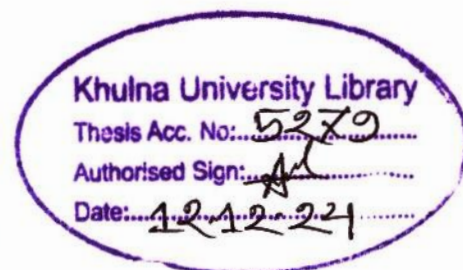


**QUALITY AND SHELF LIFE OF MANGO AND BANANA AS
INFLUENCED BY CHITIN NANOFIBER**

**A THESIS
BY
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STUDENT ID: MS-210814**



A thesis (Course No. HRT-6104) submitted to the Agrotechnology Discipline of Khulna University in partial fulfillment of the requirements for the degree of Master of Science (MS) in Horticulture



**AGROTECHNOLOGY DISCIPLINE
LIFE SCIENCE SCHOOL
KHULNA UNIVERSITY
KHULNA, BANGLADESH**

DECEMBER, 2023

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DECEMBER 2023

DEDICATION

The author dedicate this book to her Late Grandfather and beloved parents.

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THE AUTHOR



ABSTRACT

Mango and banana are two popular fruits widely cultivated in tropical and sub-tropical countries in the world. These fruits are perishable with limited shelf life resulting in considerable amount of postharvest loss every year. To minimize these problems, harvested green mature fruits (mango and banana) were treated with different concentrations of chitin nanofiber (CNF) (0.1%, 0.3% and 0.5%) and stored in ambient condition at the Horticulture Laboratory, Agrotechnology Discipline, Khulna University, Khulna. Non-treated fruits were considered as control. Two separate experiments were conducted for these fruits following factorial Completely Randomized Design (CRD) with three replications of the treatments having 10 fruits in each replication to investigate the postharvest performance of the fruits during storage. The effectiveness of the coating in extending shelf life of fruits was assessed by evaluating cumulative weight loss (%), changes in color (subjective and objective), fruit firmness, TA (%), TSS (% BRIX), vitamin C (mg/100g), disease incidence (number), disease severity (%) and shelf life (days). From the study it was observed that 0.3% CNF coating reduced the loss of cumulative weight (0.96 part) and firmness (0.73 part) of banana while 0.5% CNF was effective to reduce total weight loss (0.83 part) and TSS (0.64 part) retention of mango. Lower disease incidence was recorded in 0.3% CNF treated mango (0.93 part); however, disease severity was remarkably reduced by CNF 0.1% (1.30 times) compared to uncoated control. CNF 0.1% also retained TSS of banana compared to other treatments. This investigation showed that, 0.1% CNF prolonged shelf life of banana while CNF 0.3% enhances the extension of shelf life and performs better in maintaining physicochemical properties of mango in ambient condition.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA = Analysis of Variance

BBS = Bangladesh Bureau of Statistics

CRD = Completely Randomized Design

CNF = Chitin Nanofiber

EPA = Environmental Protection Agency

FDA = Food and Drug Administration

FAOSTAT= Food and Agriculture Organization Corporate Statistical Database

g = Gram

ha = Hectare

LSD = Least Significant Difference

1-MCP = 1-Methyl cyclopropane

ml = Mililitre

MT = Metric Ton

ppm = Parts Per Million

RH = Relative Humidity

STAR = Statistical Tools for Agricultural Research

TA= Titratable Acidity

TSS = Total Soluble Solids

°C = Degree Celsius

Chapter 1

Introduction

A variety of fruits are abundant in Bangladesh. From nutritional point of view, fruit contains adequate quantity of appreciable β -carotene, vitamin C, ascorbic acid and dietary fiber. It also contains soluble sugars and different minerals (Alam et al., 2019). Fruits are rich in naturally occurring antioxidants, including flavonoids, carotenoids, vitamins, phenols, tannins, and numerous secondary metabolites. These chemicals have been found to function as active oxygen scavengers or free radicals (Zheng and Wang, 2001). At present, the production of fruits in Bangladesh is 12.2 M tons and there has been an 11.5% annual rise in fruit production. Bangladesh now lists among the top 10 countries in the world in respect of producing seasonal fruits (The business standard, 2022). About 0.17 M ha of lands are covered with fruit cultivation depending on location and size of the gardens (FAOSTAT, 2021). Many fruits are grown in Bangladesh of which mango and banana occupies most of the areas and production (BBS, 2019).

Mango (*Mangifera indica* L.) is one of the fruits widely cultivated in the tropical and subtropical countries of the world and is rightly designated as the 'King of fruits' (Puravankara et al., 2000). It is also the most important fruit of Bangladesh, ranks 1st in the country and 7th in the World in terms of production (BBS, 2019). About 250 varieties of mangoes are grown in Bangladesh and it occupies 0.12 M ha of lands and production is 1.4 M tons in the year 2018-2019 (Shafique, 2006; FAOSTAT, 2021). Due to its wide range of adaptability, high nutritive value and richness in variety, delicious taste and excellent flavor, mango is a very popular fruit.

Banana (*Musa paradisiaca* L.) is another well-known delicious fruit and preferred by people of all ages. It is highly nutritive and also a rich source of energy (89 Kcal 100⁻¹g) (Sidhu and Zafar, 2018). Bangladesh is ranked 23rd among the banana-producing countries of the world (FAOSTAT, 2021). In Bangladesh, total banana production in the year 2018-2019 was recorded as 0.082 M tons from the cultivated areas of about 0.05 M ha (FAOSTAT, 2021).

A considerable number of fruits have been lost due to faulty postharvest handling every year. One-third of fruits in Bangladesh go to waste due to lack of postharvest management, including the lack of facilities of preservation and storage, and a proper system of transportation (The business post, 2023). Due to short shelf life and improper postharvest handling, mango exports to distant markets have been hampered (Ntsoane et al., 2019). Additionally, the quick

postharvest deterioration led to a significant economic loss every year during harvesting, transportation and marketing (Hassan, 2010). Banana is also a highly perishable fruit and its postharvest losses range from 25 to 50% during transportation and marketing due to adverse physiological changes, softening of the flesh and lack of resistance capacity against microbial attack. Hassan (2010) reported that every year 24.62% postharvest loss of banana has been recorded in Bangladesh which accounts for 0.567 billion BDT. A similar trend of fruit loss has been reported by researches for other fruit crops of Bangladesh.

The postharvest quality factors are fruit weight, color index, TSS, pH, anthocyanin content, carotenoid content, flavonoids, total phenol content, carbohydrate content, ascorbic acid, firmness of fruit, titratable acidity, decay percentage, shelf life of fruit etc. These quality standards can be maintained using a variety of ways, including edible coatings (Hossain and Iqbal, 2016), edible oil treatments (Solgi and Ghorbanpour, 2014), packing materials (Azene et al., 2014), chemical treatments (Dovel, 2018) etc. Fruits have a limited shelf life since they are perishable. Due to their high nutritional value and beneficial effects on health, fruits are in high demand in the market. But still, fruit preservation is a major problem worldwide.

Different postharvest treatments on fruits have been studied by different researchers to identify treatment and techniques that can maintain quality and extend shelf life. The treatments include the use of modified atmosphere (MA) (Irtwange, 2006), low temperature (Hernández et al., 2009), salicylic acid (Asghari and Aghdam, 2010; Win and Setha, 2022), calcium chloride (Madani et al., 2014; Dovel, 2018) etc. Two different studies by Bender et al., (2000) and Noomhorm and Tiasuwan (1995) reported that MA storage has been used to extend shelf life of mango but it is costly. It has also been observed that MA storage slows down the ripening of mangos, but it is frequently associated with excessive CO₂ and an off flavor (Gonzalez-Aguilar et al., 1997). Although lower temperatures may prolong the storage periods of fruit, they cannot entirely prevent the reduction of organic acid levels or water loss over an extended period of time (Ding et al., 1998). Therefore, applying chemical fungicides has long been a popular and successful method of extending the shelf life of harvested fruits by slowing down the decay process (Diskin et al., 2019, Swart et al., 2006). However, the use of chemical fungicides to prevent postharvest decay in harvested fruits have some detrimental effects on the environment by the formation of chemical residue, development of pathogen population resistance and advancements in global living and consumption standards (Sellitto et al., 2021).

To minimize postharvest loss of mango and banana, development of safe and environment friendly method is essential. Application of thin layer of materials that form a protective layer around edible commodity, can be consumed along with coated product and applied on fruits by dipping, spraying or wrapping and act as a protective layer against transmission of gases, vapors as well as solutes and provide mechanical protection (Wu et al., 2002; Guilbert, 1986). It may be applied on fruits and vegetables to improve appearance, delay ripening, reduce water loss and decay, and extend shelf life (Saucedo-Pompa et al., 2007). Edible film as chitin nanofiber (CNF) is one of such materials recently introduced to coat fresh commodities as a protective layer. It contains a food preservative and is a novel system for extending shelf life and preserves the quality of fruits (Amiri et al., 2020). CNF has nano-morphologies and efficient mechanical (ifuku, 2014) and biomedical properties (Ding et al., 1998). Edible coating of chitin nanofiber could be an alternative to traditional postharvest methods as a bio-preservatives. Chitin and its derivative chitosan have been suggested as a possible solution to control postharvest diseases (Abbasi et al., 2009; Azeredo et al., 2019). Chitosan is a naturally occurring edible coating that has been used due to its film-forming, biochemical, innate antifungal, and eliciting properties and to prolong the postharvest life of many fruits (Kumarihami et al., 2022).

Chitin is a high molecular weight long chain polymer of N-acetyl-D-glucosamine units through β -d-(1 $\rightarrow$ 4) linkage having the chemical formula $(C_8H_{13}NO_5)_n$. Chitin is the second-most abundant polymer in nature after cellulose and is found in the exoskeletons of prawns and shrimp, lobsters, shellfish, squid pens, crabs, insects, and fungal cell walls (No et al., 1989; Cho et al., 1998; Barber et al., 2013; Younes and Rinaudo, 2015) and has a wide range of potential applications in the agriculture and food business (Prashanth et al., 2007).

The nanofibrillation of chitin is termed as Chitin Nanofiber (CNF). Typically, fibers having aspect ratios greater than 100 and diameters smaller than 100 nm are referred to as nanofibers (Xia et al., 2003; Li and Xia, 2004). Chitin is used in agriculture, although it has some drawbacks, including the inability to dissolve in water and other popular solvents. The insolubility of chitin makes water soluble chitin derivatives like CNF a widely accepted alternative in agricultural applications (Azuma et al., 2014; Shamshina et al., 2019). Chitin have been approved as a biopesticide by the U.S. Environmental Protection Agency (EPA), and as a food additive, approved by the U.S. Food and Drug Administration (FDA). Similarly due to its bio-pesticidal nature, antibacterial activity, and ability to trigger defense responses in plant tissue, chitosan has also emerged as a potential alternative treatment for fruits and

vegetables. Chitin or chitosan has been used on several fruits to prevent postharvest infections in mango, banana, dragon fruit, strawberry, tomato etc. (Terry and Joyce, 2004; Fisk et al., 2008). However, there is a limited or no study conducted on the use of these chemicals on popular fruits in Bangladesh.

In this context, this work is designed to evaluate the efficiency of coating mango and banana fruits with CNF to increase shelf life and minimize postharvest damage by observing physicochemical properties in ambient condition with following objectives-

General Objectives

- ❖ To evaluate the effects of chitin nanofibre on mango and banana shelf life.
- ❖ To analyze the effect of chitin nanofibre on the physicochemical properties of mango and banana.

Specific Objectives

- ❖ To find out an eco-friendly chemical treatment to reduce postharvest loss of fruit during storage.



Chapter 2

Review of literature

2.1 Introduction

Chitin nanofiber (CNF) is nanofibrillation of chitin which is extracted from exoskeleton of shrimp and other crustaceans as well as insect (Cho et al., 1998; Barber et al., 2013; Younes and Rinaudo, 2015). Recently, CNF have emerged as a promising material for food preservation due to their unique physicochemical, biodegradable and biocompatible properties (El Knidri et al., 2020; Sharp, 2013; Al-Hetar et al., 2011). The consumption of fruits has been increased globally due to the growing interest in healthy and nutritious foods. However, fruits are highly perishable and have a short shelf life, which limits their quality to consumers. Different postharvest treatments on fruits have been studied by different researchers to increase quality and shelf life. The treatments include the use of chitosan (Aziz et al. 2021; Parvin et al., 2023), salicylic acid (Asghari and Aghdam, 2010; Win and Setha, 2022), cumin essential oil (Solgi and Ghorbanpour, 2014), clove extract (Nur Fatimma et al., 2018), edible coating (Gol and Ramana, 2011; Hossain and Iqbal, 2016), calcium chloride (Madani et al., 2014; Dovel, 2018), aloe vera (Shah and Hashmi, 2020; Jodhani and Nataraj, 2021) etc. However, there is no particular research based on edible coating of chitin nanofiber extracted from shrimp shell on fruits. Therefore, research on ways to increase the shelf life and maintain the quality of fruits by using chitin nanofiber has been of great interest. Some of the literatures have been reviewed here to understand the impact of CNF, one of its derivatives chitosan and other edible coating on quality and shelf life of different fruits.

2.2 Application of chitin/chitosan in agriculture

Several consumers have benefitted greatly in recent years from the use of complex compounds based on the natural biopolymer chitin and its deacetylated derivative chitosan (Malbera and Cerana, 2019). Majority of chitin and chitosan produced for agricultural purpose have significant influence on plant growth, yield, shelf life, quality, insects and disease control.

2.2.1 Use of chitin/chitosan as postharvest treatment

Chitin and its derivative chitosan can be used to prevent fruit postharvest deterioration as evaluated by Zhang et al. (2011a). They summarized the impact of chitin and chitosan on fruit postharvest degradation and emphasized the role of chitin and chitosan's molecular processes in preventing fruit postharvest deterioration. Molecular substances of chitin are being used as

effective components to produce drugs, cosmetics and food preservatives (Malerba and Cerana, 2019). Rajestary et al. (2021) reported chitosan as a unique natural compound for long-term preservation of fresh fruit. Green mature banana coated with different concentration of chitosan reduce postharvest losses (Hossain and Iqbal, 2016). Chitosan coatings on mango significantly preserved many fruits postharvest quality attributes like vitamin C content, titratable acidity, sugar content, ash, and protein content as reported by Parvin et al. (2023). Abbasi et al. (2009) observed postharvest quality of mango coated with irradiated Crab and Shrimp chitosan and un-irradiated Crab chitosan as treatment. The results demonstrated that using irradiated chitosan coatings on fresh fruit as postharvest treatment has excellent potentials. Chitosan postharvest fruit treatment significantly reduced bacterial and fungal development on strawberry. Similarly treated strawberries showed improved postharvest preservation by maintaining cellular structure as reported by El-araby et al. (2022).

2.2.2 Chitosan in fruit preservation

Both chitin and chitosan are naturally occurring biopolymers. Chitosan is the naturally generated biopolymer of chitin (Hanh et al., 2009). Chitosan are also frequently used in fruit preservation as analyzed through different tests by many scientists.

