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Effects of Storage Temperatures on different Biochemical Characteristics of 1-Methylcyclopropene Treated Mango (*Mangifera Indica* L.) Variety Khirshapat

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Abstract As a part of our present investigation to find out the effective strategy for enhancing the shelf life of mango fruits, we observed the changes in biochemical parameters of a local mango variety namely Khirshapat at the concentration of 1 & 2ppm of 1-MCP during storage at 12-13 °C and 30-32 °C. Green mature mangoes were directly collected from mango garden and hot water treatment was given for 10 minutes and then air-dried. After that the mangoes were carefully stored in a specialized store (Time lagging cooling system) at 12-13°C for 24 h and treated with ethylene inhibitors 1-MCP at the concentration of 1 & 2 ppm and incubated at same storage condition. Untreated mangoes at room temperature were considered as control. In case of control and mangoes treated with 1-MCP at the concentration of 1 & 2ppm under normal temperature the total soluble solid, pH, total sugar, amylase activity and invertase activity were increased whereas titratable acidity, vitamin-c and starch content decreased significantly but all these attributes were remained unchanged when the mangoes were treated with 1-MCP at 2ppm concentration under storage temperature (12-13 °C). The experimental variety Khirshapat showed increased pulp pH, total sugar content, starch content and invertase activity at all the storage duration. The results explored that some biochemical properties and enzyme activities along with shelf life drastically decreased from untreated mangoes as well as treated mangoes under normal temperature. Between the concentrations of 1-MCP the 2ppm concentration treatment showed better results in delaying the changes in biochemical properties and extended shelf life.

Keywords: *ethylene inhibitor, Methylcyclopropene, Time lagging cooling system, biochemical properties, shelf life*

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1. Introduction

Mango (*Mangifera indica* L.) is well known for its excellent exotic flavor and usually referred to as the king of fruit. It is a popular and economically important, nutritious fruit widely cultivated in the tropical and subtropical areas [1]. It is an attractive color, delicious taste and excellent nutritional properties, making it a world choice fruit [2]. The fruit is eaten fresh and in several others by-products, including nectar, purees, squash, pulp, juice, jam, jellies and canned [3]. The mango is a commercial crop in many countries of the Southeast Asia, namely, India, Pakistan, the Philippines, Indonesia, Malaysia, Thailand, Burma, Sri Lanka, and Bangladesh [4]. Mango ranks third among the tropical fruits grown in the world [5] and like most of the tropical fruits mango has limited postharvest life. In harsh climatic conditions, fruits become very soft within 3 to 9 days postharvest due to accelerated ripening [6]. The short shelf life of whole mango fruits limits their marketing in distant markets.

Many attempts have been taken to develop techniques to prevent postharvest loss and increase the shelf life of different perishable fruits and vegetables. Among different techniques one of the most prominent techniques is using 1-methylcyclopropene (1-MCP) as a tool to delay the ripening of fruits and vegetables [7,8].

1-methylcyclopropene is an odorless gas that has a physical similarity to ethylene it to bind to the ethylene receptors in fruits, thus inhibiting the normal action of ethylene and prolonging the storage life of fruit.1-MCP is an inhibitor of ethylene perception that can markedly affect ripening and senescence processes of many horticultural commodities [9]. Binding of 1-MCP to the ethylene receptor allows the ethylene cascade to be inactivated and protects the plant from both endogenously and exogenously produced ethylene [10]. The affinity of 1-MCP for the receptors is about 10 times greater than that of ethylene; hence, 1-MCP is active at much lower concentrations than ethylene. 1-MCP has been certified to have a nontoxic mode of action and is chemically similar to naturally occurring substances [11].

Several studies [12-16] have shown that the ripening procedure of mangoes can be delayed up to several days through the use of 1-MCP. Singh et al. [17] compared 1-MCP treatments to silver nitrate, gibberellic acid, sodium metabisulphite, and ascorbic acid treatments on 'Dashehari' mangoes and found that the 1-MCP treatment was the most effective in delaying ripening. Sozzi and Beaudry [18] note that the majority of current usage for 1-MCP is as a supplement to proper postharvest temperature management or controlled atmosphere storage. Because temperature management is the most critical factor in the management of ripening in mature green mangoes. Paull and Chen [19] indicate that holding the fruit in the temperature range of 20 to 23 °C provides the best appearance, palatability and decay control when ripening mangoes. Kader and Mitcham [20] indicate that holding the fruit between 15.5 to 18°C during ripening provides the most attractive skin color, however the flavor remains tart unless the fruit are held an additional 2-3 days at 21-24°C.

