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# Changes in organoleptic and biochemical characteristics of mango fruits treated with calcium chloride in hot water

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#### ABSTRACT

Purpose: In Bangladesh, mango fruit supply is limited in the local market as well as for export due to its short self-life and susceptibility to post-harvest diseases. This study aimed to evaluate the effects of CaCl<sub>2</sub> in hot water on organoleptic and biochemical characteristics of mango fruits for extension of shelf-life. Research Method: Mangoes were treated with different concentrations (0.0, 0.5, 1, 2, 3, 4 & 5 %) of CaCl<sub>2</sub> in hot water (50°C) for 10 min and kept at 25±2°C over 12 days. Each treatment included 20 mangoes with three replications. The physiological changes were observed and biochemical characteristics of mango fruits were analyzed. Findings: Better skin color and aroma were observed at 0.5~4.0% CaCl<sub>2</sub> and no fungal infection was found at  $3^{\sim}5\%$  CaCl<sub>2</sub> as compared with untreated control whereas taste and texture of mangoes increased significantly with the increasing concentration of CaCl<sub>2</sub>. The shelf life of treated mangoes increased 2~3 days with increasing concentration of 4~5% CaCl<sub>2</sub> but slight skin shriveling and weight loss were observed. Higher concentration of CaCl<sub>2</sub> treated fruits maintained higher values of moisture, ash, titratable acidity, vitamin-C, reducing sugar, starch, invertase activity whereas total soluble solid, total sugar, non-reducing sugar, total phenol, amylase, polyphenoloxidase activity decreased significantly. Limitations: In future, mechanism of CaCl<sub>2</sub> in hot water for extending shelf life of mangoes will be elucidated using molecular approach. **Originality/Value:** The treatment of 4% CaCl<sub>2</sub> in hot water could be used to extend the shelf life of mangoes up to 2~3 days with consumer acceptance.



# **INTRODUCTION**

Mango (Mangifera indica, L.) fruit is popular as an item of international trade due to its widespread utility, taste, unique flavor, pleasant aroma, and high nutritional contents (Hossain, 2016). Bangladeshi mango is sold at good prices in European and Middle East countries due to its unique quality. Mango fruit supply is limited in the local market as well as for export due to its highly perishable and climacteric nature; and susceptibility to postharvest diseases. The fresh mango may need 3-6 days to ripen at ambient temperature (25 °C) (Gill et al., 2017) and short shelf-life is the main reason for the limited export in the foreign market (Mahmud et al., 2015). So far there is no well-established system for long-term storage of climacteric fruits under controlled or modified atmosphere. Sometimes fruits stored in modified atmosphere exhibit unexpected characteristics such as black color, tastelessness, and bad flavors. Various chemicals are used to slow down the fruit ripening process to extend the short shelf-life of mango fruits. Calcium salts such as chloride and nitrates were reported to delay the fruit ripening process by slowing the respiration rate (Mahmud et al., 2015; Xu et al., 2019). Various dosages of calcium chloride were sprayed on the pre-harvest and postharvest mango while some other researchers dipped the fruits in calcium solution for varying times (Kazemi et al., 2013; Sharma et al., 2013) to delay the fruit ripening process. In another experiment 2, 4, 6, and 8% calcium chloride solution were used to treat fresh mango under a positive pressure at 115 kPa for 2 min and stored at 20 °C in boxes covering polyethylene. Researchers showed that CaCl<sub>2</sub> delayed fruit ripening under pressure and vacuum infiltration by approximately 12 and 8 days respectively, compared with control fruits. Different concentrations of CaCl<sub>2</sub> made the differences for delaying ripening (Anjum & Ali, 2004; Yuen et al., 1993). Ripening was delayed by CaCl<sub>2</sub> treatment in Tommy Atkins mango but not in Manila fruits (Corrales-Garcia & Lakshminarayana, 1991). Hot water treatment was the most effective for retarding the ripening process and spoilage of mango fruits of both Fazli and Ashwina cultivars up to 5-8 days of storage (Gofure et al., 1997). Moreover, it has been reported that combined treatment of hot water and CaCl<sub>2</sub> could be effective for delaying the onset of anthracnose symptoms for 5 days and improved the papaya postharvest quality (Ayon-Reyna et al., 2017). However, the underlying mechanism of delayed-ripening by CaCl<sub>2</sub> in hot water treatment is still unclear. Moreover, information about the effects of CaCl<sub>2</sub> in hot water on the organoleptic and biochemical characteristics of mango fruit is very limited. Therefore, the objective of this study was to observe the changes of organoleptic and biochemical properties of mango fruits under the treatment of calcium chloride in hot water to increase the shelf-life.

### **MATERIALS AND METHODS**

The experiments were carried out using the equipments available in the Plant Molecular Biotechnology Laboratory, Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh. All chemical reagents used for conducting the experiments were obtained from suppliers of Carl-Roth GmbH and Sigma-Aldrich, Germany.

# **Collection and treatment of mango fruits**

Fresh mature green mango fruits (Variety: Ashwina hybrid) were collected in the morning from Binodpur Bazar at Rajshahi, Bangladesh. Samples were sorted at the same maturity level based on the shape, size and length, and the diseased and/or mechanical damaged mangoes were discarded. The mango sap and mesh were cleaned with cold tap water and air-dried at  $25\pm2$  °C with ceiling fan for 1 hr. The concentration of CaCl<sub>2</sub> for the treatment of T0, T1, T2,



T3, T4, T5 & T6 were 0.0, 0.5, 1, 2, 3, 4, & 5%, respectively. Twenty fruits were put in the separate plastic net bag for each treatment and were dipped in hot water (50 °C) containing above concentrations of CaCl<sub>2</sub> in each case for 10 min. A control was also included in which fruits were dipped in fresh water for 10 min. The treated and control mangoes were further air-dried under the ceiling fan. The fruits were kept at ambient temperature ( $25\pm2$  °C) for ripening over 12 days in boxes lined and covered with newspapers. The experiments were conducted with completely randomized design with three replications per treatment. Total number of mangoes used for this study was 420 [Number of mangoes for each treatment (20) × Number treatment (7) × Number of replication (3)]. However, there was a limitation that we did not collect the mango fruits directly from trees.