2.2.2.1 Chitosan in different concentrations as fruit preservative

Fresh fruit, vegetables, or their freshly cut materials have been preserved using chitosan coating and chitosan biopolymer as edible material (Yu and Ren, 2013; Parijadi et al., 2022). Shrimp chitosan at 1.5% provided second best result in case of mango coating but at 1% delayed color change compared to other treatments (Monira et al., 2016; Aziz et al., 2021). Chitosan at 1% was more suitable in prolonging the shelf life and quality of banana during ripening and storage (Hossain and Iqbal, 2016; Sikder and Islam, 2019). Best quality in strawberries was maintained for 12 days while the fruits were coated with Chitosan (1%) (Benhabiles et al., 2013). Chitosan coating @ 4% was found as the most effective treatment for improving the postharvest quality which also prolong the shelf life of dragon fruits when stored at ambient condition (Prashanth et al., 2022). Taha et al. (2020) also assessed how edible coverings made of chitosan and chitosan nanoparticles could enhance the quality and extend the shelf life of strawberries in storage. Postharvest physiology and cell ultrastructure of apricot fruits treated with 0.75g/L of chitosan can greatly slow down fruit ripening and softening while maintaining higher levels of total soluble solids (Jiang et al., 2010).

2.2.2.2 Effect of chitosan combined with other chemicals

Treating postharvest jujube with chitosan and sodium silicate could be a noble way to stop the disease from infecting postharvest jujube (Guo et al., 2019). Carambola fruit coated with chitosan 0.3% and gum arabic 1% preserved superior visual quality than the control and other coated samples and retained superior postharvest quality (Gol et al., 2015). However, coating of 2% chitosan with deacetylation (80%) prevents the loss of fruit weight and vitamin C without deteriorating desired sensory qualities in Cavendish banana (Suseno et al., 2014). Kumar et al. (2021) reported that shelf life of mango fruits could be prolonged by using an alternate preservation technique that combines chitosan-pullulan (50:50) and pomegranate peel extract. A workable solution for maintaining quality and extending storage life in mango at 10°C is the use of all applications of chitosan coating or 1-MCP in order to maintain firmness, sucrose and starch content as stated by Wongmetha and Ke (2012).

2.2.2.3 Effect of chitosan combined with bio-preservatives

Bio-preservatives are alternatives of hazardous chemical preservatives for controlling postharvest diseases which have good efficacy, low residues, and little or no toxicity to non-target organisms (Holmes and Eckert, 1999). They effectively inhibit postharvest diseases of fruits by direct inhibition on spore germination, germ tube elongation and mycelial growth of phytopathogen (Zhang et al., 2011a).

Peppermint essential oil (0.2%) and untreated chitosan (1%) resulted in 100% marketable fruits with no peel discoloration in papaya compared to the experimental control (Picard et al., 2013). Pineapple coated with chitosan and incorporation of eugenol as well as aloe vera gel has the effectiveness to preserve quality and shelf life up to three weeks in ambient conditions (Basumatary et al., 2022). The findings from 'Cavendish' banana fruit treated with chitosan nanoparticles with different bio-preservatives demonstrated significant and desired changes in firmness, rate of ethylene production, respiration, and total phenolic content during storage (Odetayo et al., 2022). It was also reported that banana fruits coated with Aloe vera 50% + chitosan nanoparticle 2% (AV+CN) and *Moringa oleifera* 10% + chitosan nanoparticle 2% (MO+CN) also showed significantly decreased weight loss (by 23%), rate of respiration and ethylene generation.

2.2.2.4 Use of Modified chitosan on fruit

Chitosan was modified by fatty acid N-acylation to maintain the quality of the papaya and it was found that in comparison to the experimental control, the fruits treated with this formulation expresses less peel discoloration (Picard et al., 2013).

2.2.3 Crop productivity and use of chitin

Molecular compounds derived from chitin and chitosan have been utilized in plants as safe, eco-friendly materials to increase crop productivity as reported by Malerba and Cerana (2019).

2.2.4 Prevention of disease and pathogen by using CNF

Research conducted by Aditiya et al. (2020) showed that the CNF coatings significantly reduced the microbial growth on papaya fruit surface. Chitin oligomers against *Alternaria* rot in tomato were effectively used by Valle-Sotelo et al. (2022) which is a serious postharvest disease for stored fruits and vegetables. The result showed that, to reduce *Alternaria* rot in tomato fruits through an enzymatic defense mechanism, *Alternaria alternata* chitin oligomers constitute a feasible option. Parada et al. (2018) examined the potential application of CNF in agriculture, the level of chitin nanofibrillation for elicitor activity and its impact on pathogen resistance in cabbage and strawberry plants. According to the results, CNF is a promising natural substance to prevent diseases in cultivated plants and can systematically develop disease resistance in strawberry and cabbage plants. A study by He et al. (2018) investigated the effect of CNFs on the shelf life of apples. The results showed that treatment with 0.5% CNF significantly inhibited the growth of microorganisms and delayed the deterioration of the apples, resulting in a longer shelf life.

2.2.5 Nutrient management in fruit by using chitin

Chitin treatment considerably enhanced the amount of nitrogen and carbon in the entire tomato plant. Hence CNF could be a valuable material for nutrient management in tomato production and that the growth-promoting effect of chitin treatment is produced by an increase in nitrogen uptake efficiency (Egusa et al., 2020).

2.2.6 Nitrogen uptake efficiency

Chitin nanofiber treatment improved nitrogen uptake efficiency in tomato plant. Through a transcriptome analysis of tomatoes exposed to CNF, Egusa et al. (2020) reported that, the

expression level of genes involved in nitrogen uptake and assimilation, nutrient distribution, and photosynthesis were changed.

2.2.7 Use of chitin as biopesticides

Chitin has been approved as a biopesticide by the U.S. Environmental Protection Agency (EPA), and as a food additive, approved by the U.S. Food and Drug Administration (FDA). Similarly due to its biopesticidal nature, antibacterial activity, and ability to trigger defense responses in plant tissue, chitosan, a derivative of chitin, has also emerged as a potential alternative postharvest treatment for fruits as mango, banana, dragon fruit, strawberry, tomato etc. (Terry and Joyce, 2004; Fisk et al., 2008).

2.3. Effect of chitin/chitosan on physical properties of fruit

The physical properties of fruits are important for the postharvest management of fruits. Physical properties include fruit weight, firmness, color etc. These properties influence quality and shelf life of fruits. Edible coatings are used to reduce weight loss during transport and storage (Baldwin, 1994). They make a barrier in gaseous exchange and have selective permeability characteristics, especially to O₂, CO₂ and ethylene. Thus allow some control of fruit respiration and can reduce growth of microorganisms (Cuq et al., 1995).

2.3.1 Loss of fruit weight

Chitosan is used to reduce the weight loss of fruit as banana (Hossain and Iqbal, 2016) though banana fruit coated with 200 ppm solution of GA₃ experienced maximum mean fruit weight compared to chitosan coated banana during storage (Rana et al., 2018). Though, only 1% Chitin nanofiber significantly reduced weight loss in case of Strawberry as demonstrated by Li et al. (2020b). A study conducted by Ahmed et al. (2020) showed that chitin nanofiber coating reduced weight loss of mango and banana at 0.5% concentration. However, 1% chitosan-coated bananas showed less weight loss than control in case of shrimp chitosan coating but commercial chitosan reduce weight loss up to 65% in mango (Parvin et al., 2023; Sikder and Islam, 2019). The weight loss was highest (12.76%) in mango fruits covered with chitosan at 0.75% and lowest (11.23%) in fruits treated with 1.5% chitosan as evaluated by Aziz et al. (2021). Banana coated with 2% chitosan followed by deacetylation showed 80% prevention of weight loss (Suseno et al., 2014). Weight loss of strawberry was reduced by 1% CNF as examined by Li et al. (2020b) and Gómez-Mascaraque et al. (2019). Banana peel coated with 2% CNF reduced weight loss as stated by Pacheco et al. (2020). CNF coating reduced weight

reduces the pH of jujube (Zhang et al., 2019c) and peach fruit (Yang et al., 2017). Sharma et al. (2020) and Hua et al. (2018) found that chitosan coating has no significant effect on the pH of mango and kiwifruit during storage.

2.4.5 Respiration and ethylene production

Chitin nanofiber coating reduced the respiration rate and ethylene production in mangoes, which slowed down the ripening process and extended the shelf life of the fruit. The best treatment percentage was found to be 1% chitin nanofiber as reported by Amiri et al. (2020).

2.5 Storage condition and use of chitin/chitosan

2.5.1 Ambient condition

The coating formula made with 0.5% chitosan, 0.3% Aloe vera gel, and 0.2% papaya leaf extracts was the most successful for preserving postharvest quality of mango at ambient storage condition (Nga and Bac, 2021). The quality of strawberries was maintained when they were kept at ambient temperature (20–25°C) by using edible coverings as impacted by chitosan extraction procedures (Benhabiles et al., 2013). Different concentration of chitosan and Aloe vera gel were analyzed by Monira et al. (2016) on shelf life and quality of mango. They found 1.5% Aloe vera gel as the most effective treatment for the large size mangoes at room temperature. Shrimp chitosan coating extended shelf life of banana at room temperature up to 16 days (Sikder and Islam, 2019). In another study by Islam et al. (2021), mangoes were treated with CNF at different concentrations and stored at room temperature. The results showed that all CNF treatments significantly delayed the ripening process, reduced weight loss and maintained fruit firmness compared to the control. However, the 0.5% CNF treatment was found to be the most effective in preserving the quality of mangoes during storage, with a shelf life extension of up to 14 days. In addition, 1% CNF treatment was found to be the most effective in extending the shelf life of bananas, with a shelf life extension of up to 18 days at room temperature as stated by Sharma et al. (2021).

2.5.2 Temperature controlled condition

Mango fruit coated with irradiated crab and shrimp chitosan at 15±1°C and 85% relative humidity showed excellent potential on quality and shelf life of fruit (Abbasi et al., 2009). Coating of starch and chitosan on banana at low drying temperature (50°C) improve shelf life and quality up to 15 days in all coating situations (Alam et al., 2020). Low temperature influenced banana metabolism and chitosan coating prolonged shelf life of fruit at low

temperature condition (Parijadi et al., 2022). In a study by Ahmed et al. (2021), mangoes were treated with CNF at a concentration of 1% and stored at different temperatures (4°C, 10°C, and 25°C). The results showed that the CNF treatment significantly reduced weight loss, decay incidence, and respiration rate, while maintaining fruit firmness and delaying the ripening process compared to the control. The best storage temperature for preserving the quality of CNF-treated mangoes was found to be 10°C, with a shelf life extension of up to 24 days.

2.6 Use of shrimp chitosan on fruits

Use of irradiated shrimp chitosan coatings on mango fruit retain quality and has excellent potential to increase shelf life as evaluated by Abbasi et al. (2009). Effectiveness of coating banana with shrimp chitosan in extending the fruits' shelf life was determined by Sikdar and Islam (2019). Sultana et al. (2020) examined chitosan and its application achieved from locally available shrimp shell in Bangladesh. They showed that application of chitosan solution to red and green apples at a concentration of 0.25% may be a natural preservative for apple. However, edible coatings of shrimp chitosan can provide the best results for weight reduction in shrinkage, moisture content, and vitamin C during apple storage (Abriana and Laga, 2016).

A study conducted by El Ghaouth et al. (2007) showed that treating strawberries with 1% shrimp chitosan solution resulted in a significant reduction in decay and an increase in the shelf life of the fruit by up to 50%. Similarly, another study by Zhang et al. (2011b) reported that treating peaches with 1% shrimp chitosan solution resulted in reduction of weight loss, softening and decay, and an extension of shelf life by up to 9 days. Chitosan from shrimp shells delayed oxidative deterioration and extend shelf life of exotic fruit. Similarly, membrane permeability permitted oxygen exchange necessary for fruit freshness and natural physiological weight loss (Zárate-Moreno et al., 2023). A concentration of 1% shrimp chitosan was found to slow down the decay rate and maintained firmness of blueberries (Liu et al., 2019b), while a concentration of 2% w/v was effective for peaches (Zhang et al., 2011b) and minimized weight loss and delayed ripening of strawberry (Mohammadi et al., 2021). This 2% shrimp chitosan coating also significantly maintained postharvest quality in mango and extend shelf life (Ali et al., 2020b). The effect of chitosan from shrimp shells on postharvest quality and shelf life of blueberries kiwifruit and was investigated by Li et al. (2018a and 2019b). However, the best treatment percentage was found to be 0.5% chitosan solution, which resulted in a significant delay in ripening, reduced weight loss as well as decay incidence, improved fruit firmness, color and overall quality of kiwifruit and blueberries during storage. In another evaluation, 1%

chitosan resulted in reduction in decay incidence, weight loss, and maintenance of fruit firmness and color of strawberries in storage condition (Wang et al., 2019) and improved overall quality of cherry tomato (Yang et al., 2018a). However, when oranges were treated with 1.5% shrimp chitosan and stored at room temperature, significantly reduced weight loss, maintained firmness and reduced the incidence of decay in oranges were recorded during storage (Li et al., 2013b). Similar findings were report by (Pongkanjana et al., 2016) while they treated bananas with different concentrations of chitosan and stored at 13°C for 21 days. Best performance was shown by 1.5% chitosan which reduced weight loss, respiration rate, and ethylene production compared to the control group. In addition, fruit firmness and decay incidence were also reduced significantly. In blueberries, 0.5% shrimp chitosan reduced decay, maintained fruit firmness, and reduced respiration rate during storage as stated by Yang et al. (2019). Sánchez-González et al. (2011) and Devi and Jeyakumar (2018) conducted research on the impact of shrimp chitosan coatings on the fresh-cut mango quality during cold storage. According to the report, 1% chitosan coatings decreased microbial development, maintained firmness, color, weight loss, extended shelf life of up to 15 days and preserved quality attributes of mango. The impact of shrimp chitosan coatings on the shelf life of banana fruit was examined in a different study by Gómez-Estaca et al. (2012). According to the findings, 1% chitosan coatings prevented weight loss, maintained firmness, and inhibited microbial growth.

2.7 Effect of chitin/chitosan on postharvest quality and shelf life of fruits

2.7.1 Chitosan on fruit postharvest

Banana is a climacteric fruit. Due to the presence of ethylene, its ripening process is significantly influenced. Banana fruit's physiological climacteric trait causes a rapid ripening and limited shelf life. To prolong fruit shelf life, edible coatings like chitosan are applied as reported by Dwivany et al. (2020). Yu and Ren (2013) made an emerging approach by using chitosan biopolymer in banana as an edible coating that can increase fruit's shelf life and maintain quality. Green mature banana coated with different concentrations of chitosan showed the effectiveness of 1% chitosan in respect of respiration activity, delaying ripening and progress of decay due to senescence as well as quality and shelf life throughout the storage duration as found by Hossain and Iqbal (2016). Shelf life of mango fruits can be prolonged by using an alternate preservation technique that combines chitosan-pullulan (50:50) and pomegranate peel extract (Kumar et al., 2021). However, Hoque et al., (2017) analyzed that mango fruits covered in thin plastic film showed the longest shelf life. Coating formula made

with 0.5% chitosan, 0.3% Aloe vera gel, and 0.2% papaya leaf extracts was the most successful formula for preserving postharvest quality of mango as evaluated by Nga and Bac (2021).