Therefore, the aim of the study was to investigate the effects of 1-MCP and storage temperature on biochemical characteristics of two locally grown mango variety namely Khirshapat.

2. Materials and Methods

The experiments were carried out using the equipment available in the Plant Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Bangladesh. Fresh mature green mango (Khirshapat variety) undertaken for investigation were collected directly from mango cultivar of Banesshar, Puthia Upazila, Rajshahi district. The samples were transported to the laboratory immediately after harvesting. Pathogen infected and mechanical damaged mangoes were discarded. Then the mangoes were washed with tap and lime water. Hot water (52-55 °C) treatment was given for 10 minutes and then dried at room temperature by applying air. 1-Methylcyclopropene (1-MCP) was used as ethylene inhibitor which was collected from China. The mangoes were randomly divided into three groups. Group-1 & 2 were treated with 1-MCP at concentrations of 1ppm & 2ppm for a period of 24 h in a sealed container. The fruits were removed, divided into two lots and one lot was stored at 12-13 °C and another lot at normal temperature (30-32 °C). Group-3 which was not treated with 1-MCP, keep as control fruits at room temperature (30-32 °C).

In order to prepared the crude extract for biochemical analysis three mangoes were taken out from each group of samples on 6 days of regular interval of time such as on 0th, 6th, 12th, 18th and 24th day and kept in -80°C for further analysis. Fruits were cut into small pieces and homogenized by adding equal volume of extraction buffer (100 mM Tris-HCl, pH 7.0 with 0.25 M NaCl and 4 mM PMSF). The homogenized fruit was kept overnight at 4°C. After passing through four layers of muslin cloth, the extract was centrifuged at 10,000 ×g for 15 min to separate the debris. Supernatant was collected as crude extract. The various biochemical parameters were determined according to the following protocols.

2.1. Fruit pH

30g mango pulp was taken in a 100ml beaker and then it was homogenized with distilled water in a blender. The blended materials were then filtered and used pH meter to measure the pH levels of solutions.

2.2. Total Soluble Solids (TSS)

TSS was determined according to the method as described by Mazumdar and Majumder [21] using digital bench refractometer (range 0-32%). An appropriate quantity of each sample was placed on the prism-plate of the refractometer and the reading appearing on the screen was directly recorded as total soluble solids (°Brix).

2.3. Titratable Acidity (TA) of Mango Pulp

TA was determined according to the method as described by Hortwitz [22]. 10 g of mango pulp was taken in a 100 mL beaker and then it was homogenized with distilled water in a blender. The blended materials were then filtered and transferred to a 100 mL volumetric flask, and the volume was made up to the mark with distilled water. 10 mL of pulp solution was taken in conical flask. Two to three drops of phenolphthalein indicator was added, and then the conical flask was shaken vigorously. It was then filtrated immediately with 0.1 N NaOH solutions from a burette till a permanent pink color appeared. The volume of NaOH solution required for titration was recorded. Percent titratable acidity was calculated by using the following formula:

$$\text{Titratable Acidity } (\%) = \frac{\text{Volume of } 0.12\text{N NaOH} \times \text{Factor}(0.0064)}{\text{Volume of sample used}} \times 100 \quad (1)$$

2.4. Vitamin C

Approximately 2 to 3 g of mango flesh was cut into small pieces and homogenized well with 20 mL of 3% metaphosphoric acid and filtered through double layers of muslin cloth. The filtrate was centrifuged at 3,000 ×g for 10 min and the clear supernatant was titrated with 2, 6-dichlorophenol indophenol solution [23]. The amount of vitamin C in the extract was determined by comparing

with the titration curve of standard vitamin C solution. Result was expressed in mg/100 g of fresh fruit.