# Sensory evaluation and scoring

Sensory evaluation of the fruits such as skin color, shriveling, aroma, taste and texture was carried out according to the modified Hedonic scale method (Peryam & Pilgrim, 1957). Three judges were employed in the panel and requested to give the score as numbers (0, 1, 2, 3, 4) scale depending on their visual inspection on the 0, 6 and 12 days after treatment (Scoring scales are shown in the Table 1). Three mangoes were taken from each treatment for the sensory evaluation with three replications (n= 3 fruits × 3 replications = 9). Panelists put their consensus average scores (4 being the most acceptable and zero the least) of sensory evaluation of the sample mangoes for skin color, aroma, texture, taste and fungal infections according to the scale provided (Table 1) after their group discussion.

# **Biochemical analysis**

# Crude extract preparation and sampling

Sample mango fruits flesh/pulp (n= 3 replicates) were cut into small pieces and blended separately by the addition of equal volume of extraction buffer (100 mM Tris-HCl, pH 7.0 with 0.25 M NaCl and 4 mM PMSF). Each blended fruit flesh/pulp was stored over night at 4 °C. Four layers of muslin cloth were used to filter the extract that was centrifuged at  $10,000 \times$  g for 15 min to precipitate the debris. Clear supernatant fluid was collected as crude extract, which was used to determine the biochemical parameters as described below.

# Determination of pH, moisture and ash contents

The pH, moisture and ash contents of mango samples (n=3 replicates) were determined according to the methods as in AOAC (2002).

Skin color	Aroma	Taste	Texture	Fungal infection (%)
Rotten =0,	Rotten /bad= 0,	Rotten =0,	Rotten =0,	Rotten=0,
Green $= 1$ ,	No aroma=1,	Sour $= 1$ ,	Very soft $= 1$ ,	Severely infected= 1,
Slight yellow $= 2$ ,	Fair =2,	Slightly sweet $= 2$ ,	Soft = 2,	Moderately=2,
Yellow $= 3$ ,	Good $=$ 3,	Moderately sweet $=$ 3,	Little hard $= 3$ ,	Slightly $=$ 3,
Deep yellow $= 4$	Very good $= 4$	Very sweet $= 4$	Hard = 4	No infection= 4

Table 1. Scoring scale for organoleptic and fungal infection evaluation



### Total soluble solids and titratable acidity

A Digital-Bench-Refractometer (Range 0-32%) was used to estimate the total soluble solid (TSS) of mango samples (n=3 replicates) according to the method as described by Mazumdar and Majumder (2003). One drop of each sample (Crude extract) was placed on the prism-plate of the refractometer and the reading was directly recorded as total soluble solids (°Brix). Titratable acidity (TA) was measured according to the method as described by Hortwitz (1960). Ten milliliters of crude extract was titrated with 0.1N NaOH using 2-3 drops of phenolphthalein as an indicator. The percentage (%) of TA was determined by the following formula (1):

Titratable Acidity (%) =  $\frac{\text{Volume of } 0.1\text{N } \text{NaOH} \times \text{Factor } (0.0064)}{\text{volume of sample used}} \times 100$  (1)

### Vitamin C

Approximately 2~3 g of mango flesh (n=3 replicates) was homogenized well with 20 ml of 3% metephosphoric acid and filtered through double layers of muslin cloth. The filtrate was centrifuged at  $3,000 \times g$  for 10 min and the clear supernatant was to titrate with 2, 6-dichlorophenolindophenol solution as described in AOAC (2002). The vitamin C content in the sample was calculated as mg/100 g of fresh fruit by comparing with the titration curve of standard vitamin C solution.

#### Starch content

The amount of starch in mango flesh was determined according to the Anthrone method (Jayaraman, 1981). Two grams of mango sample (n= 3 replicates) was homogenized well with 20 ml distilled water to juice, which was then filtered through double layers of muslin cloth. The polysaccharide, mainly starch was precipitate from filtrate using twice volume of concentrated enthanol. Then solution was kept overnight at 4 °C and centrifuged at 3,000 × g for 15 min. The precipitate was dried and dissolved in 40 ml of 1M HCl and then reheated at 70 °C for 5 min. Then solution was transferred to a volumetric flask and diluted to 100 ml with 1M HCl. Aliquot 1 ml of the extract of each sample was taken into test tubes and 4 ml of the anthrone reagent was added to the each test tube and mixed well. The tubes were heated on a boiling water bath for 10 min and immediately cooled. A reagent blank was included by adding 1 ml of distilled water and 4 ml of anthrone reagent in a test tube and treated similarly. A colorimeter was used to measure the optical density of the blue-green solution at 680 nm. The starch content present in mango flesh was estimated from the standard curve using the different concentrations of glucose which was expressed as g/100 g of fresh fruits.

#### Total sugar, reducing sugar and non-reducing sugar

The amount of total Sugar (TS), reducing Sugar (RS) and Non-reducing Sugar (NRS) were determined according to methods as described by Hossain et al. (2014). Shorty, two grams of mango flesh (n= 3 replicates) was sliced into small pieces and dipped into 20 ml of boiling ethanol for 5-10 min. Then it was grounded in a mortar with a pestle. The homogenate was filtered through a Whatman no-41 filter paper. Then a steam bath was used to evaporate the extract and wait for 5 min to cool it. One hundred milliliters of distilled water was added to the residues and dissolved in and the resulting solution was used as sample stock for the determination of TS and RS.

*TS:* The amount of TS present in mango flesh was estimated following the Anthrone method (Jayaraman, 1981). Aliquot 1 ml of the sample stock was taken into three test tubes and 4 ml of the anthrone reagent was mixed to each tube, which was boiled on water bath for 10 min. When cooled, the absorbance was taken at 680 nm against a reagent blank. TS



content in the sample tubes were calculated using different concentrations of glucose and expressed as g/100 g of mango flesh.

*RS*: RS in the mango samples was determined by dinitrosalicylic acid (DNS) method (Miller, 1959). Three milliliters of the sample stock were taken into each of three test tubes and 3 ml of DNS reagent was mixed to each of this solution. The test tubes were heated in a boiling water bath until color development and then immediately 1 ml of 40% Rochelle salt was added. The test resulting solutions were then placed under tap water for cooling. An absorbance of the solution was measured at 575 nm in a colorimeter against a blank. RS content present in each test tube was calculated from the standard curve of glucose and expressed as g/100 g of mango flesh.

*NRS*: The formula (2) as described by Rahman et al. (2011) was used to calculate the NRS present in mango.