Chitosan coating (1%) with low-temperature storage (5°C) is the most effective strategy for improving physiological quality and prolonging shelf life of ber fruits (Hesami et al., 2021). Edible coatings of chitosan were applied to sapota fruits to prolong shelf life, prevent postharvest deterioration and quality improvement by Anu et al. (2015). When compared to control, coated sapota fruits exhibited a 9-day shelf life while they decayed in 6 days in control. However, dragon fruit coated with 4% chitosan showed best result in postharvest quality improvement and to prolong shelf life as stated by Prashanth et al. (2022). Strawberries were coated with chitosan at different concentrations and stored at 4°C for 14 days by Tunc et al. (2007). The findings demonstrated that, 1.5% chitosan coatings considerably reduced weight loss, decay incidence and firmness loss. However, 1% chitosan coating on mango showed best result by reducing weight loss, decay incidence, firmness loss and delayed ripening (Kumar and Yadav, 2009; Mali et al., 2006). It (1% chitosan) also reduced respiration rate of longan fruit as evaluated by Supapvanich et al. (2015). However, 2% chitosan coating reduced decay and extend shelf life of strawberry (Du et al., 2009), apple (Zhang et al., 2009) and grape (Oms-Oliu et al., 2011). It (2% chitosan) also maintained firmness and delayed ripening during storage, they added. Therefore, 2% chitosan coating also reduced weight loss, maintained color and improved texture of mangoes and blueberries (Saikia et al., 2021; and Li et al., 2020c). The impact of chitosan coating on the postharvest quality of sweet cherries and strawberry were studied by Zhang et al. (2019a) and Ghaouth et al. (1994). According to their study, 1% chitosan coating greatly reduced weight loss, maintained firmness and prevented mold as well as yeast growth in both fruits.

2.7.2 Chitin nanofiber CNF) on fruit postharvest

A study investigated the effect of CNF coating on the quality and shelf life of blueberries. The results showed that CNF coating delayed ripening, maintained firmness, reduced weight loss, and inhibited mold growth during storage (Liu et al., 2020c). Another study showed that CNF coating-maintained firmness and delayed the occurrence of decay in apples (Ningthoujam et al., 2018). During storage, it also delayed softening, reduced weight loss, and inhibited fungal growth of kiwifruit (Li et al., 2018b) and mango (Paniagua et al., 2018; and Roy et al., 2018). However, Li et al. (2017) found that coating apples with chitin nanofiber slower softening, as well as reduced fungal growth and higher antioxidant activity. A study by Huang et al. (2018)

also investigated the effect of different concentrations of CNFs on the shelf life of strawberries. The results showed that treatment with 0.2% CNF significantly delayed the degradation of kiwifruit and mango, resulting in a longer shelf life (Liu et al., 2019a and Li et al., 2020d). However, Chitin nanofiber at 0.5% effectively inhibited the growth of microorganisms, most effective in shelf life extension and maintained the quality of blueberries during storage (Song et al., 2019) and had higher antioxidant activity and lower respiration rates, leading to better overall quality and longer shelf life of strawberry (Yu et al., 2017). The effect of chitin nanofiber coating on the quality and shelf life of longan fruit during storage was investigated by Wang et al. (2020). The results showed that the chitin nanofiber coating significantly reduced weight loss, maintained the firmness of the fruit, and inhibited the growth of spoilage microorganisms, thereby prolonging the shelf life of the fruit. Zhan et al. (2021) reported that chitin nanofiber coating significantly extends the shelf life of mango fruit during storage. A study conducted by Jayawardena et al. (2020) showed the effect of CNF coatings on the quality and shelf life of mangoes and reported that the CNF coatings reduced the weight loss, maintained the firmness of the fruit, and enhanced the antioxidant activity, resulting in an extended shelf life of up to 12 days. In terms of banana quality and shelf life, a study by Liu et al. (2020a) showed that treating bananas with 0.1% CNF resulted in an extension in shelf life by up to 4 days. According to two different evaluations, 1% CNF coating significantly lengthen shelf life by reducing weight loss, maintained firmness, and preserved the antioxidant activity of the strawberries (Wang et al., 2017) and blueberries (Jiang et al., 2020) when stored at 4°C. However, Peaches coated with 2.5% CNF and stored at 5°C for 15 days (Etxabide et al., 2020) showed significant reduction in weight loss, delayed loss of firmness, and preservation of antioxidant activity in peaches.

2.8 Conclusion

From the reviewed literature, it could be said that, chitosan synthesized from shrimp shell and other edible coatings were effectively used by many researchers to improve quality and shelf life of fruits. However, a very little works has been done with edible coating of Chitin nanofiber on fruit postharvest. In this regard the current study was planned to find out the effect of chitin nanofiber coating on the physicochemical properties and shelf life of popular fruits of Bangladesh.

Chapter 3

Materials and Methods

This chapter describes the materials used and methods followed during conducting the experiment. It includes detailed information about experimental site, storage room, experimental design and layout, varieties and treatments with determination of physical and chemical characteristics of fruit.

3.1 Experimental Site

This experiment was conducted at Horticulture Laboratory, Agrotechnology Discipline, Khulna University, Khulna. The fruits were stored in ambient condition and the average temperature and relative humidity of the storage room was recorded every day.

Duration of experiment

The experiment was conducted from 25th July 2022 to 6th August 2022 at the Horticulture Laboratory of Agrotechnology Discipline, Khulna University, Khulna.

3.2 Storage condition

The minimum and maximum temperature of Horticulture Laboratory ranges between 28°C to 30°C with relative humidity 80-90%. The temperature and relative humidity were recorded with a digital thermo-hygrometer.

3.3 Experimental Materials

Hard green mature mango (var. Nilumbori) and banana (var. Jin) fruits were collected from farmer's orchard at Satkhira, to use as plant material in the experiments. Fruit of uniform size, free from visual symptoms of blemish and disease were collected early in the morning in a bright sunny day. The collected fruits were transported immediately to the Horticulture Laboratory of Khulna University by using air-cooled vehicle.

3.3.1 Jin

Jin is one of the popular banana fruits in Bangladesh mostly cultivated in Khulna region. During the lack period of banana in April-July it is available in the market. Bunches of this variety are pendent. Rachis and peduncle are pubescent. Each bunch contains 6 to 8 hands with 12-16 fingers. The finger is long and curved in the middle. Pedicel of the finger is short and

prominent nipple at the apex. The pericarp is medium thick. Pulp of ripe banana is soft, having fine texture and aroma (Islam and Hoque, 2003).

3.3.2 Nilumbori

Individual weight of Nilumbori mango is about 350g. The length of fruit is 9.26g and breadth is 6.18 cm. Flesh thickness is 2.10 cm while edible portion is about 87.26%. Average yield of Nilumbori mango is 10.15 kg/plant (Rahman and Akter, 2019).

3.4 Experimental Design and Layout

Two separate experiments were conducted using factorial Completely Randomized Design with mango (variety: Nilumbori) and banana (variety: Jin) cultivars. Four concentrations of Chitin nanofibre (0%, 0.1%, 0.3%, 0.5%) extracted from shrimp shell were used as treatments in three replications. For each replication of a treatment, 10 fruits of mango and banana were used.

3.5 Experimental Treatments

Factor A. Treatments

Experiment

Two separate experiments were conducted with two different fruits (mango and banana) which includes different concentration of chitin nanofiber (CNF) and as follows-

T₁= Control

T₂= Chitin nanofiber 0.1%

T₃= Chitin nanofiber 0.3%

T₄= Chitin nanofiber 0.5%

Factor B. Days after treatment (DAT)

3.6 Preparation of the treatments

Initially, chitin nanofiber was prepared (plate 3.1) from shrimp shell by a series of extraction process (Ifuku, 2014) followed by mechanical treatments using high-speed grinder in Forestry and Wood Technology Discipline laboratory, Khulna University. To prepare stock solution, 250 ml CNF and 250 ml distilled water were mixed to make volume up to 500 ml. From stock solution, 0.1%, 0.3% and 0.5% solution were prepared. Solutions were sprayed on mango and

banana fruits immediately after preparation. Each set of mango and banana were placed on newspaper separately.



Plate 3.1 Prepared chemicals of various treatment

3.7 Application of the treatments

Postharvest treatments were applied to randomly selected, visual blemish free mango and banana fruits through hand sprayer (plate 3.2). Chitin nanofiber was applied to both fruits and kept on newspaper at ambient condition. Some fruits as control were also maintained which received no treatments of CNF. Each treatment comprises 10 fruits in each of 3 replications.



Plate 3.2. Application of treatments through hand sprayer

3.8 Observation recorded

Data on physical characteristics, such as cumulative weight loss (%), changes in firmness (score on a scale of 1-6), color (subjective and objective), and shelf life (days), as well as microbiological characteristics, such as disease incidence (%) and severity (%), and chemical characteristics, such as total soluble solids (TSS) (% brix), titratable acidity (TA) (%), vitamin

C content (mg/100g) were measured using standardized techniques. Regular observations of the physical and microbiological features were made. However, data regarding chemical parameters were collected on an alternate day. Data on different parameters of mango were collected for 12 days after treating the fruits. On the other hand, data of banana was collected for 7 days after treating fruits. The gathered information was written down and also saved as Excel files on the computer for the analysis. The procedures of data collection have been described as follows-

3.9 Method of studying parameters

The physicochemical properties and shelf life of mango and banana fruits were studied using the following techniques. During the study period, data on physicochemical parameters were obtained regularly or in every alternate day.

3.9.1 Weight loss (%)

Weight loss (%) was determined according to Kader (2005). The weight of mango and banana was measured daily during storage and the weight difference was compared day to day. Mango and banana were selected at random from each treatment. The weight was measured using an electronic balance (plate 3.3) and then it was converted into a percentage value. The evaluation of weight reduction was done by using the following equation:



Plate 3.3. Electrical balance

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final Weight}}{\text{Initial weight}} \times 100$$

3.9.2 Changes in color

3.9.2.1 Visual assessment

The changes in color of banana and mango were determined using a numerical rating scale of 1-7, where 1 = green, 2 = breaker, 3 = up to 25% yellow, 4 = 25-<50% yellow, 5 = 50-<75% yellow, 6= 75-100% yellow and 7= blackened (Hassan, 2006).

3.9.2.2 Assessing Fruit color by using a Chromameter

The skin color of sample fruit was also recorded on color coordinates as L^* , a^* and b^* and hue angle (h°) from opposite positions of each fruit in Commission International de L'Eclairage (CIE) units using a chromameter (plate 3.4).

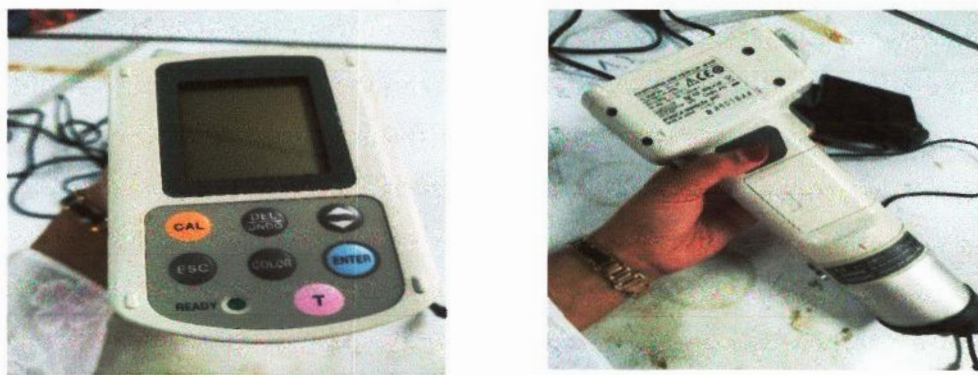


Plate 3.4. Chromameter

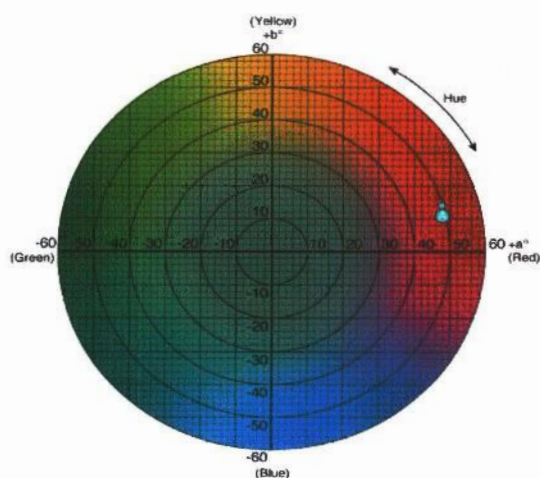


Figure 3.1 Color chart (Hunter and Harold, 1987)

Chromaticity L^* represents the lightness of the fruit color, which ranges from 0 (black) to 100 (white). Chromaticity a^* denotes the redness ($+a^*$) or greenness ($-a^*$), while chromaticity b^* denotes the yellow ($+b^*$) or blue ($-b^*$) color of fruit skin (Figure 3.1). Red was at an angle of

0° or 360° for the sake of color interpretation, yellow at 90°, green at 180°, and blue at 270°. Hue angle (h°) values were obtained using the formula: $h^\circ = \tan^{-1} b^*/a^*$. Chroma (C*) was computed as follows: $C^* = (a^{*2} + b^{*2})^{1/2}$. It was characterized as the intensity of color saturation ranging from a dull to a vibrant color (low to high values, respectively) (McGuire, 1992).

3.9.3 Fruit firmness

Firmness of banana and mango was determined by hand feeling using a numerical rating scale of 1-6 where, 1= mature hard, 2= sprung, 3= between sprung and eating ripe, 4= eating ripe, 5= over ripened 6= totally unfit for consumption. Similar method was mentioned by Hassan (2006).

3.9.4 Disease incidence

Disease incidence means the number, indicating the how many fruits in a population or sample that is affected by the disease. Black spots or strips and visible symptoms were considered as disease on banana while black spot, hole or concentric ring in mango for determining disease incidence as stated by Ullah (2007). Disease infected fruits were counted on the basis of eye estimation.

3.9.5 Disease severity

Disease severity refers to the extent or intensity of damage caused by a disease on a plant or fruit according to Mohamed et al. (2017). All the infected fruits were selected to determine percent fruit area infected and data were collected on daily basis. The percentage of fruit area diseased was measured based on eye estimation. The mean values regarding infected fruit area were calculated, presented and discussed in result and discussion chapter.

3.9.6 Titratable Acidity (TA) (%)

Both organic and inorganic acids are present in mango and banana fruits. These acids are vital elements of fruits since they, along with carbohydrates, affect the fruit's quality and flavor. When these fruits are harvested, their acid content, along with their sugar content or the amounts of soluble solids, are used to determine their maturity stage. It was determined by using the following equation as suggested by Sugri et al. (2013).

$$\text{Malic acid (\%)} = \frac{0.0067 \times \text{Vol. of NaOH} \times 30 \times 100}{5 \times 10}$$

Here,

0.0067 = Milli-equivalent weight of malic acid

30 = Total volume (mL)

5 = Extract juice sample (mL)

10 = Volume of aliquot (mL)

Reagents:

A. Sodium hydroxide (NaOH): 0.1 normality (N)

B. Indicator: Phenolphthalein ($C_{20}H_{14}O_4$) [approx. 0.5 % in 80% ethanol (W/V)]

Equipment used:

10 milliliter pipette, 25 ml burette, small glass funnel, dropper, blender, 250 ml measuring cylinder, burette stand and beaker.

