STUDY OF PHYSICO-CHEMICAL PROPERTISE OF *Aloe vera* COATED GUAVA (*Psidium guajava*) AT REFRIGERATED STORAGE CONDITION

A

THESIS

BY

DEBASHIS KUMAR DUTTA ROY
Student ID.: 1405207
Session: 2014-2015
Semester: July-December, 2015

MASTER OF SCIENCE
IN
FOOD PROCESSING AND PRESERVATION

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY DINAJPUR-5200

DECEMBER, 2015

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Submitted to the
Department of Food Processing and Preservation

Hajee Mohammad Danesh Science and Technology University, Dinajpur

In Partial Fulfillment of the Requirement for the Degree of

MASTER OF SCIENCE (MS)
IN
FOOD PROCESSING AND PRESERVATION

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Approved as to style and content by

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Supervisor	**Co-supervisor**

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Chairman of the Examination Committee
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Chairman, Department of Food Processing and Preservation

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

DECEMBER, 2015

DEDICATED
TO MY
BELOVED PARENTS

ABSTRACT

Various concentration of aloe vera based coating formulation (25%, 50%, 75%, and 100%) was applied on fresh whole guava by dipping method. The guava was stored at a refrigerated condition (4^0C) and weight loss, color, firmness, vitamin C, total phenol and pH change were observed in this research. A significant effect of aloe vera coating was found over the storage period. Aloe vera treatment lowered the weight loss, also retard the texture and color than the control sample throughout 28 days of storage periods. Vitamin C and total phenol content seemed highest value 141.4 mg/100g and 219.6 mg GAE/100g respectively in 100% aloe vera coated sample after 28 days of storage than the control. Among 25%, 50%, 75%, and 100% aloe vera based coating sample, 100% aloe vera was best coating material to prevent the physical changes in fresh guava.

ACKNOWLEDGEMENT

First and foremost, I would like to express my deepest gratitude to Almighty for the guidance and giving me the strength to complete this study successfully.

I would also like to express my profound regard to research Supervisor Md. Mojaffor Hosain, Assistant Professor Department of Food Processing and Preservation, HSTU for his constant supervision, proper guidance, encouraging suggestions, enthusiastic support and thoughtful criticism throughout this study.

I also sincerely wish to express deepest senses of appreciation, gratitude and in debtedness to respected Co-supervisor Shakti Chandra Mondal, Assistant Professor Department of Food Processing and Preservation, HSTU for his help, suggestions and assistance throughout the entire period of research work, as well as in the preparation of manuscript.

A special note of thanks to Dr. Maruf Ahmed, Associate Professor and Chairman , Department of Food Engineering and Technology, for his help, suggestion and thoughtful criticism throughout the entire period of research work.

Cordial thanks are extended to all the staff member of the Department of Food Processing and Preservation for their help and co-operation.

The author would like to express his gratefulness to National Science and Technology (NST) under the ministry of Science and Technology, Bangladesh for financial support.

Finally, the authors wishes to acknowledge his heartfelt indebtedness to his beloved parents for patience, inspirations, sacrifices, blessing and encouragement for completing the research work successfully.

The Authors

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LIST OF ABBREVIATION

AOAC	= Association of Analytical Chemists
D	= Day
et.al.	= and others
FAO	= Food and Agriculture Organization
g	= Gram
GAE	= Gallic Acid Equivalents
LDPE	= Low Density Polyethylene
mg	= Milligram
Min	= Minute
ml	= Milliliter
N	= Normality
^{o}C	= Degree Centigrade
SD	= Standard Deviation
µg	= Microgram

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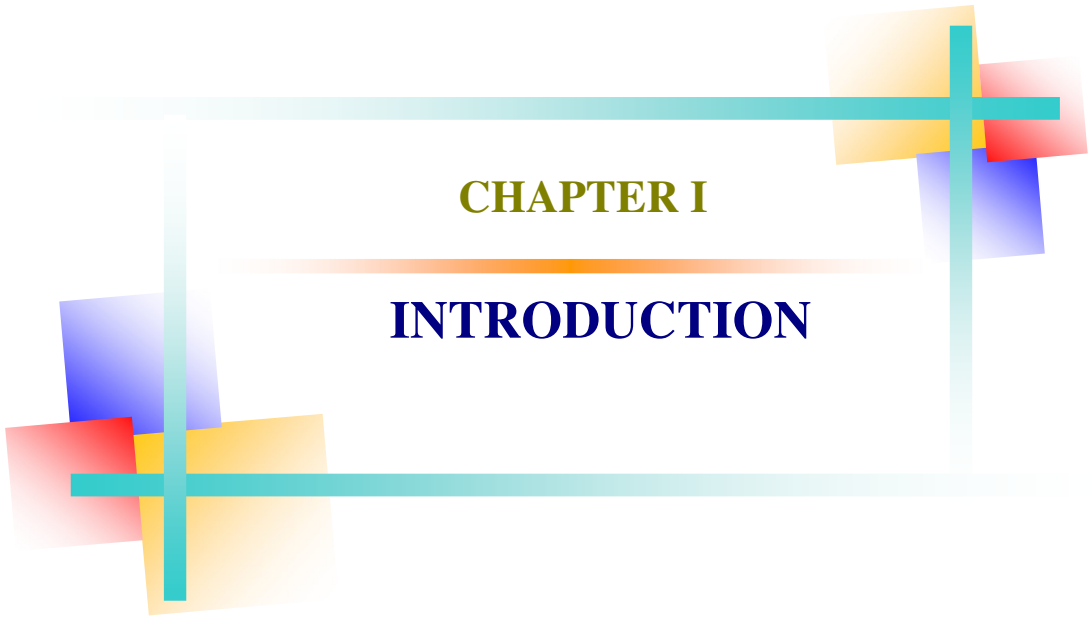

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Guava (*Psidium guajava*) is a most common tropical fruit in Bangladesh. It is locally known as Peyara or "Apple of Bengal". Basically guava is popular for its high content of vitamin C as 260 mg per 100 gm (Bose and Mitra, 2011). It is also rich in antioxidants which can retard aging by reducing oxidative damage of lipids, protein and nucleic acid (Feskanich *et al.,* 2000). According to Hossen, 2012, the annual production of guava is about 145,000 m tons in an area of about 10,000 ha per year averagely and 30-40% of it is being wasted due to several causes in Bangladesh (Source: Directorate of Agriculture Marketing, Bangladesh Agriculture Develop Council (BADC).

Guava is highly perishable and its shelf-life ranges from 3 to 10 days at room temperature (Campbel, 1994). Without any treatment guava spoiled very first because of its high respiration rates, mechanical damage and microbial decomposition. Different types of method are used as ionizing radiation, preservatives and controlled atmosphere (Silva *et al.,* 2011; Lima *et al.,* 2003; Singh and Paul, 2008).

Additionally application of edible coating can increase the shelf life by reducing the moisture and gas loss. Edible coatings can improve the quality, safety, transportation, storage, and display of a wide range of fresh and processed fruits (Baldwin *et al.,* 1995). However, edible films and coatings also may prevent moisture loss and contamination of fruits and vegetables (Park, 1999). They can act as moisture and oxygen barrier during processing, handling, transportation (Díaz-Pérez *et al.,* 2001). During storage, it helps to retard decomposition and enhance the safety of guava by improving antimicrobial functions (Vargas *et al.,* 2008). In recent studies, edible coating formulation is used as a new improved technique. The thin film can act as a packaging material. However, it has more functional properties than thermoplastic materials (Brody, 2006).

Different types of edible coating are used and aloe vera is one of them. Aloe vera gel is a polysaccharide based edible coating material,which has a commercial application in processing of fruits(Ahmed *et al.,* 2009). It has a therapeutic be an innovative and interesting means for commercial application and an alternative to the use of postharvest treatments. *Aloe* v*era* has been used for centuries for its medicinal and therapeutic, antioxidant and anti-microbial properties (Capasso*et al.,* 1998; Hamman, 2008; Chen *et*

al., 2007; and Miranda *et al.,* 2009). Various types of coating condition and different coating materials can enhance the shelf life different fruits (Chen, 2001).