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Non – reducing sugar (%) = Total Sugar (%) – Reducing sugar (%) × Factor (0.95) (2)
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# Total phenol

Total Phenol content in mango flesh (n=3 replicates) was determined by the Folin-ciocalteau reagent method (Bray & Thorpe, 1954). Aliquot 1 ml of crude extract was taken into three separate test tubes and 1 ml of Folin-ciocalteau reagent was added to each test tube and mixed well. Then 2 ml of 20% Na<sub>2</sub>CO<sub>3</sub> solution was added to each tube and mixed thoroughly, and then kept on boiling water for 2 min. After cooling at room temperature (25°C), the optical density was measured at 650 nm against a blank. Different concentrations of catechol were used to prepare a standard curve and calculated the total phenol content and expressed as mg/100 g of mango flesh.

# Estimation of amylase activity

The amylase activity (MA) of the samples (n=3 replicates) was measured according to the protocol developed by Jayaraman (1981). Here MA was measured by estimating the release of maltose from substrate (1% starch solution) hydrolyzed by amylase. Amount of maltose was calculated from the standard curve prepared with different concentrations of maltose. Enzyme activity is expressed as mg/min/ml, which was defined as amount of enzyme needed to release 1 mg of maltose per minute at 37 °C.

# Estimation of invertase activity

The invertase activity (IA) of the samples (n=3 replicates) was measured according to the modified method of Mahadevan and Sridhar (1982) where substrate was sucrose. IA was measured by estimating the release of glucose from sucrose. Different concentrations of glucose were used to prepare the standard glucose curve. Enzyme activity is expressed as mg/min/ml, which defined as the amount of enzyme needed to release 1 mg of glucose per minute at 37 °C.

# Estimation of polyphenol oxidase activity

The polyphenol oxidase (PPO) activity in mango flesh (n=3 replicates) was estimated according to the protocol as followed by Mahadevan and Sridhar (1982). In this protocol, substrate was catechol. Enzyme activity was expressed as unit where a change in absorbance of 0.001 per min per ml of crude extract was considered as one unit of enzyme. PPO activity was measured by a spectrophotometer as an initial rate of increase in optical density at 495 nm. First aliquot 2 ml of the crude extract was taken in a cuvette and 3 ml of 0.1 M phosphate buffer (pH 6.0) was added and mixed by inverting the cuvette and finally placed it in the



spectrophotometer previously set at 495 nm. Then the absorbance was adjusted to zero. One milliliter of catechol was added to the cuvette and immediately mixed by inversion. The changes in optical density at 495 nm were determined in every 3 min after placing cuvette in the spectrophotometer.

### Data analysis

The data collected was subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS). A p-value was calculated at 95 % confidence intervals around the differences between the treatments.

# **RESULTS AND DISCUSSION**

# Organoleptic observation of treated mangoes stored at 25±2 °C over 12 days

Skin color of any fruit is an important quality component in marketing that determines the level of attractiveness of the commodity to customers. At the 6th day after treatment, control mangoes showed better skin color than CaCl<sub>2</sub> in hot water treated mangoes. On the other hand, the skin color significantly increased with increasing conc.  $CaCl_2(1\sim4\%)$  in hot water at the 12th day. However, 5% CaCl<sub>2</sub> developed less color compared to other conc. (Table 2). Light yellow color development of mango was due to the reduced rate of respiration of the treated fruits by CaCl<sub>2</sub> in hot water (Anjum & Ali, 2004). It was also reported that less orange color development in Tommy Atkinson and Kent mango cubes with higher CaCl<sub>2</sub> concentration and longer dip times, indicating the ability of CaCl<sub>2</sub> to retard ripening and maintain tissue integrity (Ngamchuachit et al., 2014). At higher concentration of CaCl<sub>2</sub> (3~5%) in hot water, the fruits were not infected with fungi over 12 days of storage but at lower concentration of CaCl<sub>2</sub> (0.5%~2) in hot water and untreated control fruits were infected rapidly with fungi (Table 3). This result is good agreement with results obtained by other research group where CaCl<sub>2</sub>-hot water treatment of infected papaya delayed the onset of the anthracnose symptoms for 5 days and improved the papaya postharvest quality (Ayon-Reyna et al., 2017).

Aroma is the perception of smell and taste of fruits or foods when we perceive it by our sensory organ like tongue and nose. Regarding the effect of calcium salts in hot water on mangoes, aroma significantly decreased with the increasing conc. of CaCl<sub>2</sub> at the 6th day and fruits treated with 0.5% CaCl<sub>2</sub> had the maximum aroma followed by the control mangoes. On the other hand, at the 12th day after treatment, aroma increased significantly with the increasing conc. of CaCl<sub>2</sub> (4%). Five percent CaCl<sub>2</sub> treated mango showed higher aroma than the untreated control (Table 2). It was reported that the lower conc. of calcium chloride slightly increased the aroma of the fruits whereas the higher concentration (4~5% CaCl<sub>2</sub>) reduced the aroma of treated fruits and eating quality (Anjum & Ali, 2004). It was also reported that sugars and acids enhance human perception of specific flavor notes in mango, including aromatics (Malundo et al., 2001). The results suggest that calcium chloride in hot water affects changes the aroma of the fruits.

Taste is perceived by specialized taste buds on the tongue in the mouth. There are four different tastes such as sweet, sour, bitter and salty. Predominate tastes of fruits are sour and sweet. Sweetness depends on presence of sugars and sourness comes from organic acids in the fruits (Kays, 1991). At the 6th day of storage, maximum taste score was obtained in untreated control but score decreased significantly with increasing conc. of CaCl<sub>2</sub> (1~5%). On the other hand, the taste score increased at maximum 4.0 over 12 days of storage at higher concentration of 4% CaCl<sub>2</sub> in hot water as compared to untreated control with lower score

1.4 $\pm$ 0.4 (Table 2). Therefore, it could be speculated that CaCl<sub>2</sub> in hot water decreased the release of sugars by inactivating the carbohydrate hydrolyzing enzymes.

Texture is one of the important parameters that plays a crucial role when consumer selects the fruits. Pectins are structural polysaccharides present the cell walls of cell and responsible for the firmness of fruits and it becomes loosen during ripening or softening due to hydrolysis by pectinase. Therefore, firmness could be considered as index for maturity for harvest (Kudachikar et al., 2001). It has been shown that the mechanism by which calcium reduces the fruit ripening is related to formation of calcium pectate, which stabilizes the cell wall (Sharma et al., 2013). At the 6th day after treatment, CaCl<sub>2</sub>-hot water treatment retained the maximum texture or firmness score of treated mango at 4% CaCl<sub>2</sub> whereas untreated control fruits lost it firmness. The best texture score >3 was obtained over 12 days of storage at ambient 25±2 °C when mango was treated with 4% CaCl<sub>2</sub> in hot water as compared with untreated control (Table 2). Maintenance of fruit firmness was associated with higher concentration of calcium content in peel and mesocarp, and vice-versa. Calcium salts are better for maintaining the cell walls strength (Ernesto et al., 2017). It was reported that fresh cut Kent mangos treated with CaCl<sub>2</sub> retarded softening during storage and the retardation was greater at higher calcium concentrations (2.26%). It can be speculated that Ca<sup>2+</sup> introduced into the mango and retained the firmness of the fresh cut (Ngamchuachit et al., 2014). Combined CaCl<sub>2</sub> and heat treatment of the fruit increased the cell permeability and penetration of the calcium in papaya (Madani et al., 2016) and fresh cut Galia melon (Silveira et al., 2011).