2.5. Starch Content

The starch content of the mango flesh was determined by the anthrone method [24]. Two grams of mango was cut into small pieces and homogenized well with 20 mL water. It was then filtered through double layers of muslin cloth. Twice the volume of ethanol was added to the filtrate to precipitate the polysaccharide, mainly starch. After keeping overnight at 4°C, the precipitate was collected by centrifugation at 3,000 ×g for 15 min. The precipitate was heated to dryness and dissolved in 40 mL of 1 M HCl and then heated at 70°C for few minutes. It was then transferred to a volumetric flask and diluted to 100 mL with1 M HCl. Aliquot of 1 mL of the extract of each sample was pipetted out into test tubes and 4 mL of the anthrone reagent was added to the each test tube and mixed well. The tubes were placed in a boiling water bath for 10 min and cooled. A blank reagent was prepared by using 1 mL of water and 4 mL of anthrone reagent in a test tube and treated similarly. The absorbance of the blue-green solution was measured at 680 nm in a colorimeter. The amount of starch present in mango flesh was calculated from the standard curve of different concentrations of glucose and expressed as g/100 g of fresh fruits.

2.6. Determination of Total Sugar Content of Mango Pulp

Total sugar content of mango pulp was determined calorimetrically by the anthrone method [24]. 4 g of mango pulp was cut into small pieces and immediately plunged into boiling ethyl alcohol and was allowed to boil for 5 to 10 minutes (5 to 10 mL of alcohol was used per gram of pulp). The extract was cooled and crushed thoroughly in a mortar with pestle. Then the extract was filtered through two layers of muslin cloths and the ground tissue was reextracted for three minutes in hot 80% alcohol, using 2 to 3 mL of alcohol per gram of tissue. The second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through two layers of muslin cloth. Both of the extracts were filtered through Whatmann no. 41 filter paper. The volume of the extract was evaporated to about 25% (1/4) of the volume over a steam bath and cooled. This reduced volume of the extract was transferred to a 100 mL volumetric flask and it was made up to the mark with distilled water. Aliquot of 1 mL of pulp extract was pipetted into test tubes and 4 mL of the anthrone reagent was added to each of this solution and mixed well. Glass marbles were placed on top of each test tube to prevent loss of water through evaporation. Then the tubes were placed in a boiling water bath for 10 minutes and then cooled. A reagent blank was prepared by taking 1 mL of water and 4 mL of anthrone reagent in a tube and treated similarly. The absorbance of blue green solution was measured at 680 nm in a colorimeter.

A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mL of standard glucose solution in different test tubes containing 0.0, 10, 20, 40, 60, 80, and 100 µg of glucose, respectively, and the volume was made up to 1 mL with distilled water. Then 4 mL of anthrone reagent was added to each test tube and mixed well. All these solutions were treated similarly as described above. The absorbance was measured at 680 nm using the blank containing 1 mL of water and 4 mL of another reagent. The amount of total sugar present in the extract was calculated from the standard curve of glucose. Finally, the percentage of total sugar was determined by using the following formula:

$$\%\text{Total sugar}\left(\text{g}/100\text{ g of mango}\right) = \frac{\text{Quantity of sugar obtained}}{\text{Weight of sample used}} \times 100. \quad (2)$$

2.7. Amylase Activity Assay

Amylase activity was assayed according to the method described by Jayaraman [24]. One percent starch solution was used as substrate. The enzyme activity was measured by estimating the release of maltose calculated from the standard curve prepared with different concentrations of maltose. One unit of enzyme activity is defined as the amount of enzyme required to release 1 mg of maltose per minute at 37°C.

2.8. Invertase Activity Assay

Invertase activity was assayed by the modified method as described by Mahadevan and Sridhar [25]. Sucrose was used as substrate. The invertase activity was measured by estimating the release of glucose calculated from the standard curve prepared with different concentrations of glucose. One unit of enzyme activity is defined as the amount of enzyme required to release 1 mg of glucose per minute at 37 °C.

3. Results and Discussion

3.1. Fruit pH

Results showed (Table 1) that the pH of the mango variety increased during storage. In case of control and 1-MCP treatment at 30-32 °C a remarkable change in fruit pH was observed. On the other hand mild changing trend of pH was observed in 1-MCP treated mangoes at 12-13 °C. Between the concentrations of 1-MCP 2ppm shows the better result in case of control the changes of pH. Khirshapat shows higher production of pH during the storage period. Increase in pH would be caused by the breakup of acids with respiration during storage [26].