Though a large quantity of guava is waste during post-harvest handling, coating of guava can reduce this type of loss. Also research work regarding aloe vera coating on guava was not carried in Bangladesh. Considering the above issues this research work was under taken with the following objectives:

1. To find out suitable coating concentration of aloe vera for guava.
2. To analyse the effect of various concentrations of aloe vera on physico-chemical properties of fresh and coated guava over the storage period.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 Preamble

Review of related literature in any research is necessary because it provides a scope for reviewing the stock of knowledge, primary concept and relevant information to the proposed research. These knowledge, concept and information give a guideline in designing and conducting the research successfully. It is essential for reviewing that gives a proper instruction in designing a future research problem and validating the new findings.

2.2 History of guava

Guava is one of the most common citrus fruit. Its origin is Mexico along with the tropical countries like Bangladesh. They also found in Africa and Caribbean areas. Several species are grown commercially; apple guava and its cultivars are those most commonly traded internationally (Morton, 1987).

Guava was first introduced in Bangladesh by the Portuguese. It is one of the most popular fruit. It is known as Peyara, also fondly called the "Apple of Bengal" locally. According to an estimate of the Department of Agriculture Extension (DAE), the annual production of guava is about 145,000 m tons in an area of about 10,000 ha per year averagely and 30-40% of it is waste due to many causes (Source: Directorate of Agriculture Marketing, Bangladesh Agriculture Develop Council (BADC)).

2.3 Plant description

2.3.1 Morphology:

It has more than 150 species and some of them have commercial and horticulture value. There are generally two kinds of guava. The common guava (*P. guajava*), the most important species is Cattley Guava (*P. cattlecianum*), which is also grown commercially.

Guava is a fast growing evergreen shrub or small tree that can grow to a height of 3-15 m. It has a shallow root system. Guava produces low drooping branches from the base and

suckers from the roots. The trunk is slender, 20 cm in diameter, covered with a smooth green to red brown bark that peels off in thin flakes. Young twigs are pubescent. The leaves grow in pairs, opposite each other. The leaf blade is elliptic to oblong in shape, 5-15 cm long x 3-7 cm broad, finely pubescent and veined on the lower face, glabrous on the upper face. The flowers are white in colour, sometimes yellow and red too, about 3 cm in diameter, solitary or in 2-3 flower clusters borne at the axils of newly emerging lateral shots. The fruit is a fleshy, periform or ovoid berry that can weigh up to 500 g. Flower goes on from 25-45 days during the production season. Flowers are hermaphrodite often pollinated by air or insects. About 80-86% flowers set fruits but finally 50-60% fruits reached to maturity as initial shredding of flowers is usual. Unripe fruits are green in color and turned in yellow when it ripe (Orwa *et al.,* 2009). The skin colour is yellowish to orange. The flesh can be white, yellow, pink or red, sour to sweet, juicy and aromatic (Ecocrop, 2015 and Orwa *et al.,* 2009). The fruit contains a variable number of seeds (about 3-5 mm long) and its mesocarp is characterized by the presence of small (0.1 mm) and hard fibrous structures called stone cells (sclereids), which may cause damage to processing machinery (El Boushy *et al.,* 2000 and Weinert *et al.,* 1988).Guava cultivars display a great diversity in the tree size, bearing habit and yield, as well as in fruit size, flesh and skin colour, taste and flavour and ripening season.

2.3.2 Nutritional value of guava:

Guava is the major source of vitamin C and Pectin. Guava contains moisture 80-83%; acid 2.45%; reducing sugar 3.5-4.45%, non-reducing sugar 3.97-5.23%, TSS 9.73-14.23; Potassium 0.48%, vitamin C 260mg, carbohydrate 14.32%, protein 2.55%, Dietary fiber 5.4%, energy 285kJ per 100 g of edible potion (Rahim, 2010).

However, nutrients contents depend on variety, season, maturity etc. Guava can be eaten both as in green and ripe stages. Fresh fruits used as salad, pudding etc. Jam, jelly, juice, pickles, ice cream can be made from guava through processing. Tea can be made from leaves of guava.

El-Zorkani (1968) carried out an experiment to determine vitamin C content of pink fleshed, white and seedless guava at various stage of development. The pink fruits were found to contain more vitamin C than other varieties. The outer flesh of the fruit content

more vitamin C than inner pulp. In pink fruit, vitamin C was decreased after ripeness had been attained. The similar result obtained in seedless fruit.

Esteves *et al.* (1984) carried out an experiment and stated that vitamin C was increased in all the cultivars during ripening and decreased during senescence.

Phandis (1970) analyzed the guava fruit to find out its composition and reported that the fruit contained 260mg vitamin C per 100g fruit, which differed with the variety, stages of maturity, ripening and season.

Pozo *et al.* (1983) reported that ascorbic acid content of samples ranged from 69.28 to 74.76-mg/100g pulps.

Nag (1988) reported that Kazi piara contained (318.28mg/100g), Local (257.30) and Swarupkathi (205.58mg/100g) vitamin C at matured stage.

2.4 Changes of fruit and vegetables during processing

2.4.1 Physiological changes of fruit and vegetables during storage:

Most fresh fruits and vegetables contain from 65 to 95 percent water when harvested. Fresh produce continues to lose water after harvest and it cannot be replaced. This causes shrinkage and loss of weight. This high humidity level prevents moisture loss that may occur due to increased respiration and lowered transpiration. When the harvested produce loses 5 or 10 percent of its fresh weight, it begins to wilt and soon becomes unusable. The rate at which water is lost from plant depends on the difference between the water vapor pressure of the plant and the pressure of water vapor in the air. To keep water loss from fresh produce as low as possible, it must be kept in a moist atmosphere. Air flow helps to remove heat of respiration but must be controlled to prevent moisture loss (Elazar, 2004).

2.4.2 Change in chemical composition:

Abeles *et al.* (1992) and Brecht, (1995) said that during the climacteric ripening stage of many fruits, there is a dramatic increase in respiratory CO_2 and C_2H_4 production. Non-climacteric fruit, leafy vegetables, non-fruit vegetables as well as roots and tubers, do not have a surge in C_2H_4 production and generally have only slightly increased respiration as senescence approaches. However, if severely wounded (e.g. by fresh-cut processing), a significant stress-induced production of CO_2 and oftentimes C_2H_4 occurs.

Yusof (1990) carried out an experiment of physico-chemical character ranged from teristics of some guava varieties of Malaysia stated that moister content of the fruits ranged from 79.2 to 85.9%.

El-Buluk *et al.* (1995) conducted an experiment on biochemical and physical changes of four Guava cultivars-Ganib, Pakistani, Shambati and Shendi during growth and development. They found that moister content was increased significantly with fruit growth and development in all cultivars and maximum of 76% in CV.

Nag (1998) carried out an experiment at the Bangladesh Agricultural University and observed the highest ash content in Swaruopkathi (0.475%) followed by Kazi piara (0.46%) and Mukundopuri (0.48%), respectively at the mature stage.

Tripathi and Gangwar (1971) carried out an experiment on the bio chemical changes during maturity of guava and reported the acidity ranged from 0.342 to 0.408%.

Palaniswami and Shanmugavelu (1974), while conducting an experiment in India with 11 varieties of guava that total soluble solid (TSS) varied from 4.0% in Lucknow-49 to 12.5% in smooth green and red fleshed fruits.

Schlimme and Rooney (1994) and Watada *et al.* (1996) reported that once harvested fruits are removed from their source of water, minerals and sustenance. Fruit tissues continue to respire by using available and stored sugars and organic acids and they begin to senesce rapidly. Post-harvest quality loss is primarily a function of respiration, progression of ripening (climacteric rise), water loss (transpiration), and enzymatic discoloration of cut surfaces, decay (microbial), senescences and mechanical damages suffered during preparation, shipping, handling and processing.

2.5 Aloe vera and its functional properties

Aloe vera is a tropical and subtropical plant that has been used for centuries for its medicinal and therapeutic properties. The two major liquid sources of aloe vera are yellow latex (exudates) and a clear gel (mucilage), which proceeds from the large leaf parenchymatic cells (Ni *et al.,* 2004). It is used as a source of functional ingredients in drinks beverages, and ice creams and also applied as an edible coating according to a patent (Martinez-Romero *et al.,* 2003). The raw pulp of Aloe vera contains about 98.5% water, while the mucilage or gel consists of about 99.5% water (Eshun, 2004).