Weight loss was attributed to physiological loss in weight (PLW) due to the respiration, transpiration of water through the peel and biochemical processes taking place inside fruit during ripening. Weight loss was lower at CaCl<sub>2</sub> in hot water treated fruit (14~17%) as compared to control with higher percent of weight loss (20%) (Table 3). It was also observed that percentage of weight loss of fruit decreased with the increasing the concentration of CaCl<sub>2</sub>. There was a gradual increase in the cumulative weight loss in 'Keitt' mango fruit after the 4<sup>th</sup> day of storage and continued with rapid increase in weight until 21 days after storage (Kumah et al., 2011). 'Keitt' mango fruits treated in hot water for 52 °C for 5 min, 50 °C for 5 and 10 min, and 48 °C for 10 min showed rapid increase in fruit weight loss in comparison to control fruit (Kumah et al., 2011).

Treatments of 0.5% CaCl<sub>2</sub> in hot water, there was no significant enhancement of the shelf life of the mango. On the other hand, shelf life of mango was extended significantly up to 2-3 days when treated with  $1\sim4\%$  CaCl<sub>2</sub> in hot water (Table 3). Several studies have been conducted on the uses of CaCl<sub>2</sub> treatment for the extension of shelf life of mango fruits up to several days (Ngamchuachit et al., 2014; Singh et al., 2017; Tirmazi et al., 1981). In other studies, 4% to 6% CaCl<sub>2</sub> treatment increased the shelf life of Kensington Pride' and 'Willard' mangoes by 5 to 7 days, respectively (Mootoo, 1991) but observed skin injury when treated with 8% calcium chloride solutions. Ernesto et al. (2017) also reported the extension of shelf life of Keitt mango and Cavendish banana Fruits using hot water (55 °C) and 3% CaCl<sub>2</sub> treatment. Calcium treated fruits were better in respect to most of the attributes over control which might be due to the Ca<sup>2+</sup> ions alter intracellular and intercellular biological activity, resulting in retard ripening process (Singh et al., 2017).



**Table 2.** Changes in organoleptic characteristics of mangoes stored at  $25\pm2$  °C over 12 days after treatments with CaCl<sub>2</sub> in hot H<sub>2</sub>O

CaCl <sub>2</sub> treatment	Skin color		Aroma		Taste			Texture				
(Conc.)	0 DAT	6 DAT	12 DAT	0 DAT	6 DAT	12 DAT	0 DAT	6 DAT	12 DAT	0 DAT	6 DAT	12 DAT
T0 (0%)	1±0.0	3.0±0.0a	0.55±0.4a	1±0.0	3.2±0.6c	0.55±0.4a	1±0.0	3.8±0.4a	1.4±0.4a	4±0.0	0.7±0.4a	0.6±0.4a
T1 $(0.5\%)$	1±0.0	2.8±0.4ab	3.4±0.4b	1±0.0	3.4±0.6c	2.4±0.6b	1±0.0	3.2±0.7a	2.6±0.4b	4±0.0	1.3±0.6a	1.1±0.3ab
T2 (1%)	1±0.0	2.4±0.4b	3.6±0.4bc	1±0.0	2.1±0.5b	2.8±0.4bc	1±0.0	2.8±0.7b	3.3±0.7c	4±0.0	2.2±0.4bc	1.3±0.4b
T3 (2%)	1±0.0	2.2±0.4bc	3.7±0.4bc	1±0.0	1.9±0.5ab	3.0±0.6bc	1±0.0	2.5±0.5bc	3.5±0.5cd	4±0.0	2.6±0.4c	2.15±0.2c
T4 (3%)	1±0.0	2.1±0.6bc	3.8±0.4bc	1±0.0	1.6±0.4ab	3.2±0.7c	1±0.0	2.3±0.4bc	3.7±0.4cd	4±0.0	2.9±0.5d	2.65±0.3cd
T5 (4%)	1±0.0	2.0±0.4bc	4.0±0.0c	1±0.0	1.4±0.4ab	3.8±0.4c	1±0.0	2.2±0.4bc	4.0±0.0d	4±0.0	3.6±0.4d	3.05±0.5d
T6 (5%)	1±0.0	1.7±0.4c	3.4±0.4b	1±0.0	1.30±0.6a	2.1±0.5b	1±0.0	2.0±0.6c	4.0±0.0d	4±0.0	4±0.0d	3.15±0.2d

DAT, days after treatment; Means followed by the same letter in the same column did not differ significantly (Tukey test, p>0.05).

**Table 3.** Effects of CaCl<sub>2</sub> in hot H<sub>2</sub>O treatment on fungal infection, weight loss and shelf-life of mangoes stored at  $25\pm2$  °C over 12 days

Treatment with CaCl <sub>2</sub>	Fungal infection (%)	Weight loss (%)	Shelf-life (Days)	
(Conc.)	12 DAT	12 DAT		
T0 (0%)	0.6±0.48a	20.00±1.50a	7.33±0.47a	
T1 (0.5%)	1.4±0.53b	17.35±1.80ab	7.66±0.47a	
T2 (1%)	2.22±0.33c	17.22±1.70ab	9.20±0.60b	
T3 (2%)	3.0±0.31d	16.45±1.85ab	9.66±1.24bc	
T4 (3%)	3.45±0.41de	16.37±1.65ab	10.00±0.81bc	
T5 (4%)	3.85±0.22e	16.04±1.72ab	10.33±0.47c	
T6 (5%)	4.0±0.0e	14.34±1.58b	10.66±0.94c	

DAT, days after treatment; means followed by the same letter in the same column did not differ significantly (Tukey test, p>0.05).

