CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECT OF CASEIN EDIBLE COATING ON THE POSTHARVEST QUALITY OF FRESH GUAVA FRUITS DURING AMBIENT STORAGE CONDITIONS

Annem Muniraj Beulah[1], Kumaram Venkata Sucharitha[1✉], Sanaboyina Dheeraj[1], Palagiri Prameela[1]

[1]*Food technology division, Department of Home science, College of Sciences Sri Venkateswara University, Tirupati, Andhra Pradesh-517502, India.*
✉*suchivenkata@gmail.com*

https://doi.org/10.34302/crpjfst/2021.13.2.1

Article history:
Received:
28 September 2020
Accepted:
25 February 2021

Keywords:
Casein;
Edible coating;
Fortification;
Guava fruits;
Shelf-life.

ABSTRACT
Maintaining quality of fruits is an important task in fresh food retailing throughout the supply chain. Fruits fall under perishable foods because they quickly respond to chemical, physical, and biological changes which lead to quality aspects. Edible coatings are used to prevent the physicochemical changes in fruits during the storage and transportation. In the present study, Casein was chosen as a bio-based edible coating material, enriched with ascorbic acid and was applied on fresh guava fruits to study the delay of ripening and other quality properties. Different concentrations of casein were fortified with 1% of ascorbic acid and applied on whole guava fruits as coating. Fruits were treated with 5% and 10% casein with and without ascorbic acid, fortification process was established to maintain and enrich the vitamin C content in the fruits to reach maximum levels to the consumers. Experimental samples were coded as S1, S2, S3, S4 and sample (So) without coating is considered as control. The fruit samples were stored at (26±1°C) for a period of 16 days. Various physicochemical, biological parameters and microstructural studies were tested to evaluate freshness, nutritional status, and keeping quality during the storage. Fruit ripening, firmness and various visual quality aspects like appearance, defects, and shrinkage rates were studied to understand the physical quality of the fruits upon storage period. During storage, results shows that all casein treated samples were noted with decreased firmness, titratable acidity and delayed chlorophyll content, microbial load while the pH, TSS, carotenoids were increased along the storage when compared with control sample and all the coated samples were found glossy appearance with acceptable flavor. This study prompt that casein is an ideal, promising coating to preserve the quality and extends the post-harvest life of guava fruits.

1. Introduction

Guava (*Psidium guajava* L.) is a well-known subtropical fruit grown widely in tropical and subtropical regions of the world. Fruits are rich in vitamin C (260 mg /100g) and a fair source of calcium, phosphorus, iron, and vitamin A (Rashida et al., 1997). Due to its characteristic nature, ripened fruits are very perishable with a very short shelf life ranging from 2-3 days at ambient temperature. Simple, low-cost technologies with improved post-harvest practices will minimize the qualitative and quantitative loss of harvested fruits from field to consumption. Edible coating (EC) is such type of post-harvest practice used to coat whole fruits to reduce the physical and biological reactions responsible for the quality deterioration. Edible coatings are formed a thin layer on the surface of the fruit, can be eaten as whole along with the fruit or can be removed with gentle washing with water. A biopolymer layer that formed on the surface of the fruits as active layer can also be served as carrier for several bioactive compound-like preservatives, vitamins, mineral, biochemical markers. etc., for their specific need. Recent years, edible coatings claimed as unique advanced preservation technique due to its antimicrobial, antioxidant nature, barrier against transmission of gases (O_2, CO_2, and ethylene), vapors, and solutes. Edible coatings perform a key role in maintaining quality, Safety, storage and distribution of fresh and processed foods (Daniel et al., 2007). Past years, several researchers were worked or working on edible coatings for fruits and fruit products (Chiabrando and Giacalone, 2016). According to the published literature from scientific database, most of the research was focused on to develop edible coatings from naturally occurring sources i.e., from bio-origin polymers, and they have been used to increase shelf life, carry/transfer the nutrients, and to enhance the physical appearance of the fruits (Lin and Zhao, 2007). Applications were not only limited for fruits and vegetable, and also used for almost all fresh and processed foods like fish, poultry and meat products, dairy products, and other designer foods and flavors. etc., (Bhagath and Manjula, 2019; Krochta et al., 1994). Thorough observations on biopolymers as edible coatings, present study was planned to investigate casein as edible coating material for preservation and fortification of guava fruits.