Furthermore, it involves a number of nutrients such as vitamins, fatty acids, amino acids, sugars, minerals, and enzymes. Therefore, it can be used in different formulations as a functional ingredient for health benefits. The gel works as a barrier to O_2 and CO_2 and acts as moisture barrier, and thus reduces weight loss, browning, softening, and growth of yeast and molds. The material contains antimicrobial compounds and thus prevents decay (Valverde *et al.,* 2005). Aloe vera contains malic acid-acetylated carbohydrates (including β-1, 4-g1ucomannans) with anti-inflammatory activity (Esua, 2006). Moreover, other properties such as antiinflammatory and antibiotic activities against some diseases (diabetics, cancer, allergy, AIDS) have been reported (Eshun and He, 2004). It has also been reported that the Aloe vera extracts possessed antimicrobial activity against bacterial pathogens from gram positive and gram negative (Adetunji, 2008). Aloe vera based edible coatings have been shown to prevent loss of moisture and firmness, control respiration rate and maturation development, delay oxidative browning, and reduce microorganism proliferation in fruits such as sweet cherry, table grapes and recterones (Valverde *et al.,* 2005; Matinez-Romero *et al.,* 2005 and Ahmed *et al.,* 2009). In addition to the traditional role of edible coatings as a barrier to water loss and delaying fruit senescence, the new generation coatings are being designed for incorporation and/or for controlled release of antioxidants, nutraceuticals, chemical additives and natural antimicrobial agents (Vargas *et al.,* 2008). There are some reports on the antifungal activity of Aloe vera gel against several pathogenic fungi including *Botrytis cinerea* .There has been increasing interest in the use of Aloe vera gel in the food industry as a functional ingredient.

2.6 Edible coating

Edible coating is an application of creating a thin layer of food grade material used to control the quality, safety, with proving modified atmosphere condition during storage of fruits and vegetables (Baldwin *et al.,* 1995).

Traditionally, food companies use polymeric films (polyethylene PE, polypropylene PP, polystyrene, PS) to package fresh fruits and vegetables because of their large availability at relatively low cost and their good mechanical performance, good barrier to oxygen, carbon dioxide (Siracusa *et al.,* 2008). Nowadays, there is a growing trend in fresh fruits and vegetables packaging sector to replace the petrochemical based packaging films with more environmentally-friendly biodegradable materials (Tharanathan, 2003).

2.7 Some research Status of fresh fruits storage techniques

Response surface methodology was considered to have succeeded in minimizing the coating pickup as a function of temperature of edible coating emulsion and dipping time Zahid (2011)

A. vera gel is an innovative method for maintaining quality parameters of minimally processed arils such as firmness, colour and bioactive compounds. In addition, micro-bial spoilage was largely reduced with A. vera treatments compared with the control Domingo Martínez-Romero (2013).

Starch, a storage polysaccharide of cereals and legumes, is most commonly used in the formulation of edible coatings and films because it is inexpensive, abundant, biodegradable, and easy to use. Films based on starch have moderate gas barrier properties. Their mechanical properties are generally inferior to synthetic polymer films. When a plasticizer, such as water, is added starches exhibit thermoplastic behavior (Krochta and Mulder-Johnston, 1997).

Aloe vera coating in oranges resulted in decrease in weight loss, increase in titrability of acids and higher TSS. *Aloe vera* gel effectively preserved fruit marketability, total phenolics content, vitamin C, catalase enzyme activity and reduced decay index also (Kumar, 2014).

Coatings based on cashew gum (CG) associated with small additions of plasticizers (Gly) and carboxymethylcellulose (CMC) have been demonstrated as being effective in extending the shelf- life of guavas, both cut and uncut when stored at room temperature (Odilio, 2015).

Modified Atmosphere Packaging (MAP) has been used to extend the postharvest shelf life of fruits by reducing respiration rate and delaying senescence (Drake *et al.,* 1987). However, it causes anaerobiosis, and the fruit fails to ripen properly (El Ghaouth *et al.,* 1992b). Research has been conducted on the optimum storage atmosphere for fresh whole produce, but limited information is available on optimum atmosphere for fresh-cut produce (Gunes *et al.,* 2001).

Controlled atmosphere Packaging (CAP) is helpful in extending shelf life of several whole fruits and vegetables but cannot be used with fresh-cut products because of the

short handling period (Ahmad and Khan, 1987 and Watada *et al.,* 1996). Respiration of the product becomes anaerobic when oxygen levels decline (McHugh and Senesi, 2000; El Ghaouth *et al.,* 1992; Howard and Dewi, 1995; Li and Barth, 1998; and Nisperos-Carriedo *et al.,* 1992). Therefore, restriction of oxygen leads to accumulation of ethyl alcohol or anaerobic metabolism that leads to off flavors (Purvis, 1983).

CAP and MAP are not economically feasible in most developing countries (Li and Yu 2000), and they require the attention of skilled operators (Park *et al.,* 1994). Since these techniques often involve high capital and maintenance costs (Krochta and Mulder-Johnston, 1997) and require relatively skilled operators, it may be uneconomical to store small quantities of fruit in such stores; furthermore, regular inspection of fruit is difficult (Smith *et al.,* 1987). Once the fruit is removed, it is again subjected to air and ambient temperature, which can result in a rapid loss of quality.

2.8 Insight from the review of literature

It is clear from the review of literature that a lot of research has been carried out on edible coating and a few researches have been conducted on guava coating in abroad. From the review of literature it is proved that edible coating can enhance the shelf life of fruits and vegetable by creating a modified atmospheric condition. In Bangladesh, there is no study has not been done with aloe vera coating on guava. Therefore, the present research work was conducted to find the suitable concentration of aloe vera coating. This study is very important in terms of increasing the shelf life of guava and reduction of wastage.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

This chapter deals with the materials and method used to carry out the present research work. The materials and methods were used to make an edible coated guava and analyzed its some physico-chemical properties.

3.1 Experimental site

The study was conducted in the laboratory of Food Engineering & Technology and Food Processing & Preservation department under the Faculty of Engineering of Hajee Mohammad Danesh Science and Technology University, Dinajpur. The study was also conducted in the chemistry laboratory of this university.

3.2 Samples and chemical collection

Fresh guava and Aloe vera leaves were bought from local market, Dinajpur. Guava fruits of uniform size and shape and without any defect were selected. All analytical grade chemicals such as Glycerol, methanol, Glacial acetic acid, Meta phosphoric acid were supplied by the food processing laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

3.3 Preparation of samples and edible coating solutions

3.3.1 Preparation of aloe vera gel

Aloe vera leaves were washed with water and skins were peeled. The gels were separated, collected and ground in a blender. Then aloe vera pulp was filtered to remove the fibres (Navarro *et al.,* 2011).

3.3.2 Process of making edible coating

Edible coating material was made by adding 1% glycerol (used as plasticising agent), 5% Corn starch (used as thickening agent), 5% ethanol (used for fast dring of coating), and 1% acitic acid with Aloe vera gel and stirred evenly for 20 minutes. Then the aloe vera gel solution was heated at a temperature of 70°C for 45 minutes for pasteurized it. The gel solution was then cooled to room temperature.

3.3.3 Guava coating process

The gel solution of aloe vera was taken in a beaker. Fresh guavas then dipped into with different concentrations of aloe vera (T_0, T_1, T_2, T_3 and T_4) for 2 minutes and then dried naturally for 30 minutes. After drying the coated guavas were packed using LDPE and stored refrigerated at 4°C for 30days.

Treatments

T_0: control, untreated guava

T_1: guava was coated with 25% Aloe vera

T_2: guava was coated with 50% Aloe vera

T_3: guava was coated with 75% Aloe vera

T_4: guava was coated with 100% Aloe vera

3.4 Proximate composition of fresh Guava

3.4.1 Determination of moisture content

Moisture content of guava was determined by AOAC method (AOAC method 920.151). At first 5g of sample was taken in a clean, dry and pre-weighted crucible. Then it was moved to oven and dried at 60ºC for 10 hours. After drying the sample was cooled at desiccator and weighed.