#### **Biochemical characteristics**

Biochemical parameters such as pH, moisture, ash content, TSS, TTA, sugar, starch, PPO and carbohydrate hydrolyzing enzymes of the mango fruits have been reported to change with the increasing storage period at different temperatures (Hossain el al., 2014). Here we investigated the changes in biochemical characteristics of mango fruits after the treatment of CaCl<sub>2</sub> in hot water over 12 days of storage at  $25\pm2$  °C.

### pH, moisture and ash contents

In case of different concentration of CaCl<sub>2</sub> in hot water treated mangoes, 0.5% CaCl<sub>2</sub> showed the pH 5.60±0.08 and 5% CaCl<sub>2</sub> had the pH 5.48±0.09 over 12 days of storage. The observed pH of mangoes was inversely proportional to the increasing concentration of CaCl<sub>2</sub> due to inhibition of carbohydrate metabolism and respiration (Hossain et al., 2014). The moisture content of treated mango was increased with increasing concentration of CaCl<sub>2</sub> up to  $74.90\pm1.5\%$  whereas control mango contained ( $71.18\pm1.5\%$ ) moisture (Table 4). Five percent CaCl<sub>2</sub>-hot water treated mango possessed the highest amount of moisture  $(74.90\pm1.5\%)$ during its ripening stage (Table 4). The result indicated that moisture content was proportional to increasing concentration of CaCl<sub>2</sub> in treated mangoes which was also negatively correlated organoleptic characters such as color and skin shriveling (Table 2). CaCl<sub>2</sub>-hot water treatment increased the ash contents significantly with increasing conc. of CaCl<sub>2</sub>. CaCl<sub>2</sub>-hot water treatment delayed ripening process and hence retained higher moisture and ash content of treated fruits, which were positively correlated with results obtained in Keitt mangoes and Cavendish bananas with CaCl<sub>2</sub>-hot water treatment (Ernesto et al., 2017). Our results are in good agreement with data obtained in peppers that hot water-CaCl<sub>2</sub> treatment could effectively limit water mobility and retain high immobilized water content (Xu et al., 2019).



They also explained that  $Ca^{2+}$  could bond with water to form clathrate hydrates at certain temperature and limit water mobility.

# Total titratable acidity

The values of total titratable acidity (TAA) are significantly affected by the rate of metabolism especially respiration, which utilized organic acid and hence decreased acidity during ripening process (Clark et al., 2003). After 12 days of storage it was found that 5% CaCl<sub>2</sub> in hot water treated mango had the highest value  $(0.63\pm0.03\%)$  of TAA and 0.5% CaCl<sub>2</sub> treated mango contained the lowest value of TTA  $(0.50\pm0.02\%)$  (Table 4). Kazemi et al. (2013) found TAA increased significantly with increasing concentration of calcium and sodium in pomegranate whereas total soluble solid also gradually decreased. The results of this study suggest that calcium chloride in hot water treatment inhibited the enzymes for the change of organic acids, resulting the increase of TTA. In "Keitt" mango, the main organic acids are citric acid and malic acid, which decline in titratable acidity with the progress of ripening might be due to their utilization as substrates for respiration (Medlicott & Thompson, 1985).

# Total soluble solid

Total soluble solid (TSS) of fruits is a major quality parameter, which is correlated to the texture and composition. The TSS content of mango usually increases up to late stage of ripening due to the change in cell wall structure and degradation of complex carbohydrates into monosaccharides during ripening (Gill et al., 2017; Hossain et al., 2014). The increase and decrease in TSS are directly correlated with hydrolytic changes occurred in starch to simple sugars and could be an important index of ripening process of climacteric fruits including mangoes and further hydrolysis also decreased the TSS contents during storage (Kittur et al., 2001). The TSS content decreased significantly with the increasing concentration of CaCl<sub>2</sub> in hot water treatment after 12 days of storage (Table 4). Similar trend of TSS content was obtained when mango was treated with 3% CaCl<sub>2</sub> and hot water at 55 °C for 10 min (Ernesto et al., 2017). The results suggest that CaCl<sub>2</sub> in hot water probably delayed changes of TSS by inhibiting the glycosidase enzymes of cell walls structural carbohydrates and starch.

# Vitamin C

Mango fruit is a natural source of ascorbic acid (vitamin C) and it was reported that its level decreased during postharvest ripening and processing (Lee & Kader, 2000). At higher concentration of CaCl<sub>2</sub> (5%) in hot water treatment, mango contained significantly high level of vitamin C (88 $\pm$ 8.35 mg/100 g) whereas untreated control had the lowest amount of vitamin C (41.81 $\pm$ 5.0 mg/100 g) after 12 days of storage (Table 4). Vitamin C contents were positively correlated with the increase of CaCl<sub>2</sub> in hot water treatment. Similarly, vitamin C contents remained significantly higher levels during storage when mango fruits were dipped in CaCl<sub>2</sub>-hot water at 48 and 52 °C for 10 min (Yousef et al. 2012). Xu et al. (2019) found that hot water-CaCl<sub>2</sub> treatment significantly maintained higher values of ascorbic acid than control peppers and maintained higher antioxidant capacities. Another study reported that hot water (55 °C) and CaCl<sub>2</sub> (3%) treatment maintained significantly higher levels of vitamin C in mango fruits (Ernesto et al., 2017). These researchers also reported that the concentrations of CaCl<sub>2</sub> delayed the rapid oxidation of ascorbic acid.



