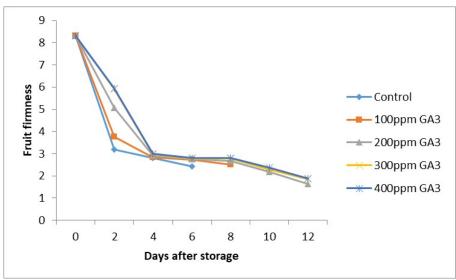


Figure 3. Gibberellic acid effect on Fruit Firmness

#### Titratable acidity (TA)

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The results of this investigation showed that over the storage period, different quantities of gibberellic acid had significantly varying Titratable Acidity values. The information on the titratable acidity of fruits treated postharvest and their mean value is shown in Table 3 and Figure 4. It is discovered that the declining rate was extremely quick from the first to the fourth day and that its tendency after that was somewhat slower, as indicated in Table 3. The fruits that were left untreated had the minimum titratable acidity, whereas @GA<sub>3</sub> 400ppm had the highest (0.1910 %).

<b>Table 3.</b> Post-harvest treatments of gibberellic acid on Titratable acidity of mango (Mangifera indica L. cv. Malda)
in Bardibas, Mahottari, 2022

Transformed		Titratable acidity								
Treatments	Initial	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS	12 DAS			
Control	1.608	0.714ª	0.2982ª	0.1608 <sup>a</sup>	-	-	-			
100 ppm GA <sub>3</sub>	1.608	0.714ª	0.3250 <sup>b</sup>	0.1675 <sup>a</sup>	0.1507 <sup>a</sup>	-	-			
200 ppm GA <sub>3</sub>	1.608	1.072 <sup>b</sup>	0.3350 <sup>b</sup>	0.1876 <sup>b</sup>	0.1809 <sup>b</sup>	0.1709ª	0.1507ª			
300 ppm GA <sub>3</sub>	1.608	1.159 <sup>b</sup>	0.3618°	0.2077°	0.2021°	0.1775 <sup>a</sup>	0.1742 <sup>b</sup>			
400 ppm GA <sub>3</sub>	1.608	1.608°	0.5796 <sup>d</sup>	0.2613 <sup>d</sup>	0.2343 <sup>d</sup>	0.2010 <sup>b</sup>	0.1910 <sup>b</sup>			
Grand Mean	1.608	1.053	0.3799	0.1970	0.1920	0.1832	0.1720			
CV%	-	6.6	2.9	4.6	5.2	3	6.1			
SEm(±)	-	0.0490	0.00783	0.00647	0.00708	0.00389	0.00741			
F-test	-	***	***	***	***	***	**			

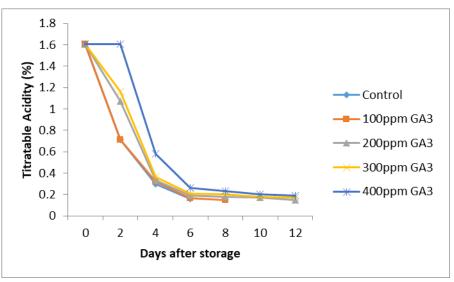


Figure 4. Gibberellic acid effect on Titratable acidity

#### Pulp pH

According to the experiment's findings, the pH was greatest in the control fruits during the whole storage time, followed by the fruits treated with 100, 200, 300 and 400 ppm of GA<sub>3</sub>.

Implementing gibberellic acid of 400ppm concentration had the lowest pH as compared to the control fruits in this study. Comparable outcomes were seen in the research of (Fatima *et al.*, 2022).