Procedure:

30 ml of fruit sample by weight was taken and extracted through. Then Extract was filtered and the filtrate was made up to 100 ml with distilled water. 10 ml fruit juice and 20 ml distilled water were diluted and made volume up to 30 ml. 5 ml of aliquot was taken from 30 ml juice. Only 1-2 drops of phenolphthalein solution were added. Titration was done through a burette filled with the sodium hydroxide (plate 3.5) and end point was determined by the appearance of pink color (plate 3.5) and its persistence for at least 10 to 15 seconds.



Plate 3.5. Conducting Titration

3.9.7 Total Soluble Sugar (TSS)

Equipment

Fruit juice was extracted from pulp through hand mortar (plate 3.6) and the value was measured using a refractometer (REF 105) (plate 3.6) and was referred to as the degree of Brix. This tested the concentration of sucrose in fruit extract. It helps to determine the concentration of sugar in the products.



Plate 3.6. Extraction of juice from pulp and hand refractometer

Procedure

A drop of juice from the fruit under study was placed on the prism of the refractometer and percentage of total soluble solid (TSS) (%) was obtained from direct reading.

3.9.8 TSS: TA ratio

TSS: TA ratio was calculated by dividing SSC (%) with the corresponding TA (%) value.

3.9.9 Assessment of Vitamin C

Principle

Titrimetric estimation of vitamin C was conventionally done by using 2,6-dichlorophenol indophenols dye solution. This dye is blue in alkaline solution and red in acidic solution. Ascorbic acid reduces the dye to a colorless form. Reaction is quantitative and specific for ascorbic acid at pH 1.0 -3.5.

Reagents

Meta-phosphoric acid: 3% in distilled water (W/V), 2,6-dichlorophenol indophenols solution. About 42 mg sodium bicarbonate was added to it. Then it was dissolved in little amount of

distilled water and the volume was made up to 200 ml. Standard ascorbic acid solution: Only 0.01% in 3 percent meta-phosphoric acid (W/V).

Procedure

30ml juice were taken after juice extraction from fruits by grinding. Then separated into 3 parts, each part contained 10 ml juice. Then it was titrated with 2,6 dichlorophenol indophenol solution. Volume of metaphosphoric acid was 5ml and standard ascorbic acid solution (0.01%).

Formula for determining Vitamin C:

According to Nerdy (2018), vitamin C was determined by the following formula-

$$\text{Vitamin C (mg/100g)} = \frac{e \times d \times b}{c \times a}$$

Here,

a = Weight of sample

b = Volume made with metaphosphoric acid

c = Volume of aliquot taken for estimation

d = Dye factor

e = Average burette reading for sample

3.9.10 Shelf life

On the basis of the physical parameters such as weight loss, change in color, firmness, disease incidence and disease severity, the shelf life of fruit was determined. These characteristics were assessed through eye observation, and the evaluation criteria were established from the perspective of the consumer to evaluate whether the fruit was suitable for purchase or not. Accordingly, the average days needed for a cumulative loss of 20%, a change from dark to light red, the retention of at least eating softness, and a minimal amount of rotten fruit (maximum 5%) were indicated as the fruits' shelf life (Tabassum et al., 2018).

3.10 Statistical analysis

The collected data were statistically evaluated using the STAR (Statistical Tool for Agricultural Research) software and the Analysis of Variance was done as one or two-way method of analysis. According to Gomez and Gomez (1984), the LSD was used to evaluate the mean differences at a 5% level of significance.



Chapter 4

Results and Discussion

In this chapter, the findings from two different study on the physicochemical characteristics of mango and banana treated with various concentrations of chitin nanofiber have been presented and discussed.

4.1 Effect of chitin nanofiber on physical characteristics of banana and mango

Data on cumulative weight loss, color, firmness, disease incidence and disease severity of mango and banana fruits treated with different concentration of chitin nanofiber were recorded every day during storage at room temperature. The findings have been presented below-

4.1.1 Effect of chitin nanofiber on cumulative weight loss of banana and mango

The total weight loss (%) of banana samples gradually increased over time (Figure 4.1). CNF coating reduced cumulative weight loss during the storage period from DAT 1 to DAT 7. As a postharvest treatment, T₃ (CNF 0.3%) coating showed lower level of weight loss (%) of banana (27.74%) which was 0.96 parts lower than average cumulative weight loss on DAT 7. It was also found that, the weight loss of T₃ treated fruit was 0.98 parts lower than T₁ treated fruit on the final day of data collection (DAT 7).

Percent weight loss during storage of mango also showed significant differences (Figure4.1). The minimum weight loss (20%) occurred in fruits treated with T₄ (CNF 0.5%) as compared with untreated fruit (T₁) (24.9%) which is 0.83 parts lower than average cumulative weight loss on DAT 10.

Increase in cumulative weight loss of fruit during storage was examined by many researchers. Khaliq et al. (2016) reported that weight loss of untreated mango fruits gradually increased during storage whereas chitosan coating significantly reduced weight loss which is similar to the findings from the current study. Chitosan-coated bananas showed less weight loss than control in case of shrimp chitosan coating but commercial chitosan reduced weight loss up to 65% in mango (Hossain and Iqbal 2016; Parvin et al., 2023; Sikder et al., 2019).

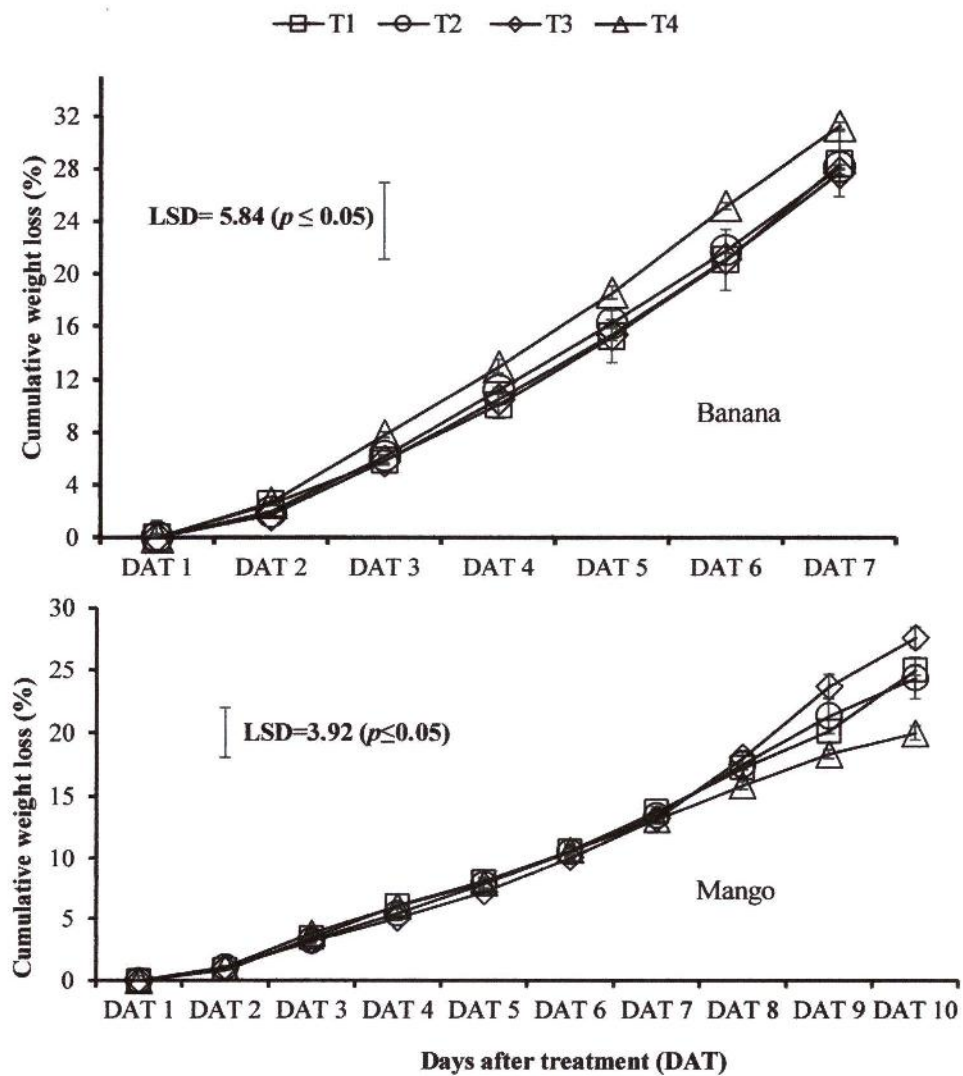


Figure 4.1: Effect of chitin nanofiber on cumulative weight loss of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

4.1.2 Effect of chitin nanofiber on firmness of banana and mango

In case of banana, rapid loss of firmness was observed from DAT 3 (average score 2.29) to DAT 7 (average score 4.11) (Figure 4.2). All the treatments except control (T_1) showed slower rate of decrease in firmness. The results associated with fruit firmness as influenced by CNF coatings showed that maximum fruit firmness was retained in fruits treated with T_3 (CNF 0.3%) (3.55) followed by the fruits treated with T_2 (CNF 0.1%) (3.77) and minimum fruit firmness was noticed in untreated control fruits (T_1) (4.89) on DAT 7. The result showed that there was significant difference ($p \leq 0.05$) in firmness loss among treated and untreated banana fruits. This observation is similar to Gómez-Estaca et al. (2012). The results showed that 1% CNF coating significantly maintained the firmness compared to the control group.

Rapid loss of firmness occurred in mango during ripening period (Figure 4.2). T_2 showed maximum loss of fruit firmness (5) and lowest in T_3 (3.67) compared to uncoated control (T_1) (3.66). Similarly, Sturzeanu (2020) did not get significant difference in case of fruit firmness among seven strawberry varieties. Aziz et al. (2021) found rapid decrease in firmness in mango when coated with 1% chitosan which is similar to our study. But mango treated with 1.5% and 2% chitosan showed slower firmness decrease rate they added.

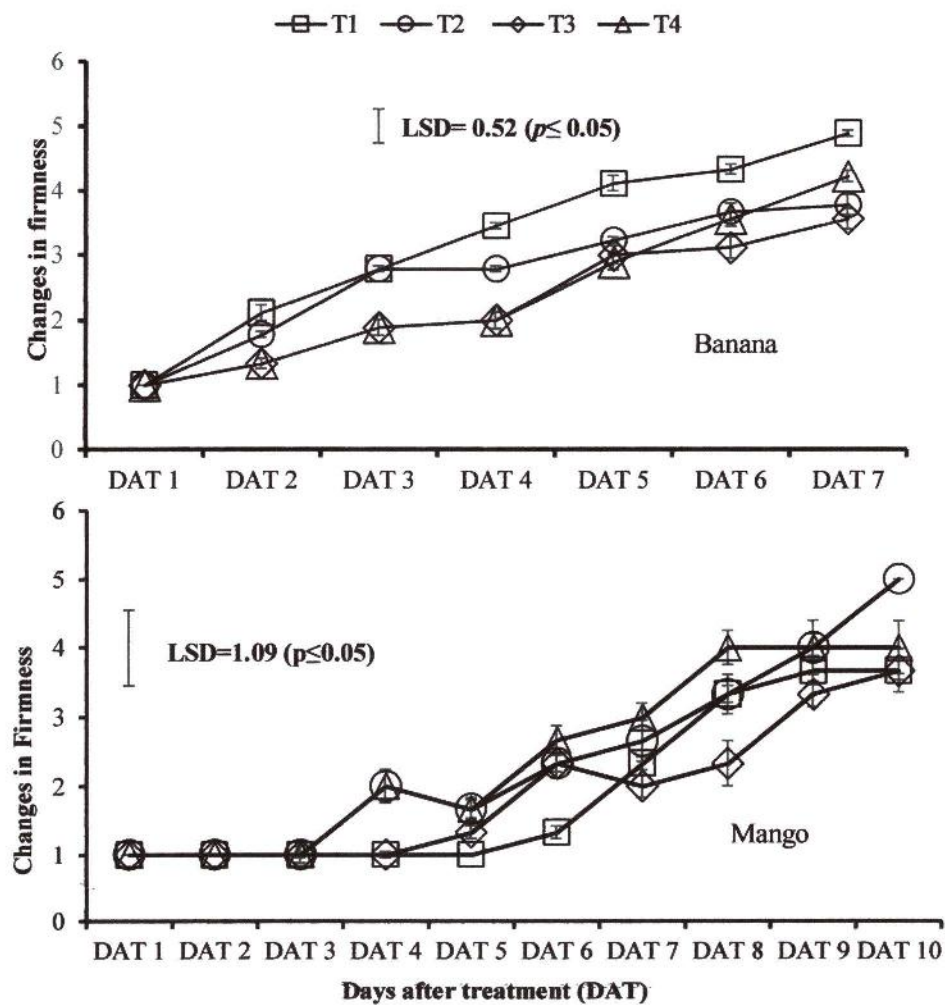


Figure 4.2: Effect of chitin nanofiber on firmness of banana and mango
 T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

4.1.3 Effect of chitin nanofiber on visual color of banana and mango

On 7th day of storage, CNF coated sample showed lower color score (4.00) in visual observation which indicates lower color development than control sample (4.89) (Figure 4.3). Among all the treatments, 0.3% CNF coating delayed the color development by 2 days in banana in comparison to the control fruits. Ali et al. (2011) also found similar result in papaya fruit treated with higher concentrations of chitosan. According to their evaluation, slow rate of respiration and decreased ethylene production could be responsible for the delayed color development, which also delayed fruit ripening and senescence.

Mango coated with CNF 0.5% showed slower rate of color change (3.36) compared to control on DAT 10 (3.63) (Figure 4.3). All the treatments delayed color development of mango except CNF 0.1%. The result of the present study is also supported by the findings of Robinson (1996) who stated that the faster rate of color change of mango under control treatment may be due to the rapid activity of some enzymes that are responsible for the color changes of mango. These findings are also similar to Doreyappa-Gowda and Huddar (2001) who reported that the green peel color of mature Alphonso and other varieties of mango turned from light green to yellow due to the breakdown of chlorophyll as a result of series of physicochemical changes during ripening.

4.1.4 Effect of chitin nanofiber on subjective color of banana and mango

4.1.4.1 Changes in color L*

Figure 4.13 shows that the effect of CNF edible coatings to chroma color L* of banana from DAT 1 to DAT 7 (Figure 4.4). Banana coated with CNF 0.3% increased fruit lightness significantly ($p \leq 0.05$) (53.88) compared to control (35.33) which is 1.13 times higher than average L* value of it on 7th DAT. Similar observation was also stated by Purwoko et al. (2002) who reported chitosan coating delayed the decreases in L* of banana during storage.

After 7 days of storage of mango, most of the treatments led to increases in L* when compared to the control, with the exception of the 0.3% CNF (Figure 4.4). Treatments with CNF 0.5% provided the best results (50.33) on 7th day of storage regarding the maintenance of fruit lightness. According to Ngo et al. (2021), a gradual decrease in color L* means dark color with ripening of fruits and beginning to spoil. It has been reported by Petriccione et al. (2015) that application of chitosan on strawberry delayed the decreases in L* compared to control which is similar to our observation.