Biochemical	Percentage of CaCl <sub>2</sub> used to treat mangoes								
parameters	T0 (0%)	T1 (0.5%)	T2 (1%)	T3 (2%)	T4 (3%)	T5 (4%)	T6 (5%)		
pН	5.64±0.08a	5.60±0.08a	5.53±0.06ab	5.40±0.12ab	5.30±0.07b	5.52±0.10ab	5.48±0.09ab		
Moisture	71.18±1.5a	72.48±1.55ab	72.74±1.55ab	72.72±1.45ab	75.62±1.53b	73.15±1.52ab	74.90±1.50ab		
Ash (%)	0.58±0.05a	0.75±0.08ab	0.77±0.10ab	0.78±0.15ab	0.78±0.05ab	0.95±0.10b	0.96±0.13b		
TTA (%)	0.49±0.02a	0.50±0.02a	0.51±0.04a	0.52±0.03a	0.53±0.02ab	0.56±0.05ab	0.63±0.03b		
TSS (°Brix)	16.00±0.66a	14.11±0.87b	13.4±0.49bc	12.66±0.66bc	12.2±0.62c	11.8±0.42c	11.5±0.47c		
VC(mg/100g)	41.81±5.0a	45.2±4.5a	63.15±4.85b	73.6±6.0bc	76.4±7.15bc	83.25±6.75c	88±8.35c		
TS (%)	17.80±0.85a	17.48±0.89a	17.26±0.79a	16.15±0.65ab	15.04±0.60bc	13.93±0.75bc	12.83±0.90c		
RS (%)	2.43±0.18a	2.74±0.14ab	2.75±0.16ab	2.95±0.15b	2.95±0.13b	2.98±0.12b	3.06±0.15b		
NRS (%)	15.49±0.67a	14.87±0.75a	14.65±0.59ab	13.34±0.50b	12.23±0.47bc	11.12±0.63c	9.92±0.75c		
Starch (%)	3.91±0.5a	3.42±0.41a	3.54±0.45a	4.06±0.55a	4.58±0.63a	6.37±0.65b	7.19±0.70b		
TP(mg/100g)	62.87±0.78a	51.25±0.82b	47.12±0.78b	40.37±0.8a	38.5±0.78a	36.0±1.00a	23.88±0.87ab		
Amylase <sup>1</sup>	0.326±0.06a	0.283±0.09ab	0.283±0.05ab	0.228±0.07ab	0.173±0.05ab	0.133±0.09b	0.133±0.05b		
Invertase <sup>2</sup>	0.011±0.005a	0.012±0.007a	0.013±0.004a	0.013±0.007a	0.024±.006ab	0.032±.008ab	0.040±0.009b		
PPO(Unit/ml)	380±15a	372+20a	371+12a	350+25ab	336+18ab	300+14b	280+19b		

**Table 4.** Changes of biochemical parameters of mango fruits treated with  $CaCl_2$  in hot  $H_2O$  after 12 days of storage at  $25\pm2$  °C

T0= Control mangoes; T1~T6, CaCl<sub>2</sub> in hot H<sub>2</sub>O treated mangoes; VC, Vitamin C; TS, Total sugar; RS, Reducing sugar; NRS, Non-reducing sugar; TP, Total phenol; Amylase<sup>1</sup> (mg/min/ml); Invertase<sup>2</sup> (mg/min/ml); Means followed by the same letter in the same column did not differ significantly (Tukey test, p>0.05).

Same row did not differ significantly (Tukey test, p>0.05).

# Total sugar and non-reducing sugar

The two main functions of carbohydrates are: as a storage form of fuel and as structural element. Glucose and fructose serve as important source of energy for biochemical processes. Some carbohydrates have highly specific function for the development of structural parts of the plants. During the ripening process carbohydrate hydrolyzing enzymes act to release sugar from structural part and loosening the texture of fruits (Hossain et al., 2014). Untreated control mango contained the highest amount of total (17.80 $\pm$ 0.85%) and non-reducing sugar (15.49 $\pm$ 0.67%) after the storage of 12 days. All treated fruits had lower values of total as well as non-reducing sugars over 12 days of storage as compared with controls fruits, suggesting that CaCl<sub>2</sub> in hot water treatment caused inactivation of hydrolyzing enzymes responsible for conversion of starch to sugars. Higher concentration (3~5%) of CaCl<sub>2</sub> treatments maintained significantly the lowers levels of total and non-reducing sugars. Results are in good agreement with those reported by Chawla et al. (2018) that 1.5% CaCl<sub>2</sub> and 1.5% chitosan coating effectively in delaying the hydrolysis of polysaccharides in the guava.

#### Starch and reducing sugar

It was also reported that starch is the main carbohydrate present in mature green mango fruits and during ripening of mango, the starch in mango-pulp is being hydrolyzed to reducing sugars (Matto et al., 1975). It was also found that the starch content at mature (unripe) stage is 3 times higher than that at ripen stage (Hossain, 2016). However, when the fruit becomes over-ripe, only traces of starch was detected (Lima et al., 2001). The untreated control fruit contained the lowest amounts of starch ( $3.91\pm0.5\%$ ) and reducing sugars ( $2.43\pm0.18$ ) over 12 days of storage but all CaCl<sub>2</sub> treated mangoes had higher values of that. Higher concentration of CaCl<sub>2</sub> treated mangoes showed significantly higher amounts of starch and reducing sugar (Table 4) than control mangoes. This might be due to the delay of hydrolysis of polysaccharide mainly starch present in the mango flesh. Similar results were also reported



from others researchers when kiwi fruits were treated with 2% CaCl<sub>2</sub> in hot water (Shahkoomahally & Ramezanian, 2013).

### Total phenol and polyphenol oxidase

Polyphenol oxidase (PPO) is a ubiquitous plant enzyme, which catalyzes the oxidation of phenolic compounds mainly causing browning of the fruits and vegetables (Hossain, 2016). It is a substrate dependant enzyme. PPO activity of untreated control mango was  $380\pm15$  unit/g while it was  $280\pm19$  unit/g when treated with 5% CaCl<sub>2</sub> in hot water over the storage of 12 days (Table 4). PPO activity was negatively correlated with increasing concentration of CaCl<sub>2</sub> in hot water. On the other hand, PPO activity is positively correlated with the total phenol contents when treated 0.5 to 5% CaCl<sub>2</sub> in hot water. A positive correlation between total phenol contents and PPO activity was found in full mature (red color) Irwin (Ueda et al., 2000) and Ashwina mango after 12 days of storage (Hossain et al., 2014). PPO is a biomarker enzyme related to browning process and used for fruit softening assay. In this experiment, since total phenol contents and PPO activity decreased with increasing concentration of CaCl<sub>2</sub> in hot water after 12 days of storage, it could suggest that CaCl<sub>2</sub> in hot water inhibits the PPO activity and delay biochemical reactions involved to release the polyphenolic compounds. A recent study on peppers reported that hot water-CaCl<sub>2</sub> treatment showed lower PPO activity but higher total phenol contents than untreated controls (Xu et al., 2019).