**Table 4.** Post-harvest treatments of gibberellic acid on pulp pH of mango (Mangifera indica L. cv. Malda) inBardibas, Mahottari, 2022

Turnet		Pulp pH								
Treatments	Initial	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS	12 DAS			
Control	4.2	4.9°	5.075°	5.125 <sup>d</sup>	-	-	-			
100 ppm GA <sub>3</sub>	4.2	4.8 <sup>bc</sup>	4.9 <sup>b</sup>	5.050 <sup>cd</sup>	5.3 <sup>b</sup>	-	-			
200 ppm GA3	4.2	4.725 <sup>b</sup>	4.750 <sup>b</sup>	4.950 <sup>bc</sup>	5ª	5.4°	7.20°			
300 ppm GA <sub>3</sub>	4.2	4.675 <sup>b</sup>	4.575ª	4.850 <sup>ab</sup>	4.975ª	5.2 <sup>b</sup>	6.97 <sup>b</sup>			
400 ppm GA <sub>3</sub>	4.2	4.325ª	4.550ª	4.750ª	4.875 <sup>a</sup>	4.8ª	6.725ª			
Grand Mean	4.2	4.685	4.770	4.945	5.037	5.133	6.965			
CV%	-	1.8	2.4	1.9	1.6	1.6	1.1			
SEm(±)	-	0.0585	0.0806	0.0652	0.0559	0.0577	0.0549			
F-test	-	***	***	***	***	***	***			

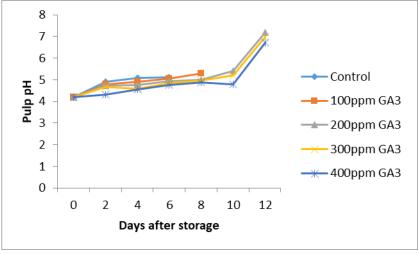


Figure 5. Gibberellic acid effect on pH

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#### Total soluble solids

The disparity in total soluble solids content in mango fruit presented in table 5 imitate the effect of post-harvest treatment of gibberellic acid. The total soluble solids increased from control mango fruits (untreated) from the initial to 6<sup>th</sup> days and then, it declined. Similarly, the other treatments such as 100 and 200 ppm gibberellic acid also increasingly provided TSS from the initial to 8<sup>th</sup> days and afterward, it decreased. Mango fruits dipping in a solution of gibberellic acid of 300 ppm and 400 ppm provided an increase in TSS from the initial to 12<sup>th</sup> days. Fruits treated with 400ppm of gibberellic acid were the slowest to reach their TSS peak. A ripening pause that prevented TSS accumulation may have caused this to occur.

**Table 5.** Post-harvest treatments of gibberellic acid on total soluble solids of mango (*Mangifera indica* L. cv.Malda) in Bardibas, Mahottari, 2022

	Total Soluble Solids								
Treatment	Initial	2DAS	4DAS	6DAS	8DAS	10DAS	12DAS		
Control	6.2	15.03 <sup>d</sup>	17°	19.40 <sup>d</sup>	-	-	-		
100 ppm GA <sub>3</sub>	6.2	14.96 <sup>d</sup>	16.75°	18°	19 <sup>d</sup>	-	-		
200 ppm GA <sub>3</sub>	6.2	13.33°	15.10 <sup>b</sup>	16.32 <sup>b</sup>	17.82°	15.07ª	13.43ª		
300 ppm GA <sub>3</sub>	6.2	11 <sup>b</sup>	14.65 <sup>b</sup>	15.30ª	17.32 <sup>b</sup>	17.38 <sup>b</sup>	18.70 <sup>b</sup>		
400 ppm GA <sub>3</sub>	6.2	9.90 <sup>a</sup>	12.32ª	15.15 <sup>a</sup>	16.25ª	18°	20.38°		
Grand Mean	6.2	12.864	15.165	16.834	17.60	16.817	17.50		
CV%	-	1.7	2.2	2.8	1.4	0.9	1.8		
SEm(±)	-	0.1528	0.2400	0.332	0.1785	0.1041	0.2230		
F-test	-	***	***	***	***	***	***		