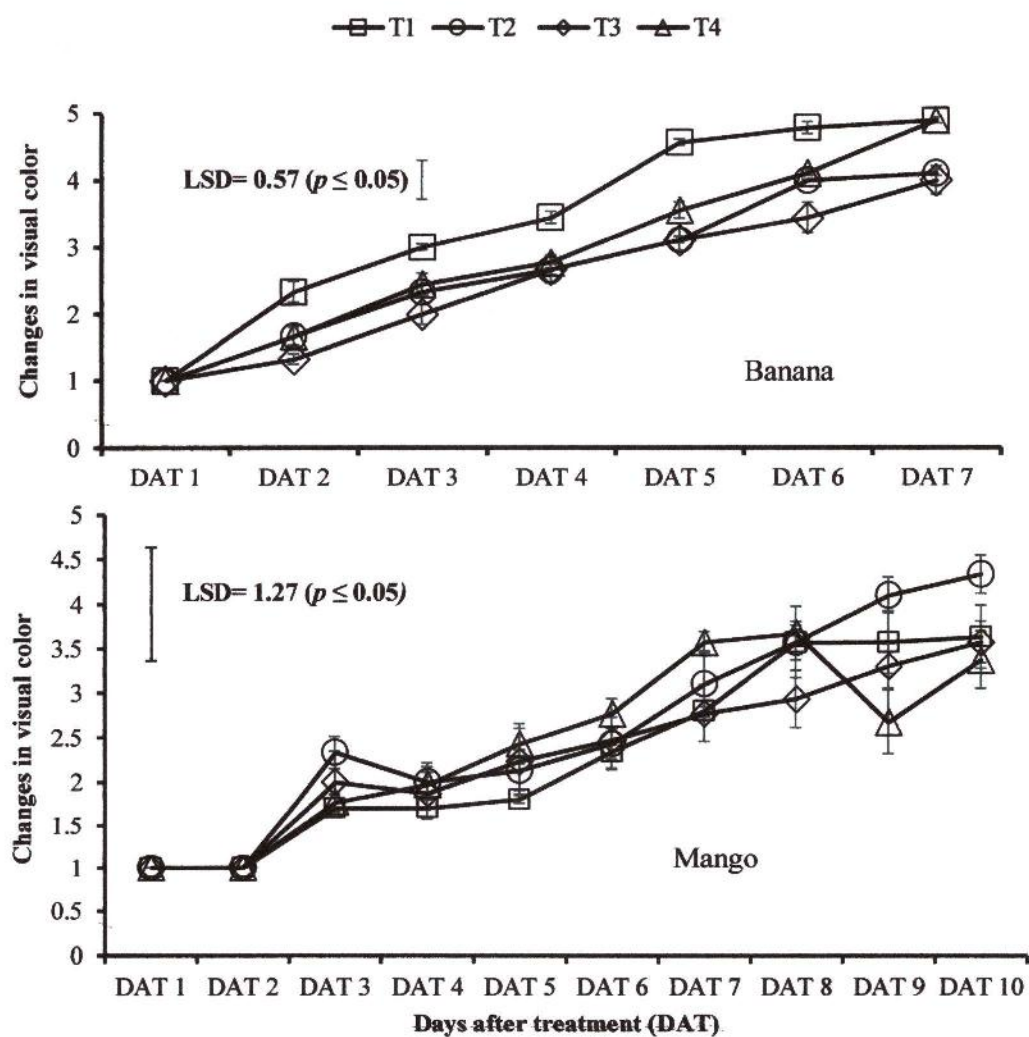


Figure.4.3: Effect of chitin nanofiber on visual color of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

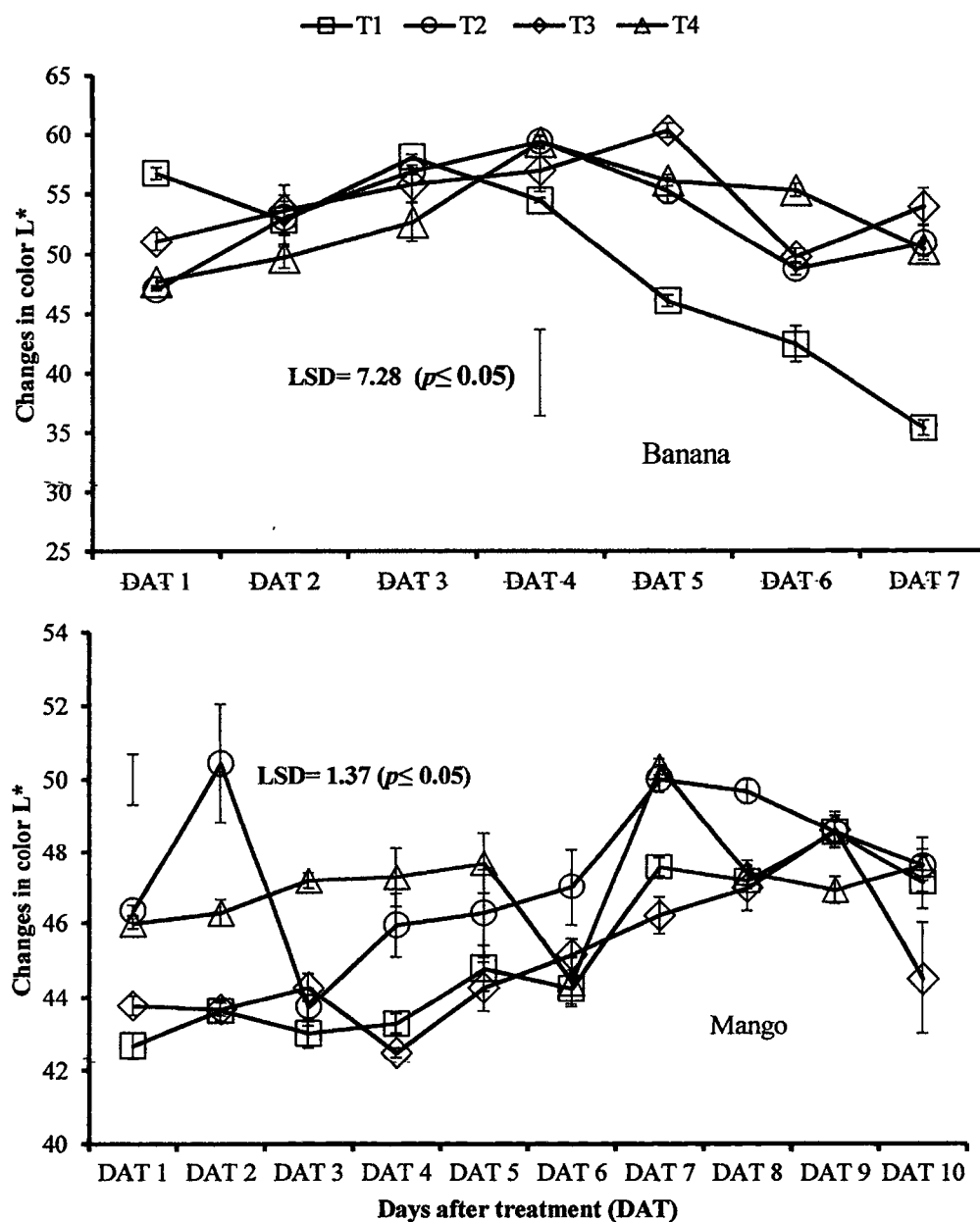


Figure 4.4: Effect of chitin nanofiber on color L* of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

4.1.4.2 Changes in color a*

All banana fruits treated with CNF showed slower decrease in color a* value from DAT 1 to DAT 7 compared to control (Figure 4.5). Value of a* was more negative in fruits coated with CNF 0.3% indicating the mango peel as greener. Mango fruits coated with CNF 0.3% also showed more negative value of a* than uncoated fruits. From DAT 1 to DAT 10, CNF 0.3% treated fruits retained maximum negative score which is the indication of green mango peel retention. Similar observation was also found by Ngo et al. (2021). According to their evaluation a* value was more negative in fruits coated with nano chitosan.

4.1.4.3 Changes in color b*

CNF 0.3% coated banana fruits showed increasing trend of changes in color b* during storage (Figure 4.6). The delayed decrease of b* value indicates a remain in yellowness of samples (Ngo et al., 2021). On DAT 7, maximum retention of color b* (25.61) was observed for banana coated with CNF 0.3% compared to control (22.50). There was no significant difference between T₁ (22.50) and T₂ (22.73) in respect of b*.

On DAT 10, mango fruits treated with CNF 0.1% retained maximum yellow color (28.90) compared to other treatments (Figure 4.6). These results are similar to the findings of Ngo et al. (2021). According to them fruits coated with nano chitosan retained yellow color compared to control up to 15 days of storage.

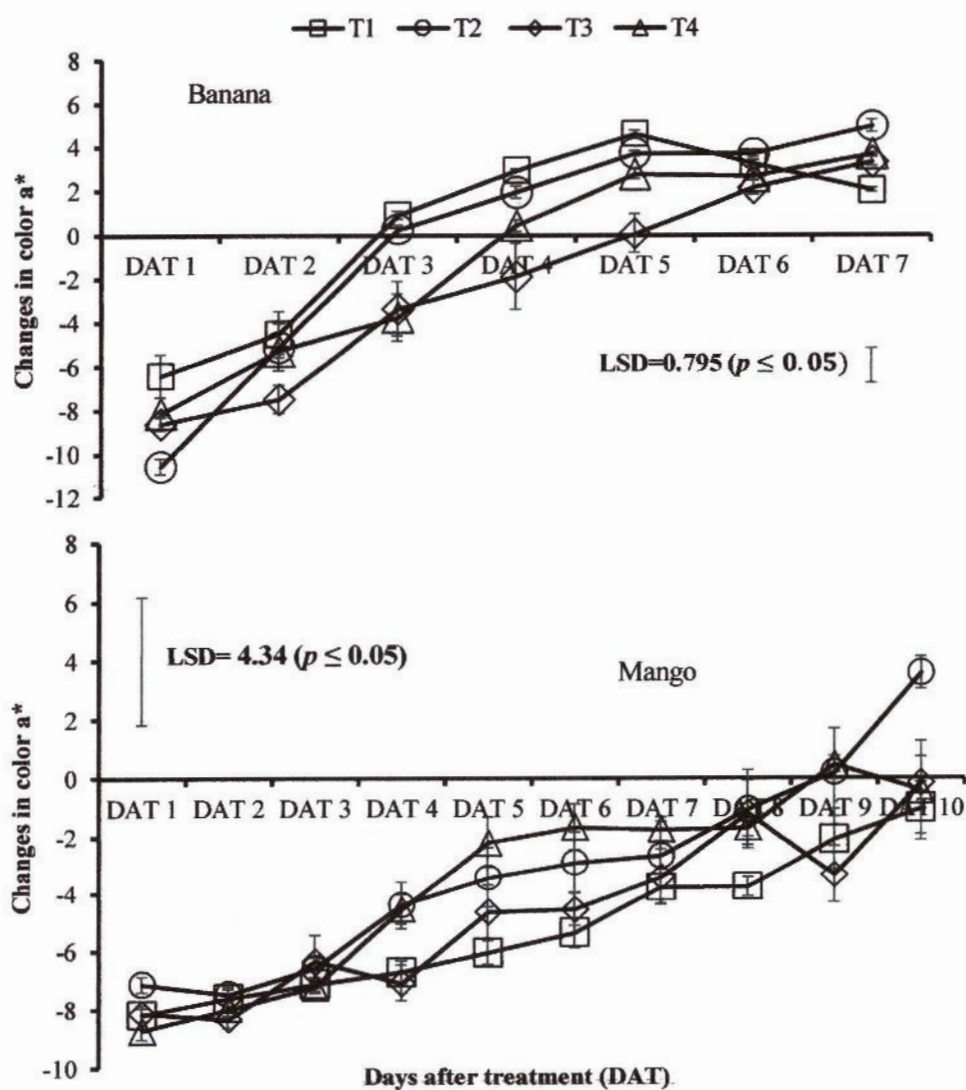


Figure 4.5: Effect of chitin nanofiber on color a^* of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

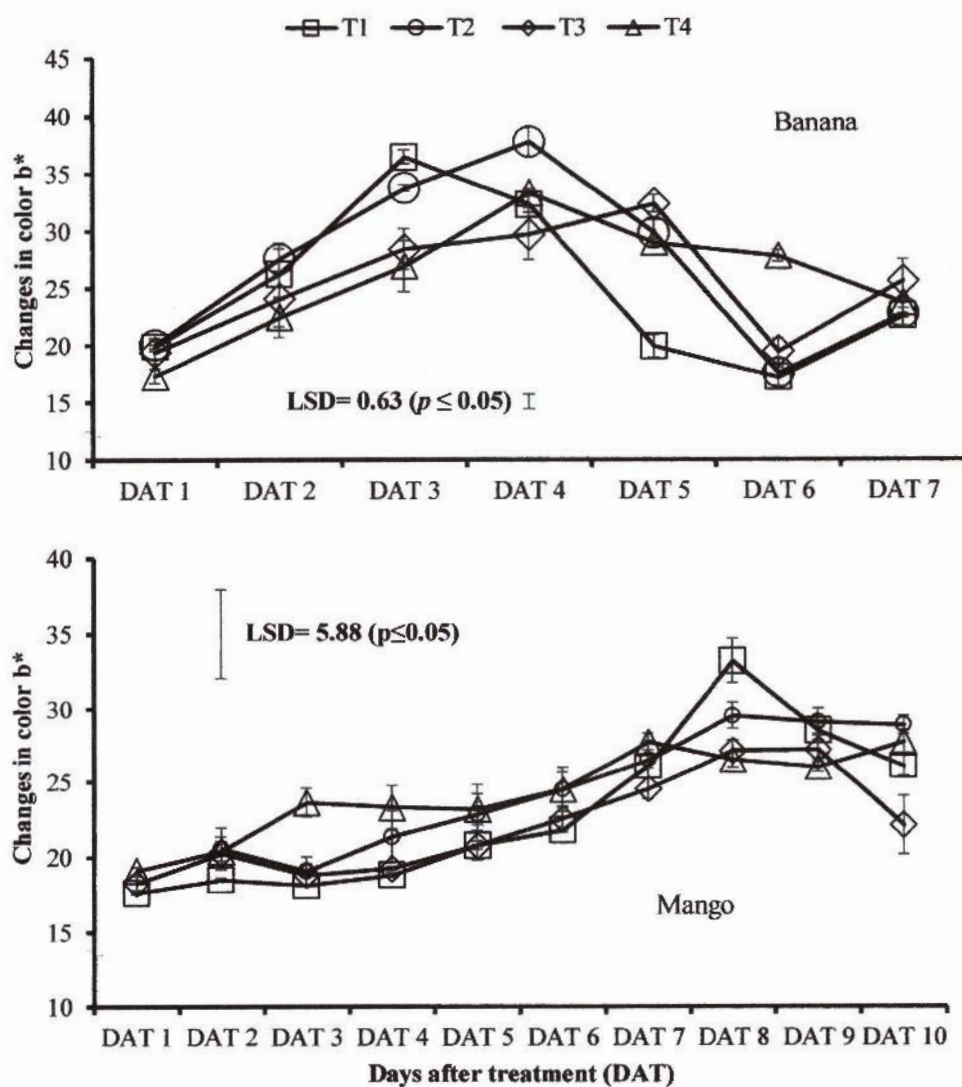


Figure 4.6: Effect of chitin nanofiber on color b* of banana and mango

T₁ = Control, T₂ = CNF 0.1%, T₃ = CNF 0.3%, T₄ = CNF 0.5%

4.1.4.4 Changes in color c^*

Changes in color c^* of banana shows increasing trend from DAT 1 to DAT 4 (Figure 4.7). On 6th day of storage, maximum value of color c^* (27.87) was recorded in T₄ (CNF 0.5%) treated fruits in comparison to all of the treated fruits including control (17.93). There were no significant differences among other treatments irrespective of days after treatment. Lower c^* value indicates less vivid as stated by Koyuncu (2004). According to his observation, the c^* value of 'Cavendish' variety decreased with increased storage time. Our evaluation is different from the study due to high temperature condition.