#### Amylase and invertase activity

Amylase is a hydrolytic enzyme, which hydrolyses starch to yield monomeric carbohydrate. The amylase activity of mango increased gradually until 8th day of storage at 30±1°C (Hossain et al., 2014) and 25 °C (Rahman et al., 2011) after that it decreased significantly for next four days of storage. The activity of amylase was higher value (0.326±0.06 mg/min/ml) in untreated control mango after 12 days of storage than that of all concentrations of CaCl<sub>2</sub> in hot water treated mangoes (Table 4). The activity of amylase decreased with increasing concentration of CaCl<sub>2</sub> treatment whereas starch content increased significantly (Table 4). It means that starch content had higher values at higher concentration of CaCl<sub>2</sub> due to the lower levels of amylase activities inhibited by CaCl<sub>2</sub>. Invertase is a hydrolytic enzyme, which hydrolyzes sucrose to glucose and fructose. The activity of invertase of untreated control mango was lower value (0.011±0.005 mg/min/ml) after 12 days of storage than that of all concentrations of CaCl<sub>2</sub> treated mangoes. Invertase activity usually increased at 4th day and then decreased significantly during the later stage of ripening of mango stored at 30±1 °C up to 12th day (Hossain et al., 2014) and its optimum temperature and pH were 60°C and 4.0; respectively (Li et al., 2017). Since the invertase activity significantly increased with CaCl<sub>2</sub> treatments, which hydrolyzed the sucrose to reducing sugars (glucose and fructose), resulting the increase in reducing sugar contents levels (Table 4). On the other hand, mangoes treated with all concentrations of CaCl<sub>2</sub> decreased the levels of non-reducing sugar contents significantly (Table 4), which were negatively correlated with increase of invertase activity as we expected. Therefore, it could be predicted that CaCl<sub>2</sub> in hot water had positive effects on invertase activity.

#### CONCLUSION

Mango export market is quite limited because of its short shelf life and infection of postharvest diseases.  $CaCl_2$  in hot water treatment showed positive and continuous effects on the organoleptic and quality attributes as well as enzymes activities of mango during storage at  $25\pm2$  °C. It extended the shelf life of mango fruits by slowing down the ripening and delaying



the onset of climacteric, which in turn maintained the compositional quality and physical integrity of the fruits during storage period. It can be concluded that 4% calcium chloride in hot water treatment could increase the shelf life of Ashwina mango with consumer acceptance. To understand exact mechanism of  $CaCl_2$  in hot water treatment on postharvest quality mango fruits, more detailed experimental research on postharvest molecular biology is needed which will be published elsewhere in future.

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# **Conflict of interest**

None declared conflict of interest.

# **REFERENCES**

- Anjum, M. A., & Ali, H. (2004). Effect of various calcium salts on ripening of mango fruits. *Journal* of Research (Science), 15(1), 45-52.
- AOAC. (2002). Association of official analytical chemists. Washington, DC, USA.
- Ayon-Reyna, L. E., Lopez-Valenzuela, J. A., Delgado-Vargas, F., Lopez-Lopez, M. E., Molina-Corral, F. J., Carrillo-Lopez, A., & Vega-Garcia, M. Q. (2017). Effect of the combination hot water-calcium chloride on the *in vitro* growth of *Colletotrichum gloeosporioides* and the postharvest quality of infected papaya. *Plant Pathology Journal*, 33(6), 572-581.
- Bray, H. G., & Thorpe, W. V. T. (1954). Analysis of phenolic compounds of interest in metabolism. *Methods of Biochemical Analysis*, 1, 27-52. https://doi.org/10.1002/9780470110171.ch2
- Chawla, S., Devi, R., & Jain, V. (2018). Changes in physicochemical characteristics of guava fruits due to chitosan and calcium chloride treatments during storage. *Journal of Pharmacognosy and Phytochemistry*, 7(3), 1035-1044.
- Clark, C. J., McGlone, V. A., & Jordan, R. B. (2003). Detection of Brownheart in "Braeburn" apple by transmission NIR spectroscopy. *Postharvest Biology and Technology*, 28(1), 87-96. https://doi.org/10.1016/s0925-5214(02)00122-9
- Corrales-Garcia, J., & Lakshminarayana, S. (1991). Response of two cultivars of mango fruits immersed in a calcium solution to cold storage at different times and temperatures. *Technical Innovations in Freezing and Refrigeration of Fruits and Vegetables*, International Institute of Refrigeration, Paris.
- Ernesto, D. V., Omwamba, M., Faraj, A., & Mahungu, S. (2017). Optimization of hot water temperature dipping and calcium chloride treatment to the selected physico-chemical parameters of Keitt mango and Cavendish Banana Fruits. *Food and Nutrition Sciences*, 8, 912-935. http://www.doi.org/10.4236/fns.2017.810066
- Gill, P. P. S., Jawandha, S. K., Kaur, N., & Singh, N. (2017). Physico-chemical changes during progressive ripening of mango (*Mangifera indica* L.) cv. Dashehari under different temperature regimes. *Journal of Food Science and Technology*, 54(7), 1964-1970. http://www.doi.org/10.1007/s13197-017-2632-6
- Gofure, A., Shafique, M. Z., Helali, M., Ibrahim, M., Rahman, M. M., & Alam, M. S. (1997). Studies on extension of post-harvest storage life of mango (*Mangifera indica* L.). Bangladesh Journal Scientific and Industrial Research, 32, 148-152.
- Hortwitz, W. (1960). Official and tentative methods of analysis. Washington DC, Springer.
- Hossain, M. A. (2016). Changes in ripening associated biochemical and enzymatic characteristics of late season mango (*Mangifera indica*, L.) of Bangladesh. *Journal of Scientific Research*, 8(3), 387-398. https://doi.org/10.3329/jsr.v8i3.27218