3.2. Total Soluble Solid (TSS)

The total soluble solids of Khirshapat variety increased gradually with increasing the storage duration at normal temperature both in control and treated with 1-MCP but did not increased in case of mangoes treated by 1-MCP under storage temperature (12-13°C). Table 2 shows that TSS content of Khirshapat variety treated by 1-MCP (2ppm) under storage temperature slightly increased 8.52

to 10.46 Brix during the 24 days of storage. On the other hand mangoes treated by 1-MCP (both 1 &2 ppm) under normal temperature the TSS content increased two times. In case of control the TSS content increased three times from mature stage to ripening stage after 18 days of storage for khirshapat variety. This probably due to the conversion of starch into soluble sugars, such as sucrose, fructose and glucose. The increase in TSS during fruit ripening is attributed to the increased activity of enzymes responsible for the hydrolysis of starch to soluble sugars [12].

3.3. Titratable Acidity (TA)

The changes in TA are significantly affected by the rate of metabolism especially respiration, which consumed organic acid and thus declined acidity during the storage (C. J. Clark et al, 2003) [27]. Table 3 shows the changes in TA of khirshapat variety with the increase of storage time in different temperatures. For Khirshapat variety TA decreased slightly from 2.58 to 1.76 (%) in case of mangoes treated by 1-MCP (2ppm) under storage temperature on day 24. On the other hand TA decreased significantly on day 24 for the mangoes treated by 1-MCP under normal temperature and in case of control TA decreased from 2.58 to 0.18 (%)on day 18 which is very remarkable. These results revealed that time and temperature are responsible for physicochemical changes of fruits and the major changes occur when fruits are stored for long time at high temperature. Low storage temperature (12°C to 13°C) and 1-MCP treatment probably inhibited the activities of the enzymes to change the TSS and TA contents. Between the concentrations of 1-MCP 2ppm is more effective than 1ppm.

3.4. Vitamin-C Content

Table 4 shows the changes of ascorbic acid of Khirshapat variety under different storage condition. There was a decline in ascorbic acid in all cases during the storage period. Vitamin-C content of Khirshapat decreased slightly 30.94 to 22.76 mg/100g fresh weight on days 24 for 1-MCP treated mangoes (concentration 2ppm) under storage temperature. On the other hand, vitamin-C content decreased sharply for 1-MCP treated mangoes under normal temperature and in case of control it decreased drastically from 30.94 to 7.28 mg/100g fresh weight on days 18. Findings clearly indicated that vitamin C content decreased with increasing days of storage during the ripening (Table 4) at ambient temperature. The reduction in vitamin C content of the fruit during ripening may be due to the susceptibility of ascorbic acid to oxidative destruction particularly at high ambient storage temperature [28].

Table 1. Effect of 1-MCP Treatment on pH of Khirshapat Variety Stored at 12-13 °C and 30-32 °C

		Storage time				
Mango variety	Concentrations of 1-MCP & Temperature	0 days	6 days	12 days	18 days	24 days
Khirshapat	Control (30-32 °C)	3.25±0.12^{a}	5.37±0.05^{a}	6.08±0.07^{a}	6.57±0.04^{a}	
	1ppm & (30-32 °C)	3.25±0.12^{a}	4.90±0.07^{b}	5.27±0.05^{b}	5.78±0.09^{b}	6.75±0.09^{b}
	2ppm & (30-32 °C)	3.25±0.12^{a}	4.39±0.17^{c}	4.90±0.17^{c}	5.29±0.22^{c}	6.13±0.22^{c}
	1 ppm & (12-13 °C)	3.25±0.12^{a}	3.94±0.07^{b}	4.21±0.05^{b}	4.84±0.09^{b}	5.54±0.09^{b}
	2 ppm & (12-13 °C)	3.25±0.12^{a}	3.37±0.17^{c}	3.89±0.05^{b}	4.15±0.09^{b}	4.46±0.09^{b}