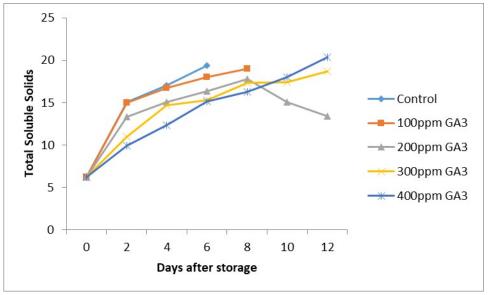


Figure 6. Gibberellic acid effect on total soluble solids

# TSS: TA ratio

As shown in Table 6 and Figure 6, the outcome demonstrated that there were substantial variations in the amounts of gibberellic acid on the TSS: TA ratio of mangoes throughout storage.

With longer storage times, a rising trend in the TSS: TA ratio was seen in mango fruits. The highest TSS: TA ratio was seen in the 400 ppm-treated fruits (124.7<sup>0</sup>Brix).

Turtur	TSS: TA ratio								
Treatments	Initial	2 DAS	4 DAS	6 DAS	8 DAS	10 DAS	12 DAS		
Control	3.855	21.18 <sup>d</sup>	52.38°	115.98 <sup>d</sup>	-	-	-		
100 ppm GA3	3.855	21.09 <sup>d</sup>	50.67°	95.15°	108.73°	-	-		
200 ppm GA <sub>3</sub>	3.855	11.53°	50°	87.25 <sup>b</sup>	95.97 <sup>b</sup>	75ª	70.4ª		
300 ppm GA <sub>3</sub>	3.855	9.31 <sup>b</sup>	40.52 <sup>b</sup>	86.92 <sup>b</sup>	94.04 <sup>b</sup>	101.48 <sup>b</sup>	117.4 <sup>b</sup>		
400 ppm GA <sub>3</sub>	3.855	6.84 <sup>a</sup>	21.28ª	58.02ª	76.13ª	101.72 <sup>b</sup>	124.7 <sup>b</sup>		
Grand Mean	3.855	13.99	42.97	88.7	93.7	92.73	104.2		
CV%	-	8.7	3.6	5.4	7	2.8	8.1		
Sem(±)	-	0.859	1.091	3.40	4.62	1.863	5.94		
F-test	-	***	***	***	***	***	***		

**Table 6.** Post-harvest treatments of gibberellic acid on the TSS: TA ratio of mango (Mangifera indica L. cv. Malda)in Bardibas, Mahottari, 2022

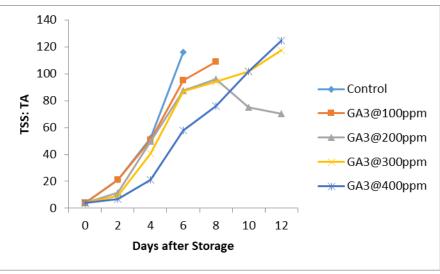


Figure 7. Gibberellic acid effect on TSS: TA ratio

#### Shelf life of fruits

As shown in Table 7 and Figure 8, the amount of GA<sub>3</sub> present during storage substantially impacted the fruits' ability to maintain their freshness. The period needed to reach peak ripeness while retaining the best marketing and eating attributes is known as the shelf life, during which time the amount of damage shouldn't rise over 50%. According to varying quantities of gibberellic acid, the shelf life varied from 8.25 to 12.75 days. The fruits treated with 400ppm GA3 had a shelf life of 12.75 days, while those treated with 300ppm, 200ppm and 100ppm had shelf lives of 12.16 days, 12.19 days and 9.50 days, respectively. The control fruit had the shortest shelf life (8.25 days).