Moisture Content was calculated using following formula:

$\%\ \text{Moisture} = (W_1 - W_2)/W_1 \times 100$

Here,

W_1 = initial weight of sample

W_2= final weight of dried sample

3.4.2 Determination of protein

AOAC method (2000) was used with some modification to determine the protein content of the guava. Usually three stages are used to determine protein content. These stages are given below:

A. Digestion

Guava (1g), Selenium powder (1g), $CuSO_4$ (0.1g), K_2SO_4 (10g) were taken into a volumetric flask. Then 25ml of H_2SO_4 (conc.) was added. After that the volumetric flask was heated at 100ºC for 3 hr and cooled for 20 minute at room temperature.

B. Distillation

After digestion 300 ml of distilled water and 125 ml of 40% NaOH were added to the volumetric flask. 25 ml of 4% boric acid solution and 2-3 drops mixed indicator were taken in a conical flask. The volumetric flask was connected with one end of the condenser and the conical flask was connected with other end. The volumetric flask was heated continuously until the conical flask was filled to 150 ml.

C. Titration

The conical flask was disconnected and was taken for titration. This was titrated against 0.2 N of H_2SO_4 solutions. The end point was indicated by orange color.

i) Calculation for N_2 content:

% of N_2 = Burette reading × Normality of H_2S0_4 × ml equivalent of N_2

Here;

Normality of H_2S0_4 = 0.2

ml equivalent of N_2 = 1.4

ii) Calculation for protein content:

% Protein = % of N_2×Protein factor

Here;

Protein factor = 6.25

3.4.3 Determination of fat

Fat content was determined by AOAC method (AOAC method 7.045, 2000). 5g sample was taken into the thimble. The thimble was attached then to the Soxhlet apparatus containing 200 ml petroleum ether in a round bottom flask. The fat was extracted for 6 hours. After that petroleum ether was evaporated at 80ºC.

Fat content was calculated by following formula:

$$\% \text{ Fat} = (W_1 - W_2)/W \times 100$$

Here,

W_1 = weight of evaporated flask with fat

W_2 = weight of empty flask

W = weight of sample

3.4.4 Determination of total ash

Total ash content was determined by AOAC method (1984). 5g of sample was taken in a clean, dry and pre-weighted crucible. Then the crucible was placed into muffle furnace at 550ºC for 5.5 hours. Then it was cooled at desiccator and weighed.

The ash content was calculated by the following formula:

$$\% \text{ Ash} = (W_1 - W_2)/W \times 100$$

Here,

W_1 = weight of ash with crucible

W_2 = weight of empty crucible

W= weight of sample

3.4.5 Determination of carbohydrate

Total carbohydrate contents of samples were calculated by difference, that the percentage of moisture, protein, fat and ash was subtracted from 100 (Pearson, 1976).

% Carbohydrate = 100 - (% Protein +% Fat +% Ash +% Moisture)

3.5 Physicochemical analysis of guava

Chemical properties

3.5.1 Vitamin C

Vitamin C content of guava was measured using AOAC method (2,6-Dichloroindophenol titrimetric method, 2006). At first 20 g of guava were grinded with 50 mL of Meta phosphoric acid acetic acid solution. Then the solution was filtered with a vacuum filter

containing a qualitative paper (Whatman No. 4). Then 10 mL of the filtered solution was taken and 2-3 drops of phenolphthalein was added and titrated with 2,6-dichloroindophenol standard solution until a light rose pink persisted for 5 s. The titration volume was recorded and used to determine vitamin C content of the sample (milligrams of ascorbic acid/g of sample, wet basis). The indophenol solution was standardized with ascorbic acid standard solution and blanks sample (Nielsen 2003).

Calculation was done by following equation:

$$\text{Vitamin C (mg/100g)} = \frac{T \times D \times V}{V1 \times W} \times 100$$

Where,

T=Titre value

D= Dye factor

V=Volume made up

V_1 =Volume of extract

W= Weight of sample.

3.5.2 Total phenol

Total phenol was determined according to the method described by Lin JY, 2007 *et al*. with slight modification. Carrot jam (1g) was extracted with 20 ml of methanol and concentrated up to 10 ml by hot plate at 60ºC. Again, volume was adjusted to 20 ml with methanol and filtered through whatman no.1. After that 1ml sample, 0.2ml of 10% Folin reagent was transferred to a test tube. After 3 minutes, 0.8 ml of 7.5% Na_2CO_3 was added and kept in dark for 1hr at room temperature. Then the absorbance was taken at 760 nm. The phenol content was determined using Gallic acid standard curve and was expressed as ppm.

Total phenol content was calculated by following formula:

$$\% \text{ Phenol} = X \text{ (ppm)} \times \frac{\text{Total volume made up}}{\text{Weight of sample(mg)}} \times 100$$

3.5.3 Determination of pH

The pH of the selected samples was determined by the conventional procedure by a pH meter.

Procedure

The electrode assembled of the pH meter was dipped into the standard buffer solution of pH 7.0 taken in a clean and dry beaker. The fine asymmetry potential knob was adjusted to pH 7.0, then in a same way at pH 4. After calibration the electrode assembled pH meter was dipped into the selected sample solution; the pH was then readout washed twice with distilled water. Again it was dipped into another sample to determine the pH. The pH of all samples was determined by the procedure.

3.6 Physical properties

3.6.1 Weight loss

Sample weight loss was determined by comparing the weights of coated guava after storage with initial weights and expressing the results as percentage (Chien *et al.*, 2007). The results calculated with following equation:

$$\text{Weight loss} = (W_1 - W_2)/ W_1 \times 100$$

Here,

W_1= Initial weight

W_2= Final weight

3.6.2 Firmness

Firmness of Guava was determined by using a penetrometer. The firmness test was done by penetrating the stainless steel probe with 3.5 mm of diameter. The probe was penetrating up to at three different locations in each sample. The data was recorded as a force express as unit of Newtons (N).

3.6.3 Color

The color property of guava was determined with a Colorimeter Minolta CM-2500d (Konica Minolta Optics, Inc. Japan). Color attributes were recorded as L* (lightness), a* (redness) and b* (yellowness). Here the change in lightness and Hue angle is calculated. The equation used for hue is as follows:

$$h = \tan^{-1} (b/a).$$

Where,

h = Hue angle

b = yellowness and

a = redness

3.7 Statistical Analysis

Each experiment included three replications. Data were analysed using statistical software (R-version 3.2.2.). A multifactorial analysis of variance was carried out. Individual effects of the factors have been calculated on a particular point of time during the study. Differences were considered to be significant at $P<0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSTION

This research was carried out to find out the effect of aloe vera coating on guava. Different concentration of aloe vera gel was applied in this study and its effect was studied. The result obtained from the research are presented and discussed in this chapter under the following headings.

4.1 Proximate analyses

The chemical composition of guava and aloe vera has shown in Table 4.1. In case of guava the result was found 83.73% moisture, 12.6% carbohydrate, 1.7% ash and 1.3% protein which nearly same reported by USDA, 1982 and Khatun, 2011. They found 80.61% moisture, 0.7% ash, 1.28% acidity, 11%carbohydrate, 19% TSS. The variation in the result may because of the different species and environmental and horticultural condition of guava production; Yusof (1990). On the other hand, in case of aloe vera the result shows 97.4% moisture, 1.3% ash and 1.7% carbohydrate. Pierce (1983) and Rowe (1941) also found 98.5% moisture and 0.3% carbohydrate which are nearly similar.

Table 4.1 Proximate composition of guava and aloe vera

Sample	**Composition (%)**						
	Moisture	Protein	Fat	Carbohydrate	Ash	TSS	pH
Guava	83.73±0.73	1.3±0.04	-	12.6±0.24	1.7±0.16	18.6±0.45	4.63±0.21
Aloe vera	97.4±0.52	-	-	1.7±0.07	1.3±0.08	1.2±0.36	4.76±0.16

*Values are mean of triplicate analysis with standard mean error

4.2 Physical property

4.2.1 Weight loss

The weight loss was measured up to 35 days, here the data showed for 28 days in Figure 4.1. The change of weight loss was increased through the storage periods. Highest weight loss was found in the control sample compare with the coated one. The change is the control sample drastically high as 24.17% at 28 days and low as 6.36 in 100% aloe vera coated sample. However different amount of weight loss was observed in different treatment.