In a column values having the same letter (s) do not differ significantly as per DMRT at 5% level

Table 2. Effect of 1-MCP Treatment on TSS of Khirshapat Variety Stored at 12-13 °C and 30-32 °C

		Storage time				
Mango variety	Concentrations of 1-MCP & Temperature	0 days	6 days	12 days	18 days	24 days
Khirshapat	Control (30-32 °C)	8.52±0.10^{a}	15.37±0.24^{a}	19.8±0.17^{a}	24.7±0.38^{a}	
	1ppm & (30-32 °C)	8.52±0.10^{a}	13.90±0.17^{b}	17.27±0.28^{b}	15.78±0.37^{b}	18.5±0.73^{b}
	2ppm & (30-32 °C)	8.52±0.10^{a}	12.3±0.15^{c}	15.85±0.16^{c}	14.49±0.33^{c}	17.73±0.40^{c}
	1 ppm & (12-13 °C)	8.52±0.10^{a}	10.94±0.26^{b}	11.21±0.19^{b}	12.24±0.38^{b}	14.64±0.33^{b}
	2 ppm & (12-13 °C)	8.52±0.10^{a}	8.97±0.17^{c}	9.34±0.15^{b}	10.15±0.19^{b}	10.46±0.27^{b}

In a column values having the same letter (s) do not differ significantly as per DMRT at 5% level

Table 3. Effect of 1-MCP Treatment on TA of Khirshapat Variety Stored at 12-13 °C and 30-32 °C

		Storage time				
Mango variety	Concentrations of 1-MCP & Temperature	0 days	6 days	12 days	18 days	24 days
Khirshapat	Control (30-32 °C)	2.58±0.06^{a}	0.76±0.04^{a}	0.32±0.07^{a}	0.18±0.08^{a}	
	1ppm & (30-32 °C)	2.58±0.06^{a}	0.98±0.07^{b}	0.62±0.08^{b}	0.48±0.07^{b}	0.25±0.07^{b}
	2ppm & (30-32 °C)	2.58±0.06^{a}	1.13±0.15^{c}	0.85±0.16^{c}	0.69±0.20^{c}	0.43±0.20^{c}
	1 ppm & (12-13 °C)	2.58±0.06^{a}	1.94±0.06^{b}	1.27±0.06^{b}	1.04±0.08^{b}	0.94±0.08^{b}
	2 ppm & (12-13 °C)	2.58±0.06^{a}	2.27±0.17^{c}	2.09±0.05^{b}	1.85±0.09^{b}	1.76±0.09^{b}

In a column values having the same letter (s) do not differ significantly as per DMRT at 5% level

Table 4. Effect of 1-MCP Treatment on Ascorbic Acid (mg/100ml) of Two Mango Varieties at Normal (30-32 °C) and Storage Temperature (12-13 °C)

		Storage time				
Mango variety	Concentrations of 1-MCP & Temperature	0 days	6 days	12 days	18 days	24 days
Khirshapat	Control (30-32 °C)	30.94±0.36^{a}	18.22±1.68^{a}	9.61±1.53^{a}	7.28±0.18^{a}	
	1ppm & (30-32 °C)	30.94±0.36^{a}	20.98±0.57^{b}	12.32±0.48^{b}	10.78±0.27^{b}	7.55±0.22^{b}
	2ppm & (30-32 °C)	30.94±0.36^{a}	21.13±0.45^{c}	13.85±0.36^{c}	11.69±0.20^{c}	8.43±0.23^{c}
	1 ppm & (12-13 °C)	30.94±0.36^{a}	24.64±0.56^{b}	19.27±0.26^{b}	14.04±0.18^{b}	12.94±0.08^{b}
	2 ppm & (12-13 °C)	30.94±0.36^{a}	28.72±0.57^{c}	26.09±0.25^{b}	24.85±0.29^{b}	22.76±0.19^{b}

In a column values having the same letter (s) do not differ significantly as per DMRT at 5% level

3.5. Starch Content

The starch is the main carbohydrate present in mango fruits and the starch in mango pulp is hydrolyzed during ripening by amylase [29]. The starch content of mango pulp of Khirshapat variety was found to decrease significantly from 15.8% to 3.64% with the increasing storage period at 30 to 32 °C till 18th day of storage and 15.8% to 3.26% & 3.98% in case of khirshapat variety treated by 1-MCP at the concentration of 1 & 2ppm under normal temperature (Figure 1). On the other hand, the starch content decreased slightly in case of mangoes treated with 1-MCP at the concentration of 1ppm but starch content remained unchanged even after 24 days of storage at 12 to 13°C and treated with 1-MCP at the concentration of 2ppm. This was due to inactivation of mango amylase in lower temperature [30].