From DAT 1 to DAT 10, mango fruits treated with CNF led to increase in color c^* . After 10 days of storage, 0.1% CNF treated fruits retained maximum color c^* when compared to control (Figure 4.7). Lowest retention of color c^* was observed in T₃ treated fruits. Pelayo et al. (2003) found that values of c^* decreased in strawberries during the storage period. This observation is not similar to our study due to varietal differences and different environmental conditions.

4.1.4.5 Changes in color h°

Regarding the hue angle of banana (h°), CNF coated fruits delayed development of red color during storage. On 7th day of storage, 0.3% CNF coated fruits retained maximum h° value (81.91) compared to control (75.47) (Figure 4.8). All the treatments showed decreasing trend during storage period. According to Koyuncu (2004) lower h° value indicates more red color. He also found higher h° values of strawberry with increased storage time which is similar to our study.

On DAT 10, maximum value of color h° was observed in mango treated with CNF 0.1% (Figure 4.8). Color h° showed increasing trend during storage period which is not similar to Koyuncu (2004) due to varietal differences and high storage temperature.

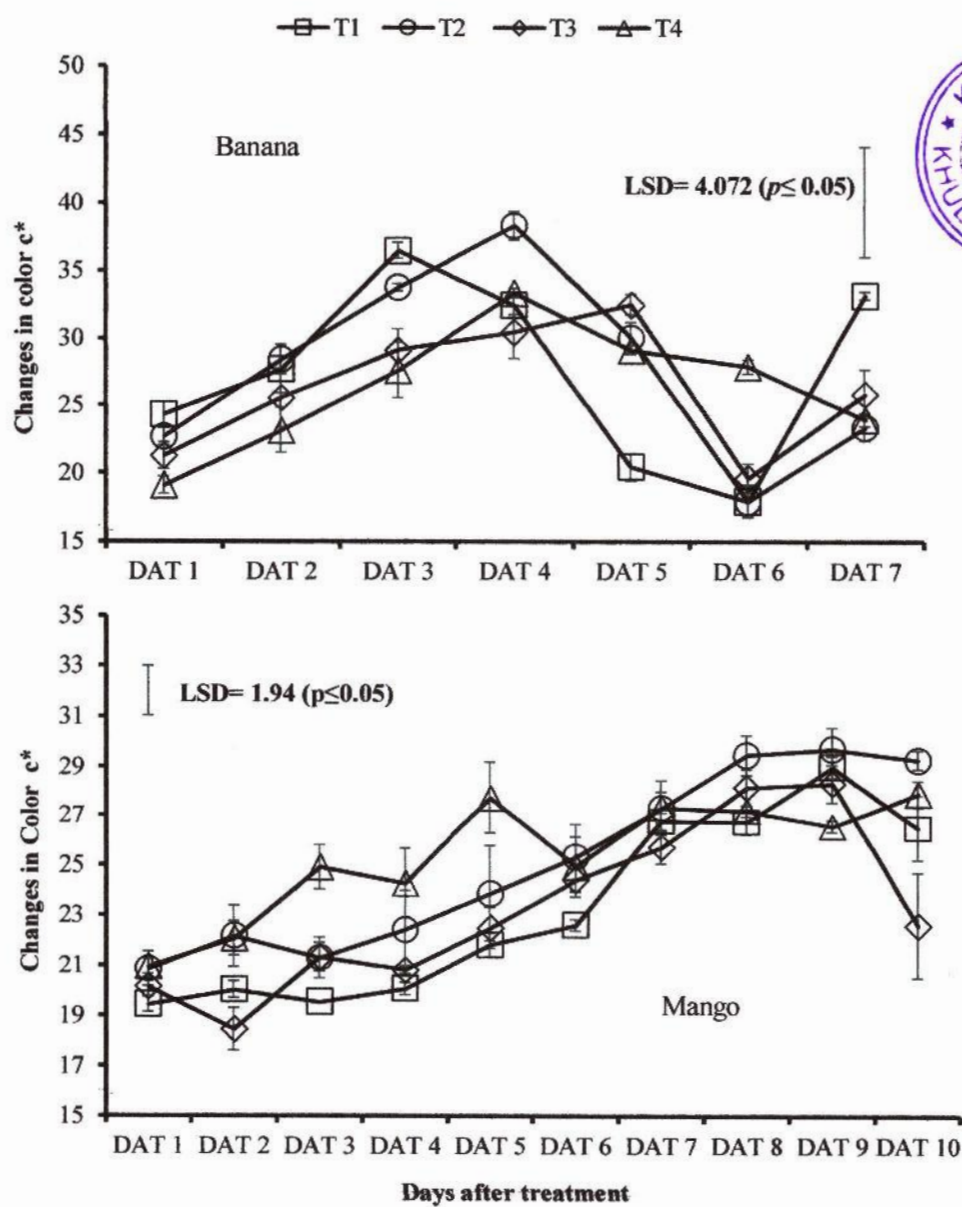


Figure 4.7: Effect of chitin nanofiber on color c^* of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

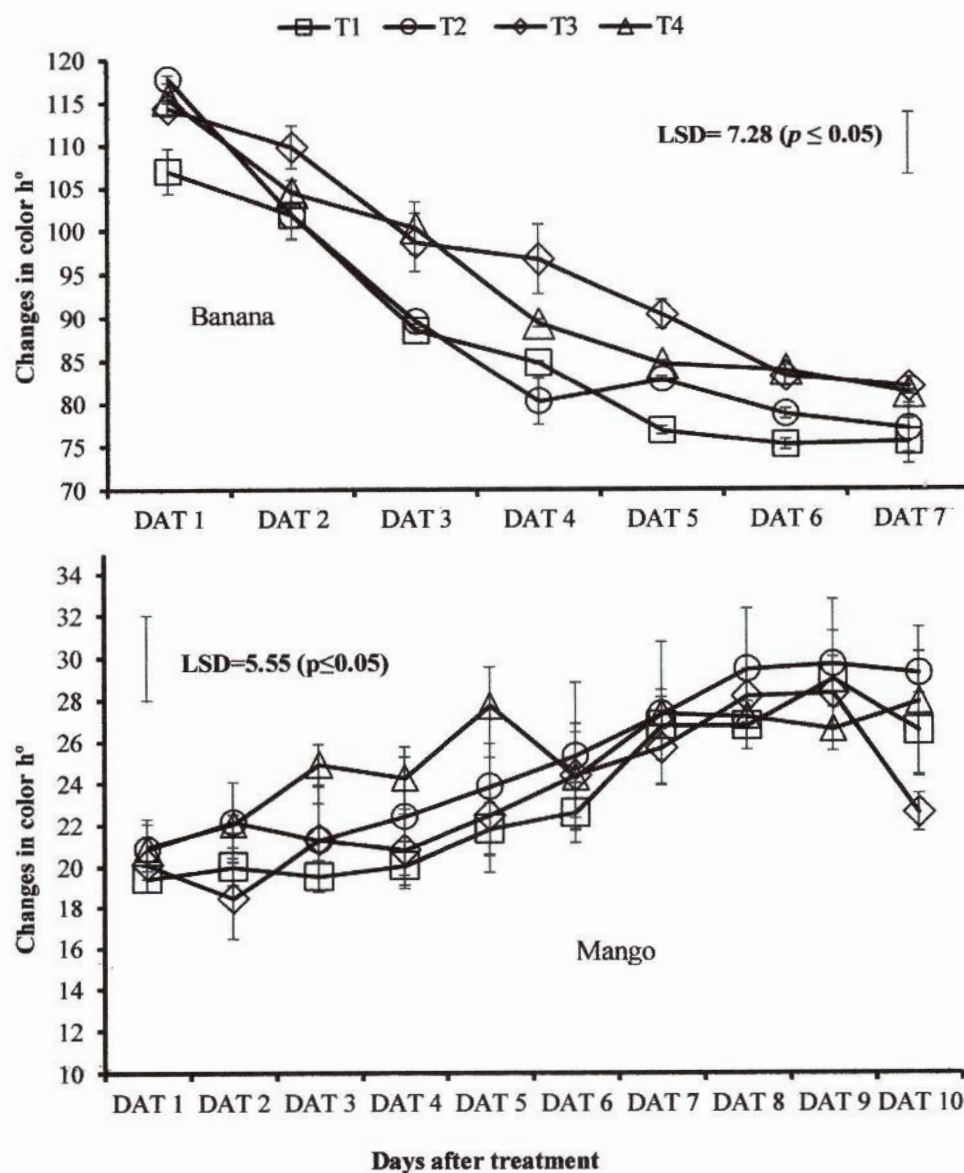


Figure 4.8: Effect of chitin nanofiber on changes in color h° banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

4.2 Effect of chitin nanofiber on microbial characteristics of mango

4.2.1 Effect of chitin nanofiber (CNF) on disease incidence of mango

CNF treatments was found effective against disease incidence in mango during postharvest storage period (Figure 4.9). Disease incidence was mostly observed from DAT 5 to DAT 10. The control fruits (T_1) were affected 8.67%, after 10 days of storage while T_3 (CNF 0.3%) coated fruits were affected 8% which is 0.93 parts lower than average disease incidence on that day. A study by Azeredo et al. (2019) evaluated the effect of CNF coatings on mangoes. The results showed that 1% CNF coatings significantly reduced the decay rate compared to control. Our study is not similar to the result due to the variation in concentration of CNF. Higher concentration may effectively reduce disease incidence of mango during storage.

4.2.2 Effect of chitin nanofiber on disease severity of mango

Disease severity of the samples gradually increased from the first day to the entire storage period of 10 days. Initiation of disease was noticed mostly from DAT 5. The trend of severity has been shown in Figure 4.10. As a postharvest treatment, 0.1% CNF coating showed a significant effect (at $p < 0.05$) on reducing the disease severity of mango (62%) than uncoated samples which showed 1.30 times higher rate of severity (80.87%) during the entire storage period. The research conducted by Ali et al. (2021) also demonstrated that chitin nanofiber-based coatings effectively control postharvest disease severity and extend the shelf life of mango fruits. Singh et al. (2019) also reported that CNF coatings effectively reduce disease severity of mango.

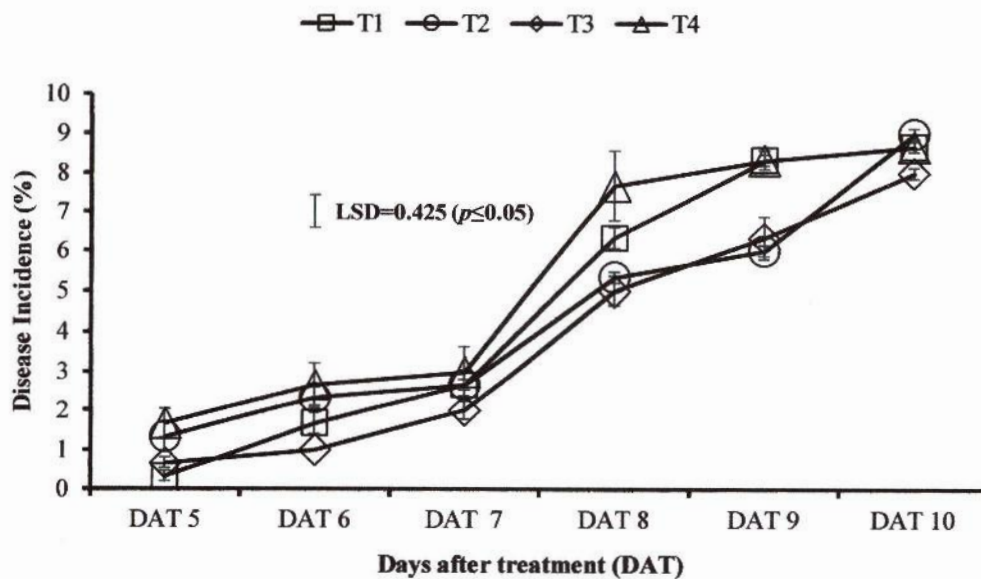


Figure.4.9: Effect of chitin nanofiber on disease incidence of mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

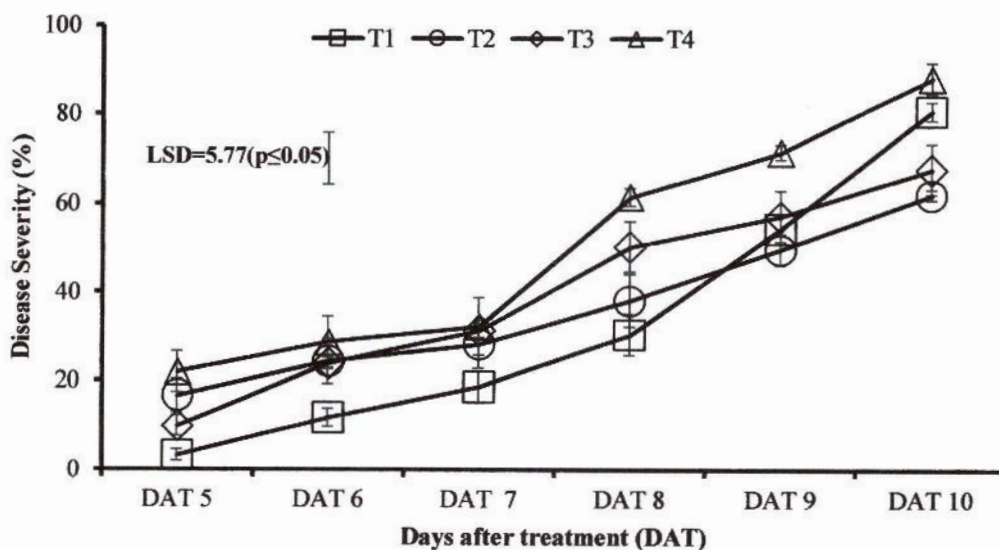


Figure.4.10: Effect of chitin nanofiber on disease severity of mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

4.3 Effect of chitin nanofiber on chemical characteristics of banana and mango

TA (Titratable Acidity), TSS (Total Soluble Sugar), TA:TSS ratio and Vitamin C were included in chemical characteristics which were measured on every alternate day. The result has been presented as follows-

4.3.1 Effect of chitin nanofiber on titratable acidity (TA) of banana and mango

The optimal value of TA gradually reduced in banana from DAT 1 to DAT 5 regardless of any treatment (Figure 4.11). Better retention of titratable acidity was observed in T₂ (0.1% chitin nanofiber coated bananas) than other treatments which is 1.23 times higher in respect of average TA value on DAT 7. Compared to uncoated sample of green mature banana fruit, 1% chitosan coated banana also showed a decreasing trend in changes in titratable acidity (Hossain and Iqbal, 2016; Sikdar and Islam, 2019).