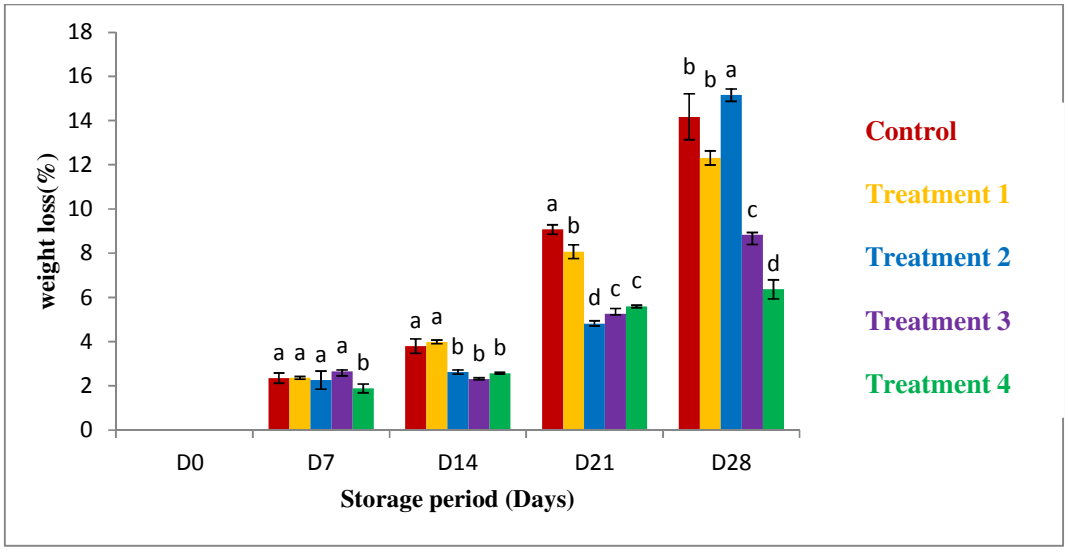

Figure 4.1 Effect of coating on Weight loss

*Values are mean of triplicate analysis with standard mean error.
*Abbreviation of treatment 1, treatment 2, treatment 3, treatment 4, treatment 5 are given in 3.3.3

The weight loss was minimized by aloe vera coating. The concentration of different aloe vera juice is caused that Dorria (2007). The result we found is nearly same to Zuraidah (2015). The weight loss takes place because of dehydration, respiration. The physical cell disruption is also one of the causes of weight (Vazquez-Ochoa, 1990).

4.2.2 Firmness

Weight loss can affect the firmness of guava. Softness of guava also affects by ethylene activity and in room temperature it changes within 4 days at room temperature (Azzolini *et al.,* 2005; Brown & Wills, 1983).

Fig.4.2 shows that the firmness is gradually lower by increasing the storage period. In case of coated sample the firmness is higher than the uncoated ones and 100% aloe vera coating restrict the firmness loss at the 28 days of storage period. Martinez –Romero *et al.,* (2006) also found that aloe vera gel has a power to lower the weight loss which also influences the retarding of firmness. The result has little bit variation to Zuraidah *et al.* (2015). The possible reason may be the use of higher concentration of aloe vera in this study. The higher concentration of aloe vera retards the firm loss and improved quality.

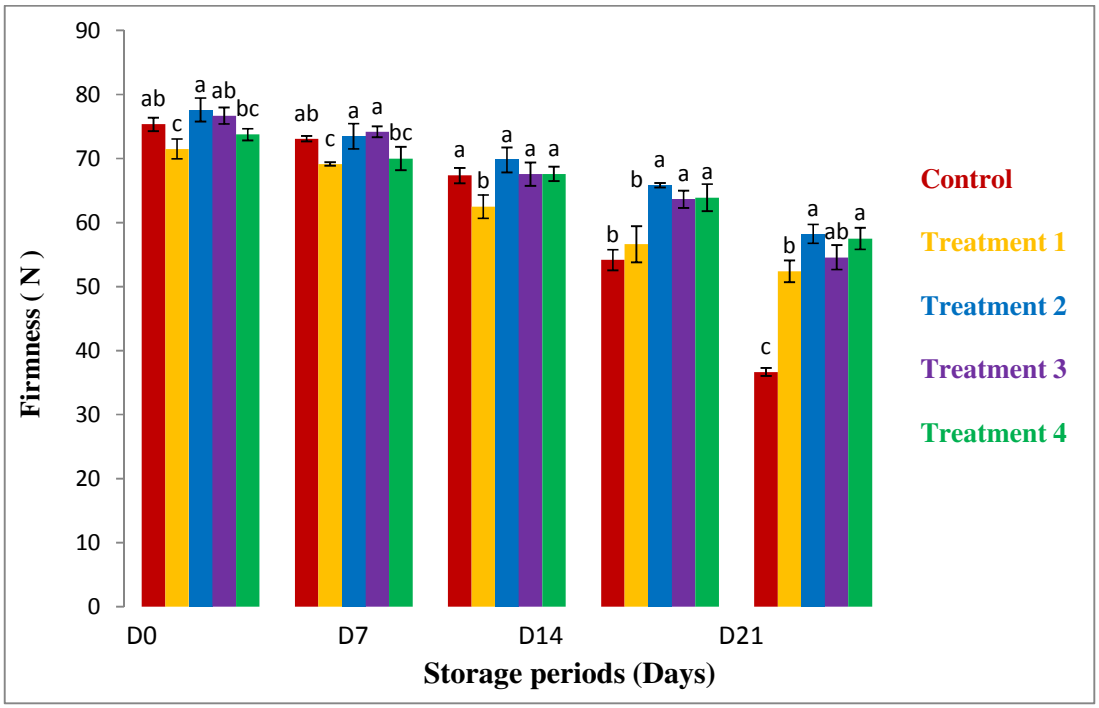

Figure 4.2 Changes in Firmness

*Values are mean of triplicate analysis with standard mean error.
*Abbreviation of treatment 1, treatment 2, treatment 3, treatment 4, treatment 5 are given in 3.3.3

4.2.3 Change in color attribute of guava

The change in lightness, redness, and yellowness are shown in Fig.4.3 and Table 4.2. The value showed negative in case of a, because guava is generally green in color. When storage time was increased lightness of guava decreased (highest 47.15 to 26.94 in controlled and 52.32 to 31.57 in 100% aloe vera coated sample). But the coated sample has higher lightness than uncoated sample. At day 28, 100% aloe vera coated sample had highest value than others. One of the possible reasons of change in lightness is coating material. It acts as a modified atmosphere packaging materials which delay the degradation of color (Ergun and Satici, 2012). According to Baldwin *et al.* (1995) edible coating also delay color change, weight loss in tropical fruit and improve appearance.