3.6. Total Sugar Content

From Figure 2 it is clear that the total sugar content of mango pulp gathered in a continuous stream with expanding of storage duration. This gathering trend was more or less hastily from initial to 18th days in both control and 1-MCP treated mangoes (1 & 2ppm) under normal temperature. During 18 days duration the control provided 4 times more total sugar content (6.27 to 25.06 %) for Khirshapat variety, whereas the 1-MCP treated mangoes (1 & 2ppm) under normal temperature provided 3 times more total sugar content. Upadhyay et al. [31] reported that total sugar content was expanded gradually, when stored for 6 days at room temperature. Sugar content increased during ripening. These results are in conformity with the findings of Shahjahan et al. [32]. The increase in TSC might be possible due to conversion of complex starch or carbohydrate into simple compound. On the other hand mangoes treated with 1-MCP at the concentration of 2ppm and stored under storage temperature (12-13°C) exhibited nonsignificant variation of total sugar content at different days after storage. The Figure 2 shows that the total sugar content increased slowly up to 24 days of storage 6.27 to 9.08% for Khirshapat variety. This is due to the effect of 1-MCP and storage temperature (12-13°C).

3.7. Amylase Activity

Amylase is an enzyme that hydrolyses starch to yield monosaccharides. Figure 3 shows that at initial stage the activity of amylase was found to be 1.23unit/mL of crude extract of Khirshapat. The activity of the enzyme remained almost constant from 0th day to 24th day of storage at (12-13 °C) for 1-MCP treated mangoes at the concentration of 1 & 2ppm. This result showed good correlation with the changes of starch content during storage at 12 -13°C (Figure 1). In case of control and 1-MCP treated mangoes under normal temperature the amylase activity was found to be increased at the middle stage of ripening on day 12th and after that decreased abruptly in case of control but other case the decreasing rate comparatively slow (Figure 3). Rahman and his associates also found similar trends of amylase activity in Fazli and Khirshapat mangoes [33].

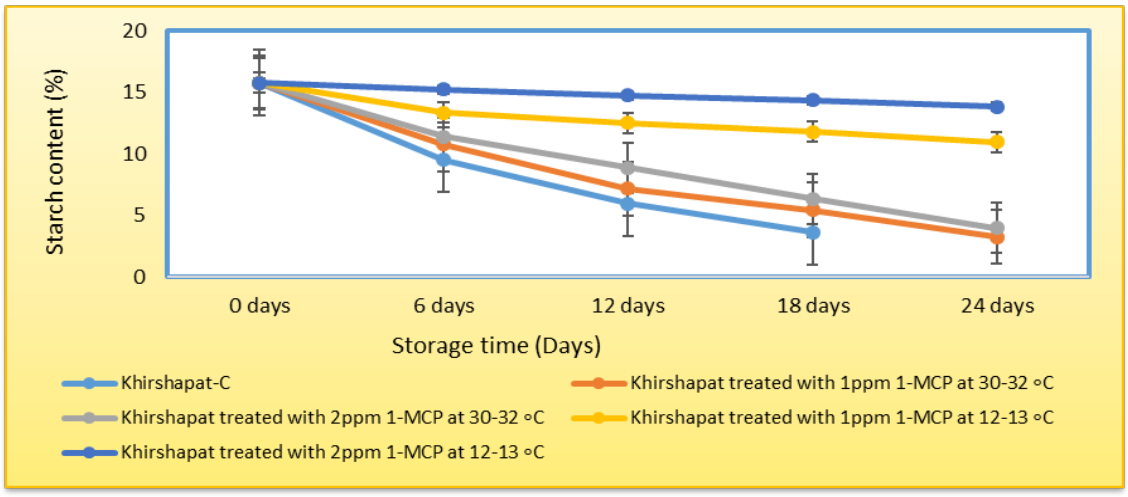

Figure 1. Changes in starch content in Khirshapat variety treated with 1-MCP at 12-13 °C and normal temperature 30-32 °C. Vertical bars represent ±SE