The titratable acidity of mango decreased along with increased storage time in both coated and uncoated fruits (Figure 4.11). These results agreed with those reported by Garcia et al. (1998). According to them, the decrease of acidity during storage demonstrated fruit senescence. Similar findings observed by Wang et al. (2006) as they reported that TA of mango did not change significantly after 1-MCP treatment.

4.3.2 Effect of chitin nanofiber on Total soluble solids (TSS) on mango and banana

The total soluble solids of the samples gradually increased over time (Figure 4.12). The increase in total soluble solids could be attributed to the conversion of polysaccharides into simple sugars and the degradation of pectic substances in soluble solids during storage (Moazzem et al., 2019). The effect of different CNF coating on total soluble solids during the storage period is depicted in figure 4.12. As a postharvest treatment, 0.1% CNF coating retained maximum total soluble solids in banana (15.50) than other CNF treatments during the entire storage period while lowest retention was observed in untreated control samples (22.67). Similarly, 1% chitosan coating with low storage temperature retained TSS of banana fruits as reported by Sikdar and Islam (2019).

Chitin nanofiber coating as postharvest treatment on mango showed significant effect on total soluble solids during storage (Figure 4.12). CNF 0.5% retained maximum TSS of mango (11.67) while lowest retention was found in untreated control (25.76) on DAT 10. The TSS value of CNF 0.5% treated fruit was 0.63 parts lower than average value on 10th DAT which

indicates lower respiration and metabolic activity of treated fruits compared to control (Jiang and Li, 2001).

4.3.3 Effect of chitin nanofiber on Vitamin C of banana and mango

The vitamin C content of uncoated banana fruits (T_1) increased on the 3rd day of storage after treatment (Figure 4.13). This result is in agreement with the reports of Aina (1990) and Hossain et al. (2014), who reported that vitamin C content peaked on the 4th day of storage and decreased afterwards. Vitamin C content of the 0.1% CNF coated bananas was higher (7mg/100g) than those of the uncoated fruits (6.2mg/100g) after 7 days of storage (Figure 4.13). The coatings were effective in reducing vitamin C loss in banana during storage. However, Suseno et al. (2014) reported reduced loss of vitamin C in Cavendish banana while they used 2% chitosan edible coating with 80% deacetylation.

At DAT 10, the highest amount of vitamin C (40mg/100g) was observed for control mangoes, whereas a decrease in Vitamin C content was recorded in CNF-treated mangoes on 10th DAT. According to Parvin et al. (2023), 0.1% chitosan coatings greatly improved vitamin C retention in mango fruit compared to control. However, due to varietal differences and high storage temperature, our evaluation is not similar to their study.

4.3.4 Effect of chitin nanofiber on TA:TSS ratio of banana and mango

Banana fruits showed decreasing trend of TA:TSS ratio from DAT 1 to DAT 7 irrespective of the treatments (Figure 4.14). On 7th day of storage, maximum value of TA/TSS was observed in 0.1% CNF treated fruits (0.091). There were no significant differences among treated and untreated fruits during storage. Suriyan (2011) found slight decline in TA:TSS ratio of intact fruit which was not statistically significant during storage. This evaluation is similar to our study.

TA:TSS ratio of mango fruits treated with CNF also decreased during storage period. On DAT 6, statistically significant differences ($p \leq 0.05$) in TA:TSS ratio were found among coated and uncoated fruits. On 10th DAT, maximum retention of TA:TSS ratio (0.017) was observed in 0.5% CNF treated fruits in comparison to other treated fruits including control. Voča et al. (2008) observed statistically significant differences in TA:TSS ratio of strawberry fruits which is similar to our study.

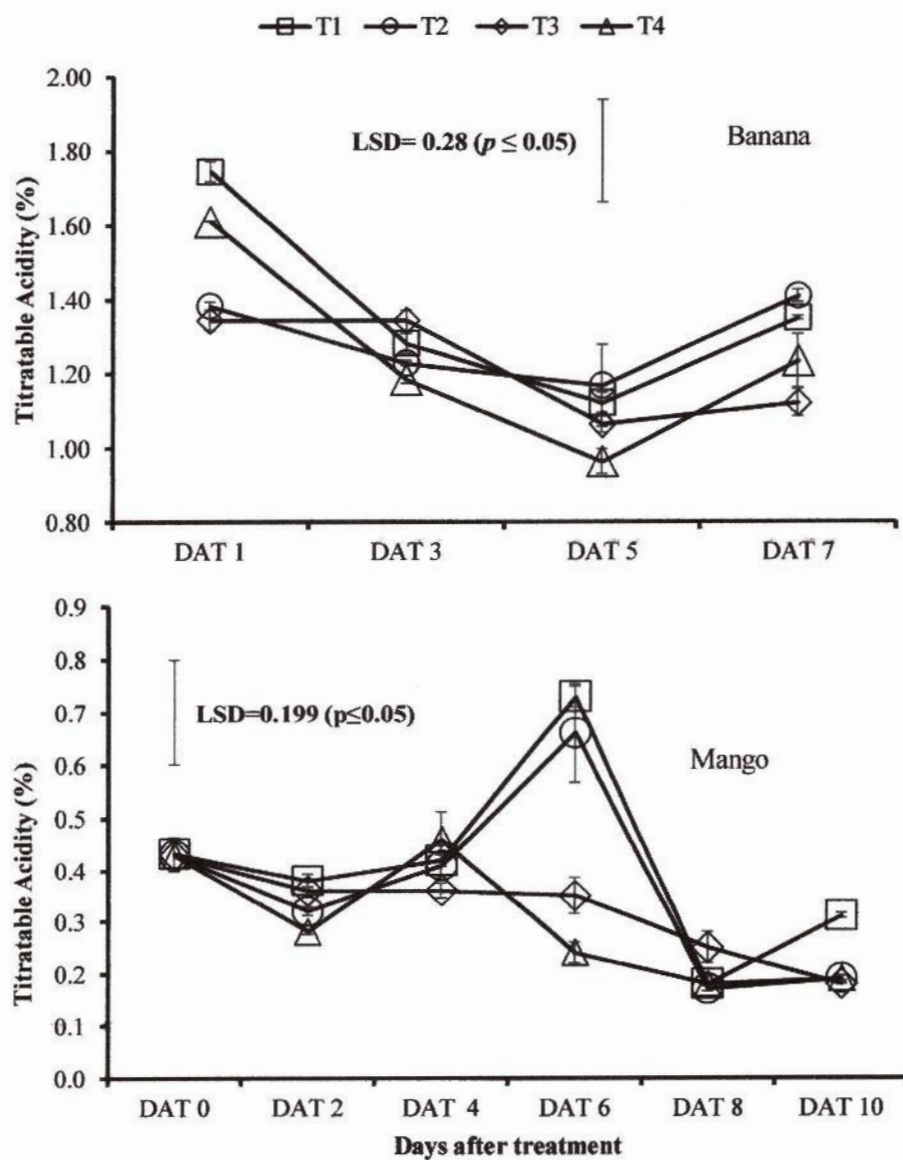


Figure.4.11: Effect of chitin nanofiber on Titratable acidity of banana and mango
 T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

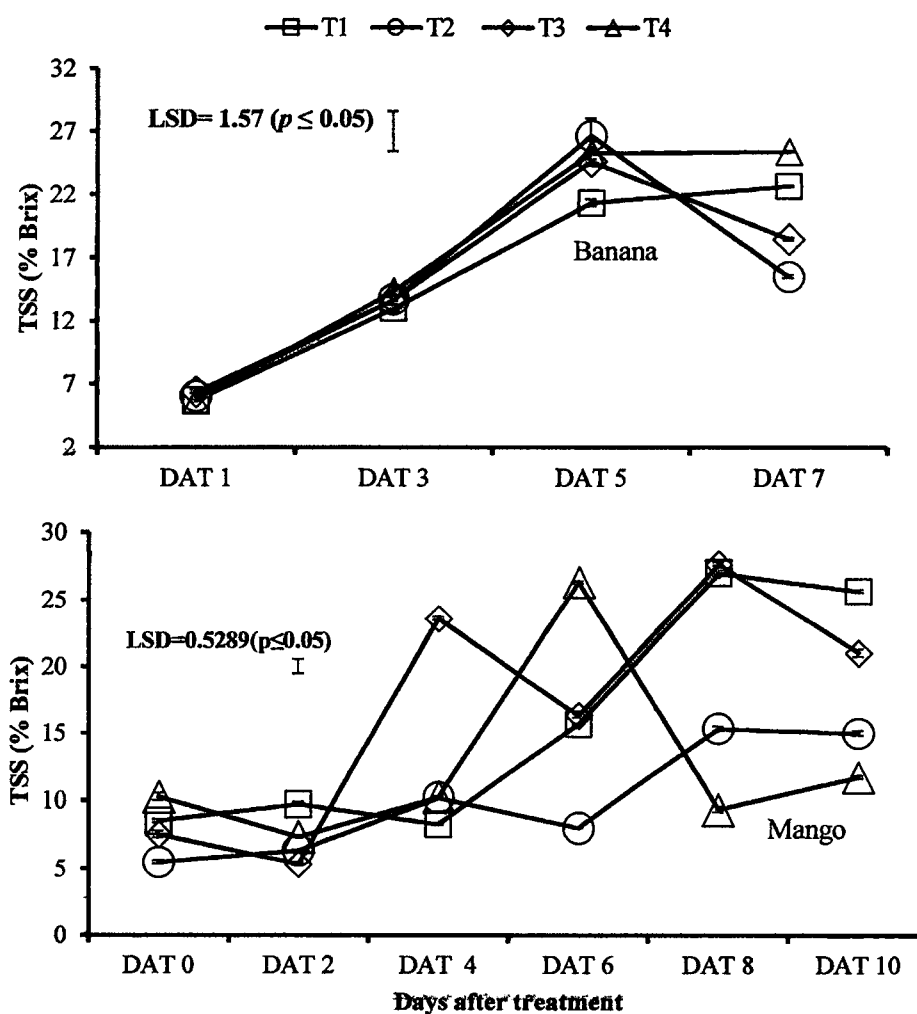


Figure.4.12: Effect of chitin nanofiber on Total soluble sugar of banana and mango

T1= Control, T2= CNF 0.1%, T3= CNF 0.3%, T4= CNF 0.5%

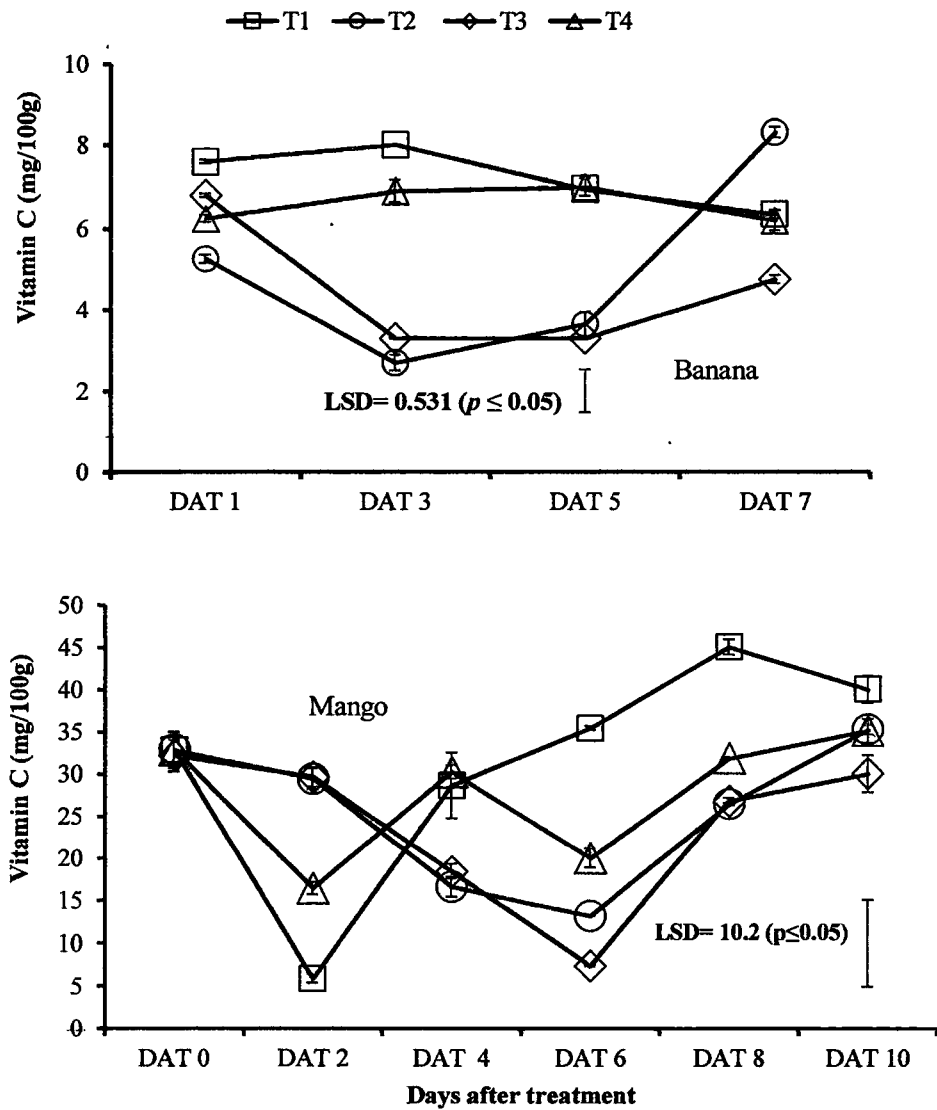


Figure.4.13: Effect of chitin nanofiber on Vitamin C of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