- Hossain, M. A., Rana, M. M., Kimura, Y., & Roslan, H. A. (2014). Changes in biochemical characteristics and activities of ripening associated enzymes in mango fruit during the storage at different temperatures. *BioMed Research International*, Article ID 232969, 11 p. https://doi.org/10.1155/2014/232969
- Jayaraman, J. (1981). Laboratory manual in biochemistry. New Delhi: John Wiley & Sons.
- Kays, S. J. (1991). *Post harvest physiology of perishable plant products*. Vas Nostrand Rein Hold Book. Westport: Avi Publication Co.
- Kazemi, F., Jafararpoor, M., & Golparvar, A. (2013). Effects of sodium and calcium treatments on the shelf life and quality of Pomegranate. *International Journal of Farming and Allied Sciences*, 2, 1375-1378.
- Kittur, F. S., Saroja, N. S., & Habibunnisa-Tharanathan, R. N. (2001). Polysaccharide based composite coating formulations for shelf-life extension of fresh banana and mango. *European Food Research and Technology*, 213, 306-311. https://doi.org/10.1007/s002170100363
- Kudachikar, V. B., Kulkarni, S. G., Prakash, M. N. K., Vasantha, M. S., Prasad, B. A., & Ramana, K. V. R. (2001). Physico-chemical changes during maturity of mango (*Mangifera indica* L.) Variety "Neelum". *Journal of Food Science & Technology, Mysore, 38*, 540-542.
- Kumah, P., Appiah, F., & Opoku-Debrah, J. K. (2011). Effect of hot water treatment on quality and shelf life of Keitt mango. Agriculture and Biology Journal of North America, 2(5), 806-817. https://doi.org/10.5251/abjna.2011.2.5.806.817
- Lee, S. K. & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology & Technology*, 20(3), 207-220. https://doi.org/10.1016/s0925-5214(00)00133-2
- Li, R. J., Li, J. Y., Liao, X. J., & Wang, Y. T. (2017). Purification and characterization of soluble acid invertase from mango fruits. *International Journal of Food Science & Technology*, *52*, 906-915. https://doi.org/10.1111/ijfs.13354
- Lima, L. C. O., Chitarra, A. B., & Chitarra, M. I. F. (2001). Changes in amylase activity starch and sugars contents in mango fruits pulp cv. Tommy Atkins with spongy tissue, *Brazilian Archives of Biology and Technology*, 44 (1), 59-62. https://doi.org/10.1590/S1516-89132001000100008
- Madani, B., Mirshekari, A., & Yahia, E. (2016). Effect of calcium chloride treatments on calcium content, anthracnose severity and antioxidant activity in papaya fruit during ambient storage. *Journal of Science, Food and Agriculture, 96*, 2963-2968. https://doi.org/10.1002/jsfa.7462
- Mahadevan, A., & Sridhar, M. (1982). *Methods of physiological plant pathology*. Madras: Sivakasi Publication.
- Mahmud, A., Shibly, A. Z., Hossain, M. M., Islam, M. S., & Islam, R. (2015). The effects of CaC<sub>2</sub> and different calcium salt on mango fruits ripening in Bangladesh. *Journal of Pharmacognosy and Phytochemistry*, 4(1), 210-215.
- Malundo, T. M. M., Shewfelt, R. L., Ware, G. O., & Baldwin, E. A. (2001). Sugars and acids influence flavor properties of mango (*Mangifera indica L.*). Journal of the American Society of Horticultural Science, 126, 115-121. https://doi.org/10.21273/jashs.126.1.115
- Matto, A. K., Murata, T., Pantastico, E. B., Ogata, K., & Phan, C. T. (1975). *Chemical changes during ripening and senescence in post-harvest physiology, handling and utilization of tropical and subtropical fruits and vegetables.* Westport: Avi Publication Co.
- Mazumdar, B. C., & Majumder, K. (2003). *Methods on physicochemical analysis of fruits*. India: University College of Agriculture Calcutta University.
- Medlicott, A. P., & Thompson, A. K. (1985). Analysis of sugars and organic acids in ripening of mango fruit (*Mangifera indica* L. var Keitt) by high-performance liquid chromatography, *Journal* of the Science of Food and Agriculture, 36, 561-566. https://doi.org/10.1002/jsfa.2740360707
- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, 31(3), 426-428. https://doi.org/10.1021/ac60147a030
- Mootoo, A. (1991). Effects of postharvest calcium chloride dips on ripening changes in "Julie"mangoes. *Tropical Science*, *31*, 243-248.
- Ngamchuachit, P., Sivertsen, H. K., Mitcham, E. J., & Barrett, D. M. (2014). Effectiveness of calcium chloride and calcium lactate on maintenance of textural and sensory qualities of fresh-cut mangos. *Journal of Food Science*. 00, 0, C1-C9. https://doi.org/10.1111/1750-3841.12446\_

- Peryam, D. R., & Pilgrim, F. J. (1957). Hedonic scale method of measuring food preference. *Food Technology*, 11, 9-14.
- Rahman, M. M., Rahman, M. M., Absar, N., & Ahsan, M. A. (2011). Correlation of carbohydrate content with the changes in amylase, invertase and β-galactosidase activity of ripe mango pulp during storage under different temperatures. *Bangladesh Journal Scientific & Industrial Research*, 46(4), 443-446.
- Shahkoomahally, S., & Ramezanian, A. (2013). The effect of hot water and calcium solution dipping on quality in Kiwi fruit during storage. *American-Eurasian Journal of Agriculture & Environmental Science*, 13(10), 1351-1356.
- Sharma, R. R., Singh, D., & Pal, R. K. (2013). Synergistic influence of pre-harvest calcium sprays and postharvest hot water treatment on fruit firmness, decay, bitter pit incidence and postharvest quality of royal delicious apples (*Malus x domestica* Borkh). *American Journal of Plant Science*, 4, 153-159. http://www.doi.org/10.4236/ajps.2013.41020
- Silveira, A. C., Aguayo, E., Chisari, M., & Artés, F. (2011). Calcium salts and heat treatment for quality retention of fresh-cut 'Galia' melon. *Postharvest Biology and Technology*, 62, 77-84. https://doi.org/10.1016/j.postharvbio.2011.04.009
- Singh, V., Pandey, G., Sarolia, D. K., Kaushik, R. A., & Gora, J. S. (2017). Influence of pre harvest application of Calcium on shelf life and fruit quality of Mango (*Mangifera indica* L.) cultivars. *International Journal of Current Microbiology and Applied Sciences*, 6(4), 1366-1372. http://www.doi.org/10.20546/ijcmas.2017.604.167
- Tirmazi, S. I. H., & Wills, R. B. H. (1981). Retardation of ripening of mangoes by post-harvest application of calcium. *Tropical Agriculture*, *58*, 137-141.
- Ueda, M., Sasaki, K., Inaba, K., & Shimabayashi, Y. (2000). Variation in total polyphenol and polyphenol oxidase activity during maturation of mango fruits (*Mangifera indica* L. 'Irwin') cultured in plastic house. *Food Science and Technology Research*, 6(1), 59-61. https://doi.org/10.3136/fstr.6.59
- Xu, H., Ding, S., Zhou, H., Yi, Y., Deng, F., & Wang, R. (2019). Quality attributes and related enzyme activities in peppers during storage: effect of hydrothermal and calcium chloride treatment, *International Journal of Food Properties*, 22(1), 1475-1491. http://www.doi/10.1080/10942912.2019.1653909
- Yousef, A. R. M., Emam, H. S., & Ahmed, D. M. M. (2012). Storage and hot water treatments on post storage quality of mango fruit (*Mangifera indica* L.) variety Copania. *Australian Journal of Basic and Applied Sciences*, 6(13), 490-496.
- Yuen, C. M. C., Tan, S. C., Jovce, D., & Chettri, P. (1993). Effect of postharvest calcium and polymeric film on ripening and peel injury in Kensington Pride mango. ASEAN Food Journal, 8, 110-113.