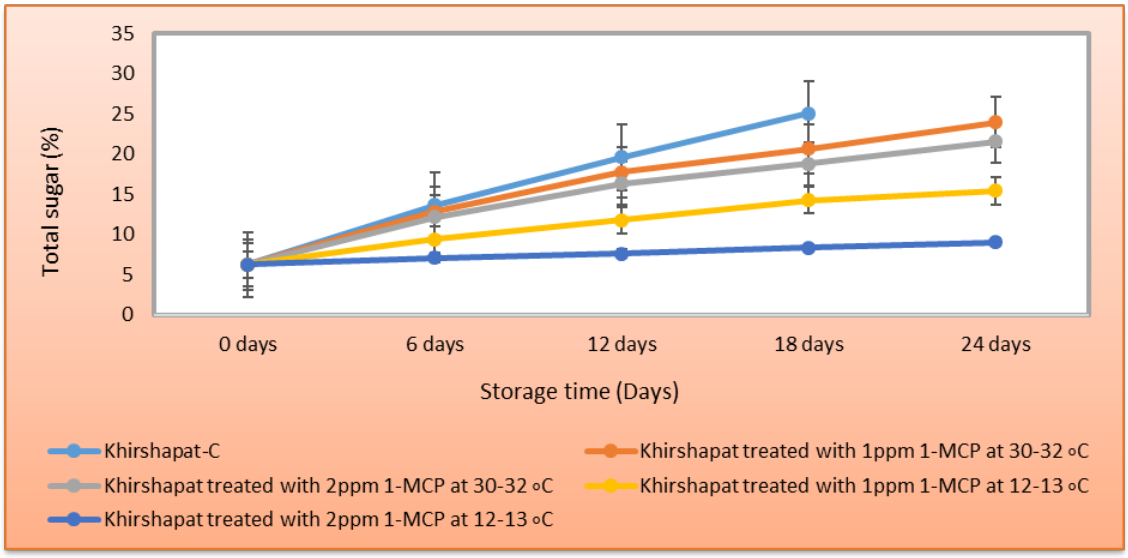

Figure 2. Changes in total sugar content in Khirshapat variety treated with 1-MCP at 12-13 °C and normal temperature 30-32 °C. Vertical bars represent ±SE

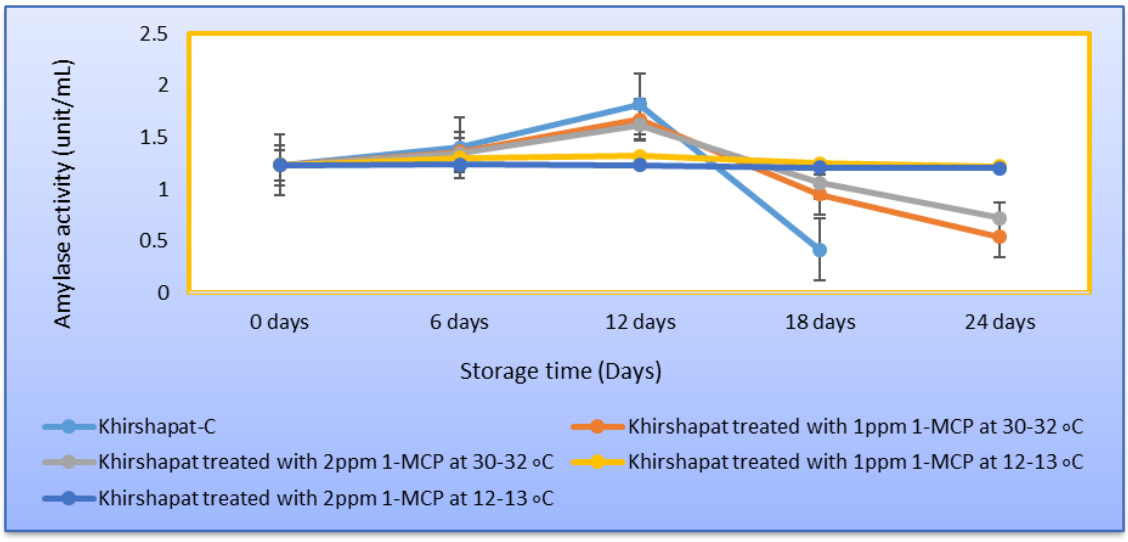

Figure 3. Changes in amylase activity in Khirshapat variety treated with 1-MCP at 12-13°C and normal temperature 30-32°C. Vertical bars represent ±SE