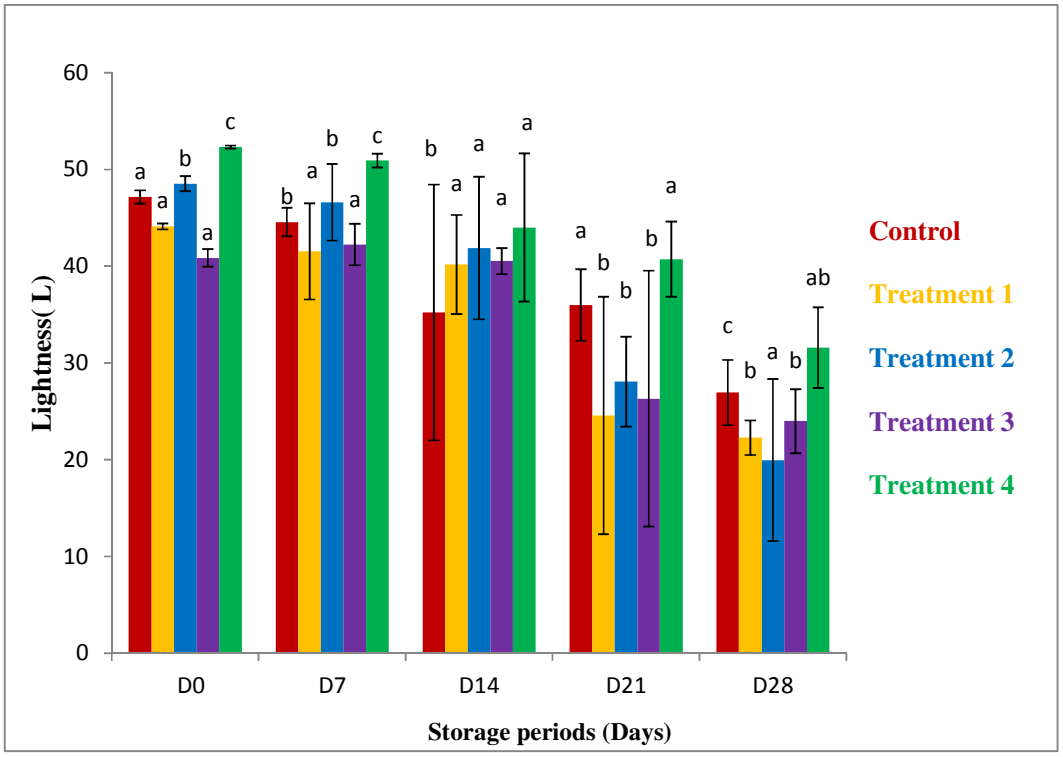

Figure 4.3 Effect of coating on lightness

*Values are mean of triplicate analysis with standard mean error.
*Abbreviation of treatment 1, treatment 2, treatment 3, treatment 4, treatment 5 are given in 3.3.3

In case of green ness, it was lowered throughout the storage period. The green color changed to light yellow to dark yellow. The controlled sample changed its color very first because of its high respiration rate than the other coated sample. Previous study also showed the same result, (Siqueira *et al.,* 2011; González *et al.,* 2011).

With the change of time the hue angles were increased that shown in the Table 4.2. This indicates also the changing of green to yellow color. The reduction of greenness (-8.08 to -16.01) is nearly similar to Xing *et al.* (2011), who used a poly saccharide based chitosan coating.

Table 4.2 Changes of color

Treatment	Storage periods (Days)				
	D_0	D_7	D_{14}	D_{21}	D_{28}
a value					
T_0	-8.86±2.05^{a}	-25.9±1.41^{a}	-31.79±2.94^{c}	-27.12±7.70^{b}	-3.85±2.11^{b}
T_1	-6.64±2.05^{a}	-15.46±0.5^{ab}	-21.45±7.61^{a}	-12.03±5.41^{a}	-4.30±1.23^{b}
T_2	-8.98±2.14^{a}	-19.92±2.1^{ab}	-29.81±1.7^{ab}	-13.16±3.23^{a}	-13.99±0.65^{a}
T_3	-5.97±1.91^{a}	-21.56±8.64^{a}	-13.5±1.54^{ab}	-20.1±0.79^{ab}	-10.60±0.37^{b}
T_4	-8.08±2.12^{a}	-8.30±0.21^{a}	-25.14±9.5^{b}	-32.11±5.25^{b}	-16.1±0.81^{b}
b value					
T_0	32.65±0.40^{a}	9.54±1.41^{b}	14.85±1.15^{ab}	14.30±2.99^{b}	13.63±4.6^{b}
T_1	28.34±0.28^{b}	6.17±2.25^{b}	26.33±4.56^{a}	24.96±9.34^{a}	18.44±2.52^{a}
T_2	31.55±0.40^{a}	17.26±2.25^{c}	3.11±2.16^{ab}	15.805±0.86^{b}	17.94±7.21^{a}
T_3	27.91±1.19^{b}	32.46±1.26^{a}	24.12±1.22^{a}	20.04±6.39^{a}	17.33±0.62^{a}
T_4	31.64±0.27^{a}	19.45±0.17^{c}	0.80±2.48^{b}	18.36±4.54^{a}	17.74±0.94^{a}
Hue angle (h^0)					
T_0	-74.85±3.14^{a}	-23.87±1.02^{b}	-34.97±2.83^{a}	-28.83±1.17^{a}	-21.72±5.02^{c}
T_1	-74.64±0.71^{a}	-43.73±1.5^{bc}	-51.05±1.4^{ab}	-62.255±1.8^{b}	-64.72±1.81^{b}
T_2	-64.1±1.07^{ab}	-40.9±0.74^{bc}	-52.1±4.1^{b}	-49.08±7.5^{ab}	-50.66±1.03^{a}
T_3	-78.02±3.40^{b}	-57.12±9.61^{c}	-58±3.21^{ab}	-44.13±8.1^{ab}	-58.54±0.03^{ab}
T_4	-75.68±3.73^{a}	-66.88±0.73^{a}	-36.19±8.72^{a}	-29.56±2.11^{a}	-47.77±0.08^{a}

*Values are mean of triplicate analysis with standard mean error
*Means with the same column with different letters are significantly different ($P<0.05$)
*T_0= Control, T_1 = 25% Aloe vera, T_2 =50% Aloe vera, T_3 =75% Aloe vera, T_4 =100% Aloe vera.

4.3 Chemical property

4.3.1 pH

The pH content of guava was decreased gradually with the increasing of storage time. The result has shown in Figure 4.4. 100% aloe vera coated sample shows the lowest pH 3.62 at day 28 compared with the others with significantly different (P>0.05). That means 100% aloe vera more control the microbial growth of microbs, Miranda *et al., (*2009). This pH change can be the organic acid substances or conversion of sugar into acid and metabolic processes, (Keditsu *et al.,* 2003).

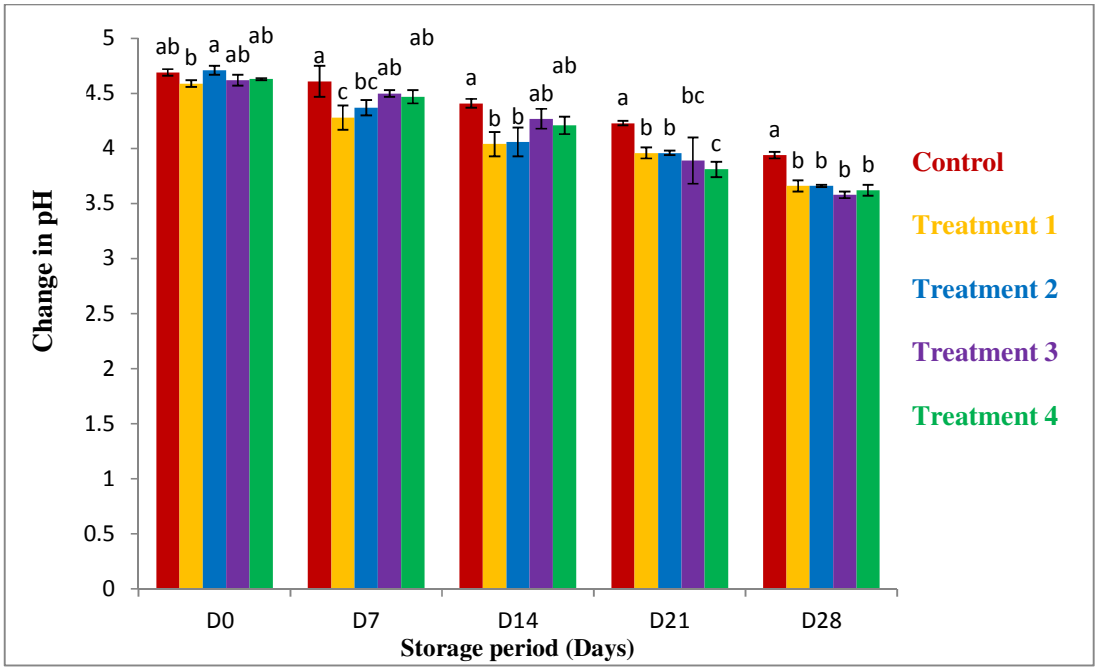

Figure 4.4 Change in pH

*Values are mean of triplicate analysis with standard mean error.
*Abbreviation of treatment 1, treatment 2, treatment 3, treatment 4, treatment 5 are given in 3.3.3

4.3.2 Change in vitamin C

Vitamin C is an important factor for guava. The change in vitamin C of guava is shown in Figure 4.5. In this study, the result shows vitamin c content of raw guava was 167.25 mg/100g. The result is similar to Vila *et al.* (2007) 168.5 mg/100g at day 1.