**Table 7.** Post-harvest treatments of gibberellic acid on the Shelf life of mango (*Mangifera indica* L. cv. Malda) inBardibas, Mahottari, 2022

Treatments	Shelf life
Control	8.25ª
100 ppm GA <sub>3</sub>	9.50 <sup>b</sup>
200 ppm GA <sub>3</sub>	12.16 <sup>c</sup>
300 ppm GA <sub>3</sub>	12.19°
400 ppm GA <sub>3</sub>	12.75 <sup>d</sup>
Grand Mean	10.970
CV%	2.2
Sem(±)	0.1672
F-test	***

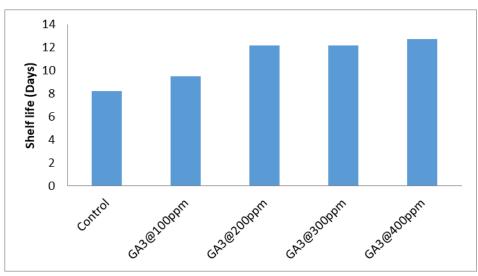


Figure 8. Gibberellic acid effect on Shelf life

#### Discussion

According to Jaw et al., (2012), Gibberellin increasing the membrane permeability following the respiratory climacteric could result in loss of moisture through the peel increase during the retention period increasing the weight loss. By preventing CO<sub>2</sub> synthesis and lowering tissue permeability, gibberellic acid slows down the respiration rate and lowers the water loss rate by evaporation (Choudhary, 2014). Gibberellic acid reduced weight loss by reducing metabolic and enzymatic activities Error! Bookmark not defined.(Islam et al., 2013). Gibberellic acid slows down some aspects of fruit ripening and prevents some changes brought on by ethylene, slowing down the rate of fruit maturation. At longer storage times, mango fruit hardness drastically declined. In mango, similar outcomes were found by Porat et al., (2001) and Reddy et al., (2014). The greater acidity in pulp offers better keeping and storage quality, making it a desirable characteristic. Reducing organic acids ultimately reduces titratable acidity because organic acids are converted into sugar and used in respiration. The current analysis found a reduction in TA. This outcome is consistent with that of Penyimpanan & L.T.S., (2013). The action of  $GA_3$  on fruits, which caused a decrease in respiration, may be the cause of fruits treated with gibberellic acid maintaining lower pH levels. Acid oxidation during storage causes a decrease in pulp pH in this event (Jain & Mukherjee, 2000). The early rise in TSS of fruits was caused by the conversion of starch and other insoluble carbs into sugar, whereas a minor decline in the latter stage was brought on by the use of soluble solids in the respiratory process (Reni et al., 2000). The slowest fruits to achieve their TSS peak were those given a 400 ppm gibberellic acid treatment. A ripening pause that prevented TSS accumulation may have caused this to occur. The greatest mean value was achieved in the control group as a result of the hydrolysis of starch into soluble sugars and the storage of these sugars in the fruits (Palmer & J.K., 1971). Gibberellic acid acts as an anti-senescent and anti-respirant, which inhibited catabolic activities and consequently reduce water loss and fruit weight loss during storage (Hu et al., 2018). Gibberellic acid therapy resulted in more delay of fruit ripening as it is known for its anti-senescing properties and ethylene antagonist and extension of the mean life of fruits (Ghimire et al., 2021).

#### Conclusion

According to the results of this study, physiological weight loss, total soluble solids, pulp pH and the TSS: TA ratio was all swiftly raised, whereas titratable acidity and fruit firmness were lowered more quickly in control fruits than in treated fruits. According to the findings of this study, mangoes can have their shelf life prolonged by using gibberellic acid in a variety of concentrations because it postpones senescence, slows the generation of ethylene, which slows down fruit ripening and extends fruit shelf life from 8.25 to 12.75 days. Gibberellic acid at 400 ppm produced superior outcomes in preventing the post-harvest quality change and extending mango's shelf life.

#### Declaration of conflict of interest and ethical approval

Bidhya Poudel Chhetri participated in the conception, execution and manuscript production of the study. Sudip Ghimire was in charge of overseeing the effort, evaluating and interpreting the findings. The authors declare no conflict of interest regarding the publication of this manuscript.

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