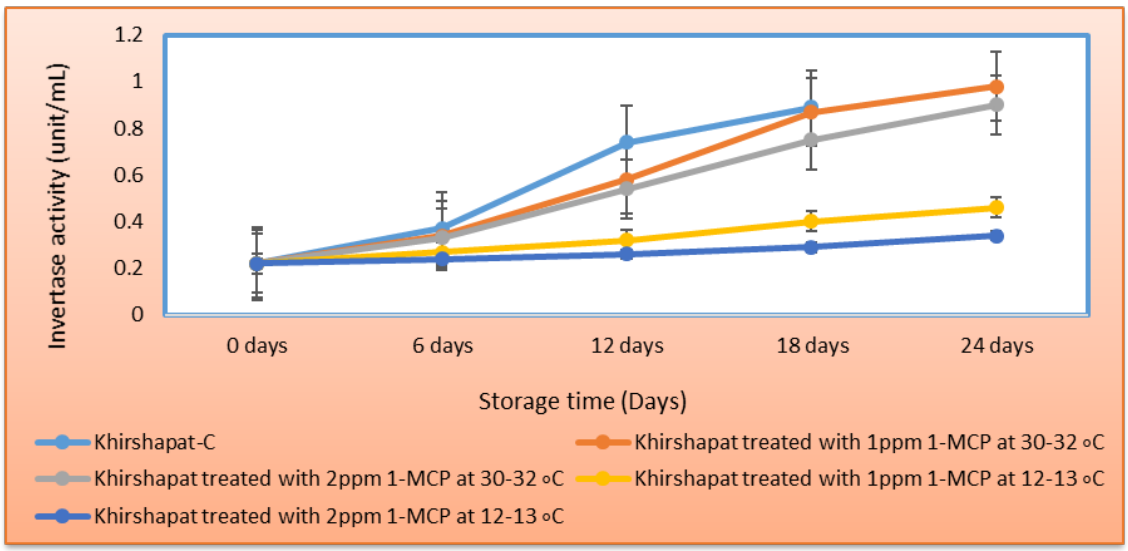

Figure 4. Changes in invertase activity in Khirshapat variety treated with 1-MCP at 12-13°C and normal temperature 30-32°C. Vertical bars represent ±SE

3.8. Invertase Activity

Sweetness of mango increases during ripening because sucrose hydrolyzes and converted to glucose and fructose due to the activity of invertase enzyme. Figure 4 shows that the invertase activity of mangoes treated with 1-MCP at the concentration of 2ppm under storage temperature (12-13℃) remained almost constant but at the concentration of 1ppm invertase activity increased slightly during 24 days of storage and reached at 0.46 unit/mL for Khirshapat variety. On the other hand the invertase activity of mangoes treated under normal temperature shows increasing trend up to 24 days of storage, meanwhile in case of control fruits invertase activity increased very sharply up to 12th day of storage but after that the increasing rate of invertase activity decreased because of the decay of the fruits.

4. Conclusion

In conclusion, the research indicates that the mango variety Khirshapat response to 1-MCP was better at lower storage temperature (12-13°C) than at higher temperature (30-32°C). In case of concentration, the 2ppm of 1-MCP treatment at (12-13℃) shows comparatively better results than the others and the fruits were firmer, greener and a prolonged storage life up to 24 days. The effects of 1-MCP on mango fruit shelf life was dependent on storage temperature. There was no remarkable shelf-life extension in 1-MCP-treated fruits stored at (30-32°C), as both treated and untreated fruits ripened with optimum eating qualities on the same day during the storage period.

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