During storage period vitamin C content of guava is decreased in all samples. The loss of vitamin C in control sample is 96.75mg/100g and in coated sample were 49.46, 42.55, 30.55, 29.05mg/100g respectively. The result shows that vitamin C loss was higher in fresh sample than the coated sample. Vitamin C loss may occur because of phenol oxidase and ascorbic acid oxidase (Yaman and Bayoundurh, 2002). Also the presence of light, heat and oxygen can reduce the vitamin C content (Davey *et al.,* 2000). On the other hand modified packaging (aloe vera coating) creates an aerobic condition to the guava surface. Due to this oxidation of ascorbic acid is influenced and lowering the ascorbic acid content Lee and Kader, (2000).

This study concluded that the loss of vitamin C in aloe vera coated sample is lower than the controlled and higher the concentration of aloe vera has higher efficiency. Here 100% coating concentration shows higher result. Serrano *et al.* (2006) also found that aloe vera gel coating for grapes was able to control the ascorbic acid content loss during storage.

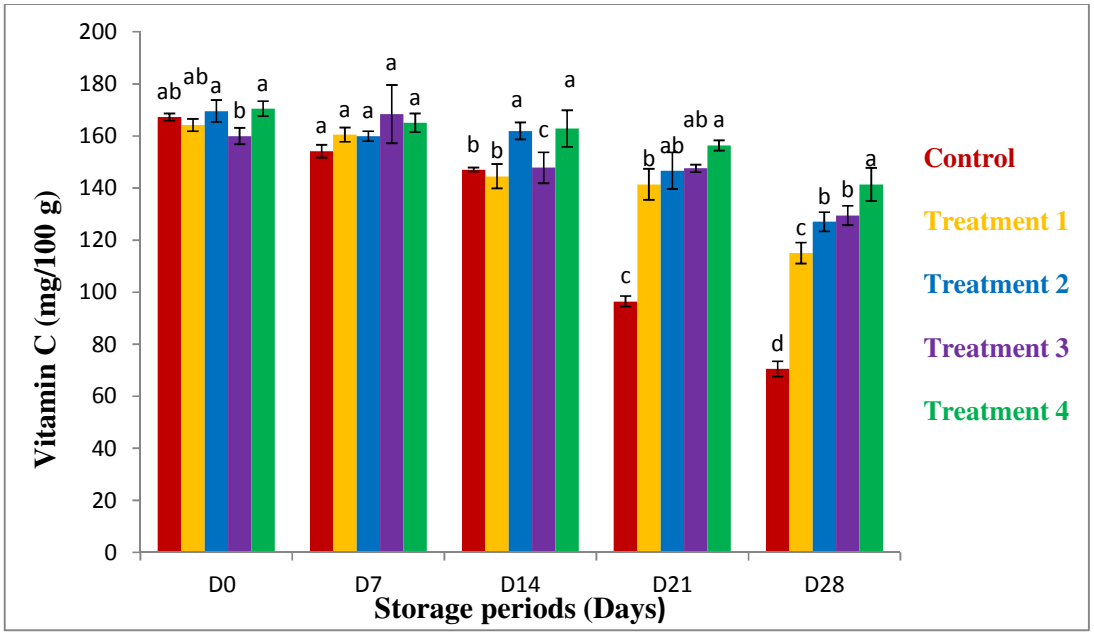

Figure 4.5 Change in vitamin C

*Values are mean of triplicate analysis with standard mean error.
*Abbreviation of treatment 1, treatment 2, treatment 3, treatment 4, treatment 5 are given in 3.3.3

4.3.3 Change in total phenol content

Total phenol is also an important factor for maturity index. Table 4.3 shows the change in total phenol throughout the storage periods. The result showed that total phenol content of guava in fresh sample was 265.3-271.1 mg GAE per 100 g. This result is similar to Thaipong, K. *et al.* (2006), and Hagen *et al.* (2007) who reported that it could be 170–344 mg GAE per 100g.

During the storage periods total phenol content is reduced 265.3 to 133.85 for control and 271.1 to 219.6 mg GAE per 100g for 100% aloe vera coated sample. The loss in control sample was 131.45 and in coated sample were 83.3, 72.9, 54.8 and 51.5 mg GAE per 100g respectively. The change may due to many factors as variety, species, ripening and environmental condition, Iqbal, *et al.* (2006). According to Taylor (1993), the lowering in total phenol content can reduce the astringency during storage period. More over some monomeric component can convert to polymeric components; these also cause the reduction of total phenol, (Iversen, 1999 Ochoa *et al.,* 1999).

This study shows that the loss of total phenol is reduced by the application of aloe vera coating. This is also proven by Serrano *et al.* (2006).

Table 4.3 Effect of coating on total phenol (mg GAE per 100 g)

Treatment	Storage periods (Day)				
	D_0	D_7	D_{14}	D_{21}	D_{28}
T_0	265.3 ± 0.56^{ab}	255.6 ± 4.66^{d}	193.6 ± 1.83^{d}	172.7 ± 5.79^{c}	133.85 ± 6.29^{d}
T_1	256.45 ± 2.61^{c}	242.7 ± 4.10^{b}	211.65 ± 1.34^{c}	196.75 ± 3.60^{b}	173.15 ± 5.30^{c}
T_2	263.25 ± 2.62^{b}	251.85 ± 4.59^{ab}	230.55 ± 2.61^{b}	239.25 ± 9.12^{a}	190.35 ± 3.46^{b}
T_3	249.2 ± 3.39^{d}	242.05 ± 4.45^{b}	225 ± 5.09^{b}	212.65 ± 8.83^{b}	194.4 ± 2.82^{b}
T_4	271.1 ± 1.83^{a}	256.95 ± 2.33^{d}	245.3 ± 1.97^{a}	230.4 ± 3.26^{a}	219.6 ± 2.40^{a}

*Values are mean of triplicate analysis with standard mean error
*Means with the same column with different letters are significantly different ($P<0.05$).
*T_0, T_1, T_2, T_3, T_4, See ligand Table 4.2

4.4 Storage studies of guava

Organoleptic properties of all the samples of guava were observed during storage periods at refrigeration temperature (4^0C), on the basis of color, flavour, texture, and visual fungal growth. This was recorded at every 7 days interval up to 35 days. The data are shown in table 4.5.In case of control sample there was no change in the properties up to 7 days. At

day 14 the color was staring to change in dark green to green and slightly fungal growth. At day 21 it was changed its all property and organolaptically unaccepted.