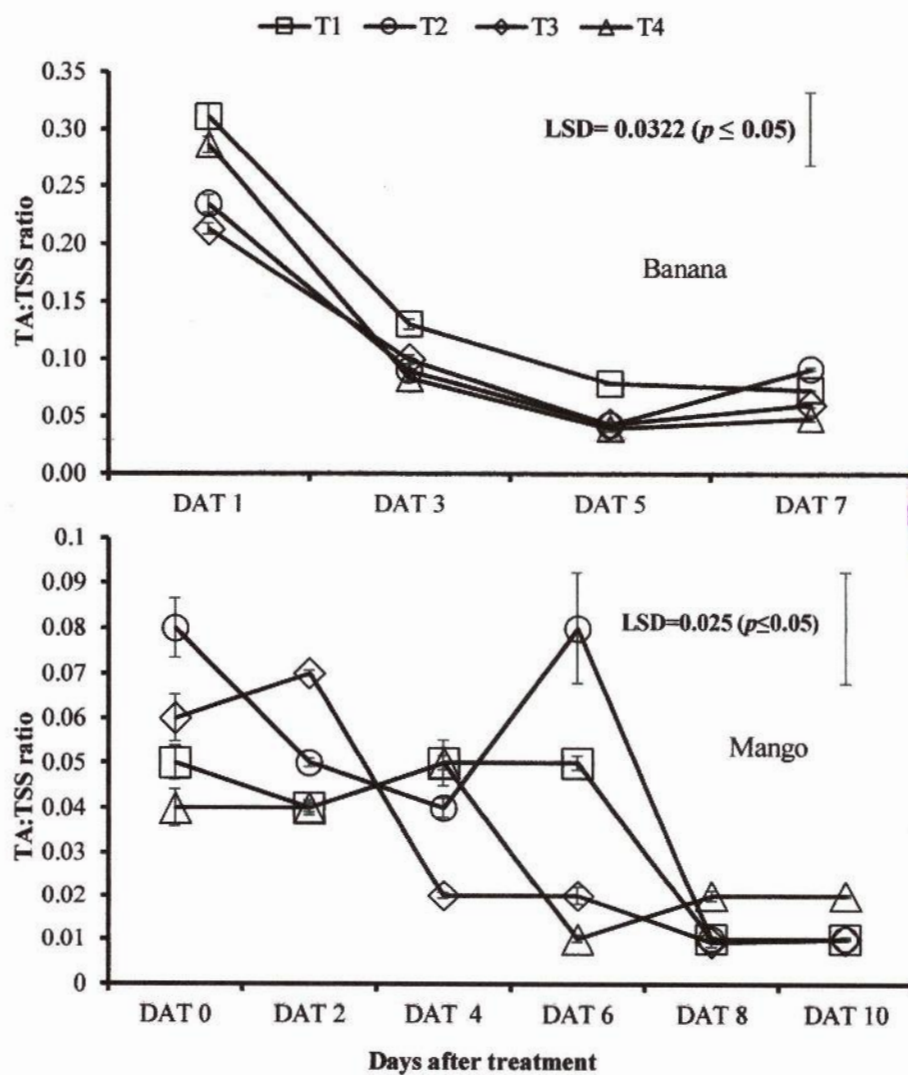


Figure 4.14: Effect of chitin nanofiber on TA:TSS ratio of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

4.4 Effect of chitin nanofiber on shelf life of banana and mango

The effect of different chitin nanofiber coating on the shelf life of banana during storage has been shown in figure 4.15. The maximum shelf life of banana was recorded to be 5.75 days in 0.1% CNF coated bananas while the shortest shelf life was observed in untreated fruits (4.75 days). The obtained results led to the conclusion that CNF coating influenced the shelf life of banana and 0.1% CNF coating was more suitable for extending the shelf life of bananas (1 day) than other treatments. According to Hossain and Iqbal (2016), shelf life of banana fruits was affected by the different postharvest treatments of chitosan concentration and chitosan 1% was more suitable for extending shelf life slightly during storage which is similar to our observation.

Shelf life of mango was influenced by higher concentration of CNF treatments during storage (Figure 4.15). The longest shelf life (8.5 days) was recorded in mangoes coated with CNF 0.3% followed by CNF 0.5% (8.33 days) at ambient condition while lowest shelf life was recorded in untreated fruits (6.93 days). Similar results were found by Salunkhe and Desai (1984) where they recorded longest shelf life (10 days) in mangoes coated with the highest concentration of chitosan (2.0%) followed by 1.5% (9.67 days).

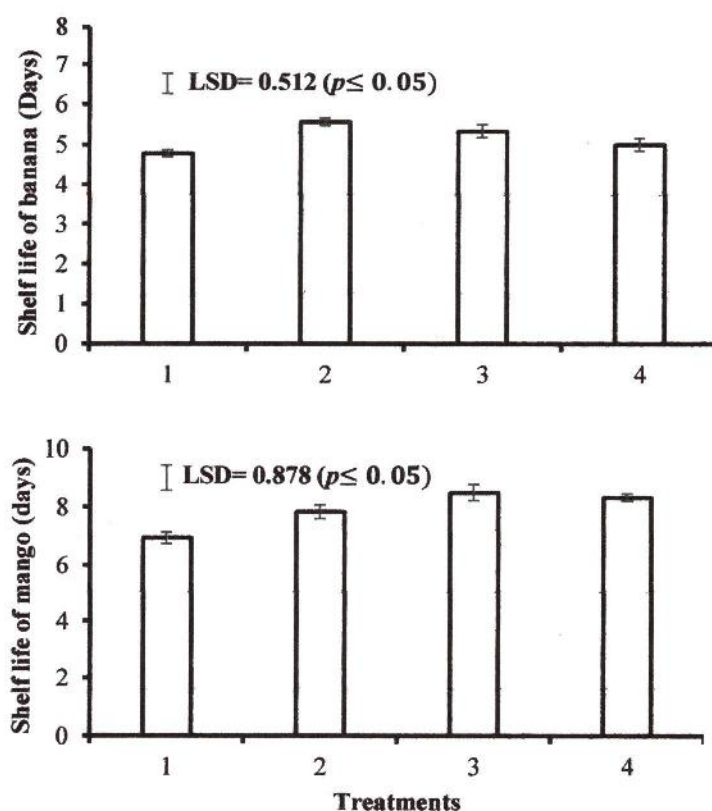


Figure 4.15: Effect of chitin nanofiber on Shelf life of banana and mango

T₁= Control, T₂= CNF 0.1%, T₃= CNF 0.3%, T₄= CNF 0.5%

Chapter 5

Summary, Conclusion and Recommendation

The current study was conducted because of the effect of chitin nanofiber on physicochemical properties of mango and banana in ambient condition. The main objective of the study was to identify suitable ecofriendly postharvest treatment for maintaining quality of 'Nilumbori' mango and 'Jin' banana. The study was conducted at Horticulture Laboratory, Agrotechnology Discipline, Khulna University, Khulna during the period from 25th July 2022 to 6th August 2022. Mango (Nilumbori) was collected from Satkhira and Banana (Jin) was collected from Khulna.

The two-factor experiment was conducted in this study following factorial completely randomized design (CRD) with three replications of treatments containing 10 fruits in each replication. The temperature of Horticulture Laboratory ranged from 26°C to 31°C with relative humidity 80-90%. The postharvest treatments were applied to randomly selected fruits. Mango (Nilumbori) and banana (Jin) fruits were treated with different concentrations of chitin nanofiber (CNF) as 0.1%, 0.3% and 0.5%. Fruits without treatments were considered as control. Both treated and untreated fruits were kept at ambient temperature. Observations were made during the storage period on various physical (weight loss, firmness, fruit color [subjective and objective], disease incidence, disease severity and shelflife) and chemical (TA, TSS, TA-TSS ratio and vitamin C) parameters of mango and banana fruits. Physical parameters were recorded everyday while chemical parameters on every alternate day.

From the experiment of banana, it was observed that cumulative weight loss, firmness and color of T₃ (CNF 0.3%) treated fruits performed better than T₂ (CNF 0.1%) and T₄ (CNF 0.5%). But in case of TA, TSS and vitamin C value, T₂ (CNF 0.1%) showed better results. The T₃ treated fruits showed lower rate of cumulative weight loss (0.96-part), firmness (0.73-part) and color (0.22-part). However, T₂ treated fruits showed better results in vitamin C (0.27-part), TSS (0.75-part) and TA (1.23-times) than the control fruits. The highest shelf life of banana was recorded 5.75 days in T₂ (CNF 0.1%) treated fruits while shelf life of control was 4.75 days. Lower concentration of CNF might have the capacity to inhibit ripening process and suitable for banana postharvest.

In case of mango, cumulative weight loss, color and TSS of T₄ (CNF 0.5%) treated fruits showed better result in comparison to control while T₃ (CNF 0.3%) treated fruits performed

well in disease incidence and shelf life. Lower rate of cumulative weight loss (0.83-part), color (0.23-part) and TSS (0.64-part) was observed in T₄ (CNF 0.5%) treated fruits while CNF 0.3% performed better in firmness (0.90-part) and disease incidence (0.93-part) in comparison to control. The highest shelf life of mango was recorded as 8.5 days in T₃ (CNF 0.3%) treated fruits while control fruits exhibited only 7 days. CNF treatment extend shelf life of mango about 1.5 days during postharvest period. This result indicates that moderate concentration of CNF might have the capacity to breakdown the inhibitory effect of higher ambient temperature on ripening process and is suitable for the mango fruits.

From the current study, it could be recommended that in banana, lower concentration of CNF (0.1% to 0.3%) and in mango moderate concentration of CNF (0.3% to 0.5%) might be a suitable treatment for maintaining physicochemical properties and shelf life in ambient condition. In this regard, further experiments could be conducted with other varieties of banana by considering lower concentrations and other varieties of mango by considering higher concentrations of CNF.

Chapter 6

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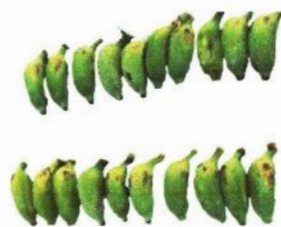
Chapter 7

List of appendices

Pictures of the performed study



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T₂



T₃



T₄

Plate 7.1: Change in color of banana on DAT 1



T₁



T₂



T₃



T₄

Plate 7.2: Change in color of banana on DAT 3

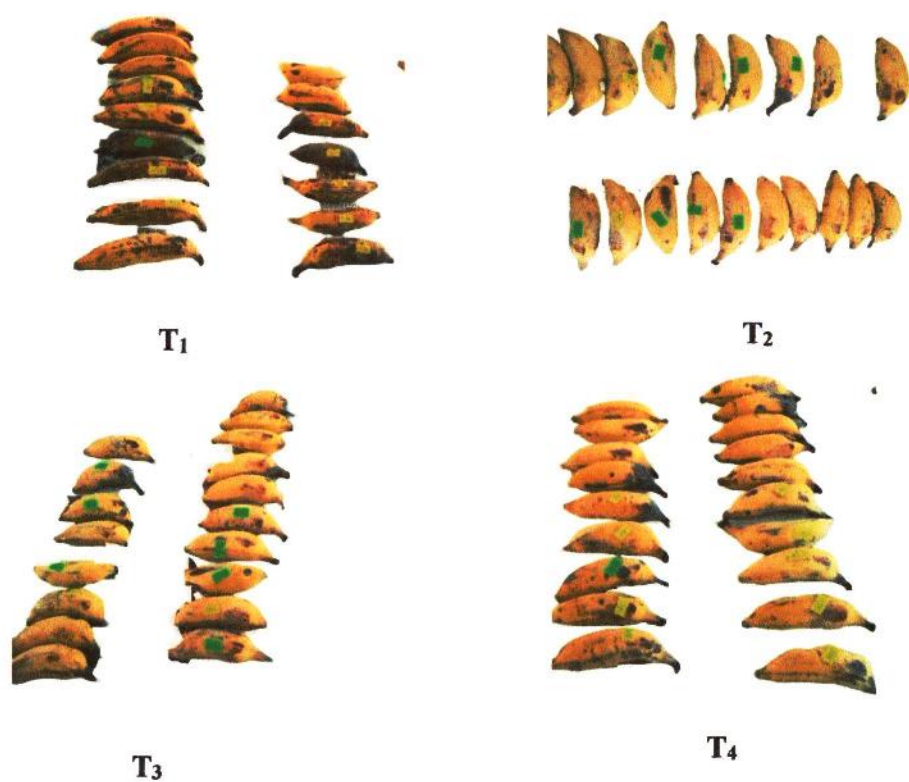


Plate 7.3: Change in color of banana on DAT 5

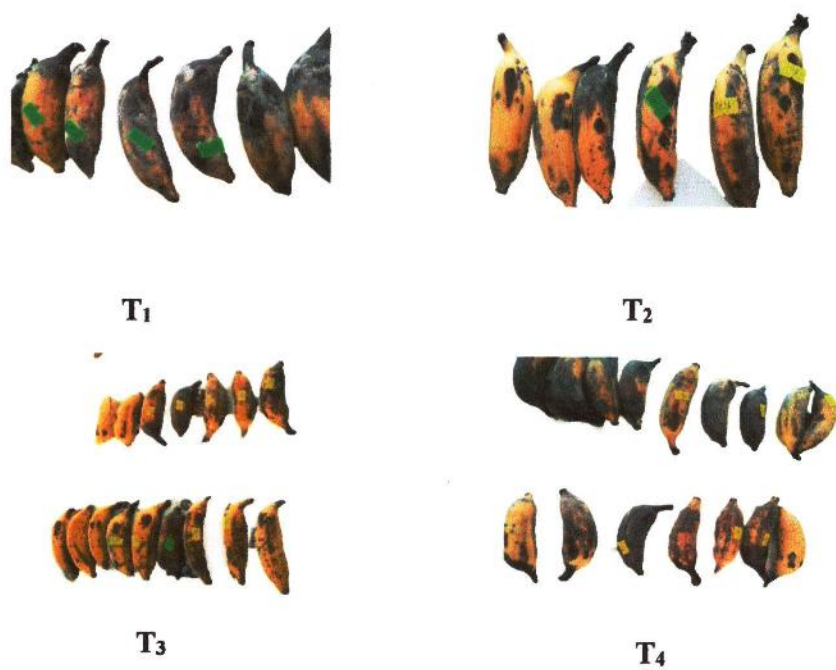


Plate 7.4: Change in color of banana on DAT 7



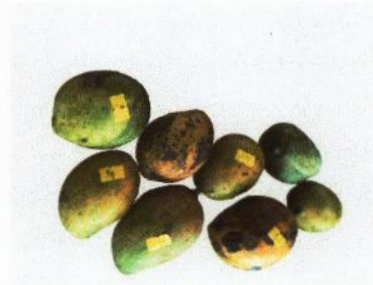
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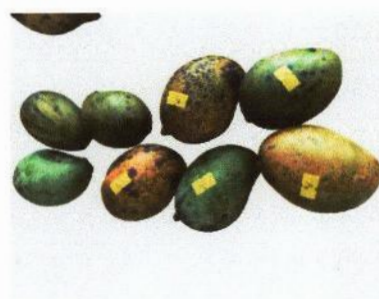


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Plate 7.5: Changes in color of mango on DAT 4



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Plate 7.6: Changes in color of mango on DAT 6



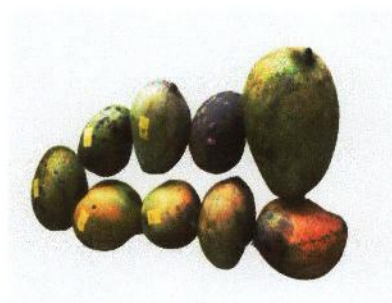
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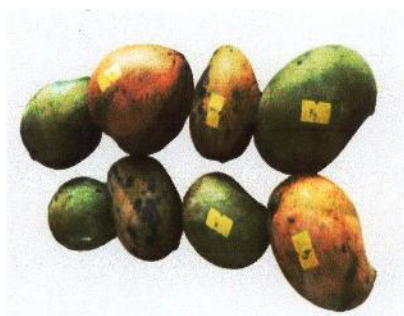
Plate 7.7: Changes in color of mango on DAT 8



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Plate 7.8: Changes in color of mango on DAT 10