Table-4.4 Sensory studies of guava

Storage period	Sample	Color	Flavor	Texture	Visual fungal growth	Remarks
Day 0	T_0	Dark Green	Good	Hard	No growth	Good
	T_1	Dark Green	Good	Hard	No growth	Good
	T_2	Dark Green	Good	Hard	No growth	Good
	T_3	Dark Green	Good	Hard	No growth	Good
	T_4	Dark Green	Good	Hard	No growth	Good
Day 7	T_0	Dark Green	Good	Hard	No growth	Good
	T_1	Dark Green	Good	Hard	No growth	Good
	T_2	Dark Green	Good	Hard	No growth	Good
	T_3	Dark Green	Good	Hard	No growth	Good
	T_4	Dark Green	Good	Hard	No growth	Good
Day 14	T_0	Green	Good	Semi Soft	Slightly growth	Good
	T_1	Dark Green	Good	Semi Soft	No growth	Good
	T_2	Dark Green	Good	Semi Soft	No growth	Good
	T_3	Dark Green	Good	Semi Soft	No growth	Good
	T_4	Dark Green	Good	Semi Soft	No growth	Good
Day 21	T_0	Slight yellowish	Off flavor	Soft	Highly growth	Spoiled
	T_1	Green	Good	Semi Soft	No growth	Good
	T_2	Green	Good	Semi Soft	No growth	Good
	T_3	Green	Good	Semi Soft	No growth	Good
	T_4	Green	Good	Semi Soft	No growth	Good
Day 28	T_0	Fully yellowish	Off flavor	No texture	Excessive growth	Spoiled
	T_1	Slight yellowish	Off flavor	Soft	Excessive growth	Spoiled
	T_2	Slight yellowish	Off flavor	Soft	Excessive growth	Slightly Spoiled
	T_3	Slight yellowish	Off flavor	Semi Soft	Medium growth	Slightly spoiled
	T_4	Slight yellowish	Off flavor	Semi Soft	Medium growth	Slightly spoiled
Day 35	T_0	Black	Off flavor	No texture	Excessive growth	Spoiled
	T_1	Full yellow	Off flavor	No texture	Excessive growth	Spoiled
	T_2	Full yellow	Off flavor	Semi Soft	Excessive growth	Spoiled
	T_3	Full yellow	Off flavor	Semi Soft	Medium growth	Spoiled
	T_4	Full yellow	Off flavor	Semi Soft	Medium growth	Spoiled

*T_0, T_1, T_2, T_3, T_4, See ligand Table 4.2

In case of coated sample there was no change up to 21 days only a little bit softness. At day 28, 25% and 50% aloe vera coated sample was unaccepted because it change it properties. On the other hand 75% and 100% coated sample had better quality than the other sample and organolaptically accepted up to 28 days. But the entire sample was spoiled at day 35.

Kendall and Sofos (2007) found that if the sample processed properly and stored in cool dry place it will retain good quality up to several months.

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

This study was conducted to introduce a novel processing technique, edible coating by aloe vera gel on guava with the objectives to document the physic-chemical properties and find out the suitable concentration of coating during storage periods.

Fresh guava and aloe vera leaf was collected from local market. The coating method was done by dipping method. Four different concentration of aloe vera gel was used as 25%, 50%, 75% and 100%. The quality and physico- chemical properties were done by different established method. Significant different ($P<0.05$) was found between the control and coated sample during 35days of storage period.

The salient points of the study are summarized below:

- The analyzed proximate composition of guava and aloe vera gel were 83.73% moisture, 1.3% protein, 12.6% carbohydrate, 1.7% ash and 97.4% moisture, 1.2% ash, 0.7% carbohydrate respectively.
- The weight loss and soft texture was found higher than the coated sample.
- The vitamin C content of guava was higher (141.4mg) in coated sample than the fresh guava (70.5mg) at the 28^{th} day of storage periods.
- The color change (yellowness) was also lower in coated sample and had a better appearance and acceptances.
- The total phenol content loss was also controlled by the application of coating material.

Therefore it is concluded that application of aloe vera coating can increase the shelf life of guava. Also the higher concentration of coating material has the higher quality. Among all the treatment 100% aloe vera coating was best. As it is increases the shelf life and lowering the loss of nutritional and quality parameter so it can be used as a natural preservation technique.

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APPENDICES

Appendix- I

Effect of aloe vera coating on weight loss at refrigerated condition

Treatment	Storage period (Day)				
	D_0	D_7	D_{14}	D_{21}	D_{28}
T_0	0	2.35	3.79	9.07	14.17
		2.68	3.32	8.76	12.69
		2.38	3.72	9.21	15.89
T_1	0	2.36	3.98	8.07	12.31
		2.45	3.86	7.63	12.75
		2.57	3.64	8.89	11.72
T_2	0	2.25	2.62	4.82	15.16
		2.83	2.48	4.65	14.76
		2.49	2.76	4.92	15.58
T_3	0	2.65	2.31	5.26	8.83
		2.74	2.39	5.60	8.67
		2.47	2.13	4.93	9.03
T_4	0	1.88	2.57	5.59	6.36
		1.59	2.63	5.65	6.96
		2.06	2.49	5.51	5.72

Appendix – II

Effect of aloe vera coating on firmness at refrigerated condition

Treatment	**Storage period (Day)**				
	D_0	D_7	D_{14}	D_{21}	D_{28}
T_0	76.1	72.8	66.5	53	37.1
	74.6	73.4	68.2	55.3	36.2
	75.3	73.1	67.3	54.1	35.9
T_1	72.6	68.9	63.8	58.6	53.6
	70.4	69.3	61.2	54.6	51.2
	71.5	70.2	60.6	54.3	49.6
T_2	78.9	74.9	71.2	65.6	57.2
	76.3	72.1	68.4	66.1	59.3
	77.6	73.5	69.8	65.3	58.1
T_3	77.6	74.8	68.6	64.6	55.9
	75.8	73.6	66.5	62.7	53.2
	76.5	74.1	67.5	63.2	54.1
T_4	74.4	71.3	68.4	65.4	56.3
	73.1	68.7	66.8	62.4	58.7
	73.8	69.6	66.7	62.9	57.2

Appendix - III

Effect of aloe vera coating on lightness at refrigerated condition

Treatment	**Storage period (Day)**				
L value	D_0	D_7	D_{14}	D_{21}	D_{28}
T_0	47.64	45.61	44.56	36.01	24.56
	46.67	43.52	25.89	41.25	29.33
	47.15	44.56	35.22	38.63	26.94
T_1	44.34	45.05	43.80	33.26	21.01
	43.89	38.00	36.57	15.89	23.54
	44.06	41.92	40.36	25.62	19.68
T_2	49.07	49.42	47.10	31.36	14.03
	47.97	43.80	36.65	24.77	25.89
	48.52	46.80	41.78	28.12	19.36
T_3	41.51	43.76	41.49	35.67	21.64
	40.20	40.72	39.58	16.95	26.32
	40.65	42.26	40.38	26.32	23.98
T_4	52.20	51.43	49.42	43.48	28.62
	52.44	50.42	38.59	37.96	34.52
	51.96	53.21	44.36	70.72	31.59

Appendix - IV

Effect of aloe vera coating on pH at refrigerated condition

Treatment	**Storage period (Day)**				
	D_0	D_7	D_{14}	D_{21}	D_{28}
T_0	4.72	4.6	4.38	4.21	3.92
	4.67	4.62	4.44	4.25	3.96
	4.69	4.61	4.47	4.19	3.97
T_1	4.57	4.2	3.96	3.92	3.7
	4.62	4.36	4.12	4.0	3.62
	4.60	4.28	4.16	3.96	3.66
T_2	4.68	4.32	4.16	3.94	3.66
	4.75	4.42	3.94	3.98	3.67
	4.71	4.37	4.06	3.92	3.61
T_3	4.58	4.48	4.34	3.91	3.56
	4.66	4.52	4.21	3.87	3.61
	4.63	4.57	4.27	3.89	3.58
T_4	4.64	4.43	4.27	3.76	3.58
	4.62	4.52	4.15	3.86	3.66
	4.63	4.49	4.21	3.81	3.62

Appendix - V

Effect of aloe vera coating vitamin C at refrigerated condition

Treatment	**Storage period (Day)**				
	D_0	D_7	D_{14}	D_{21}	D_{28}
T_0	168.2	152.4	146.5	97.9	68.4
	166.3	155.9	147.6	95	72.6
	167.9	154.3	147.05	96.3	70.5
T_1	162.5	158.6	147.8	137.2	112.2
	165.9	162.4	141.2	145.6	117.9
	164.2	160.5	144.5	141.4	115.05
T_2	166.6	161.3	164.2	141.6	124.4
	172.6	158.6	159.6	151.6	129.7
	168.9	158.8	161.7	146.3	127.05
T_3	162.2	160.4	152.0	148.6	126.8
	157.8	176.3	143.6	146.5	132.1
	159.7	168.3	147.2	147.5	128.6
T_4	172.5	167.6	167.8	155.0	136.9
	168.4	162.5	157.9	157.8	145.9
	169.7	165.06	161.3	156.2	141.9