Casein, a milk protein performs strong mechanical and barrier properties than polysaccharides with its unique coat forming capability. Addition of ascorbic acid (AA) to the casein as a fortificant plays a vital role to improve antioxidant nature of the coatings as well total to maintain antioxidant capacity of the whole fruits as an essential vitamin for humans throughout the supply chain. In general, ascorbic acid content is gradually decreases during senescence in fruits due to the activity of ascorbic acid oxidase so, fortification of coatings with AA is recommended to meet optimum range within the fruits (Hosseini et al., 2018). According to the published data, effects of different edible coatings have been tested on guava fruits and few of them are milk-proteins (Cerqueira and Alleoni, 2011), starch and chitosan solutions (Soares et al., 2011), coating based on potato starch and pectin (Quezada Gallo et al., 2005); cassava starch (2%) formulation with chitosan (2%) and Lippa gracilis Schauer genotypes (Aquino et al., 2015); cassava starch (2%) with cinnamon essential oil (0.01%) (Botelho et al., 2016); 2% chitosan coating extended 12 days shelf-life of guava at 11° C (Hong et al., 2012). Cashew gum and carboxymethyl cellulose based coatings extend the shelf-life of guava for two weeks at ambient temperature (Forato et al., 2015). McGuire and Hallman. (1995) extended shelf life up to 7 days at 12° C by using hydroxypropyl cellulose or carnauba wax coating. Guava coated with xanthan gum and carnauba wax improved storage stability to about 30 days at 10°C (Zambrano-Zaragoza et al., 2013). Additionally, edible coatings such as carnauba wax (Jacomino et al., 2003); candelilla wax (Salinas-Hernández et al., 2010), wax coating (Pal et al., 2004); and miscellaneous formulations like gelatin, triacetin and lauric acid (Fakhouri et al., 2003) have also been tested on guava fruits. However, to the best of our knowledge, there is no report on the use of casein-based coatings to maintain the quality and extend the storage life of guava fruits. Based on the thorough investigation, casein was selected as coat forming proteinaceous substance for the preservation of fresh guava fruits because of its availability, safety and versatility. Various quality parameters were assessed periodically to understand the qualitative and quantitative changes in guava fruits during the storage. Therefore, the chief objective of our research was to assess the potential effect of casein coatings on the storage life of fresh guava fruits during ambient temperature.

2. Materials and methods

2.1. Materials

Fresh and matured green colored guava fruits (*Psidium guajava* L.) were procured directly from the farm (single farm) with maximum uniform identity. Immediately fruits were brought to the laboratory in carton boxes with straw, where they were sorted out to remove any further immature, misshaped, bruised, diseased, and insect infested fruits. Casein powder was purchased from HiMedia Laboratories, Mumbai and citric acid, ascorbic acid and glycerol were purchased from Merck spl.Ltd, Mumbai, India. All chemicals were maintained as analytical grade and restricted for a single brand.

2.2. Preparation of edible coating solution

Two different casein coating dispersions were prepared, based on concentration and fortification of casein. Among the treatments four different samples were developed as 5% and 10% casein coatings without and with fortification of ascorbic acid. The sample without coating is kept as a control. The casein-based edible coatings were produced by dissolving 5g (5%) and 10g (10%) of casein, 10 g of citric acid and 5ml of glycerol (as plasticizer) in 100 ml of distilled water. The prepared solution was kept in autoclave 15 lbs for 15 min. For fortified samples 10g (10%) of ascorbic acid was added to the previously prepared cooled casein coating solution under stirring and further it is used to coat on the fruit.

2.3. Preparation of sample

Fruits were cleaned, washed with water and treated with 1ppm chlorinated water solution for 10 minutes and air-dried at room temperature. The Randomly selected guava fruits were divided into five lots (S0, S1, S2, S3, and S4). Each treatment contained 64 fruits. S1, S2, S3, and S4 were dipped in casein coating solution at 5% and 10% respectively without and with fortification for 1 minute. One lot (C) was left without casein treatment as control. The treated fruits were air dried (Hussain et al., 2012) and kept in ambient storage conditions for 2 hrs to set a coat of edible casein on their surface. The coated and control samples were wrapped in blotting paper (filter paper) and each was coded as sample S1, S2, S3, S4 and control(C) with respect to the days on blotting paper and stored at ambient temperature (26± 1ºC) for a period of 16 days for shelf-life studies in the laboratory.

2.4. Determination of weight loss

Guava fruits of coated and control samples were weighed with the electronic weighing balance (Shimadzu- ELB300 NO: D515711067, Japan) at an interval of 4 days for the total 16 days storage period and the results were expressed as percent weight loss by using the following formula.

$$\text{Weight loss}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \qquad (1)$$

2.5. Measurement of fruit firmness

Firmness of whole fruit was measured in the middle on two opposite sides of each fruit using a penetrometer (Digital fruit firmness tester, T.R.Turoni srl, Italy) with a 8-mm diameter cylindrical plunger was used to determine tissue firmness of the whole guava fruits. The mean values for maximum force were reported in Newtons laws (N). Three guavas were used on each replication. The results of three replicates were averaged to produce a single value.

2.6. Visual quality

The visual quality of the guava fruit was evaluated by subjective method using a rating scale of 5 points (Thumula, 2006). The parameters for evaluation of the quality of guavas are shown below:

Visual Appearance (1- very poor, 2- Poor, 3- Fair, 4- Good, 5- Very good)

Defects* (5- None, 4- 10%, 3- 10-25%, 2- 25-50%, 1 >50%)

Shrinkage (5- None, 4- 10%, 3-25%, 2-50%, 1 >50%)

*Defects include microbial spoilage, discoloration, pitting and softening

2.7. Determination of pH, total soluble solids, titratable acidity, and ascorbic acid

The pH, total soluble solids (TSS) and titratable acidity (TA) have been measured by Islas-osuna et al. (2010) method with slight modifications. 5g of guava pulp was homogenized in 25 ml of distilled water. Then the mixture was filtered using muslin cloth. An aliquot sample was used to measure pH with a pH meter (Eutech instruments, prod-ECPH70042SEU, Singapore). The TSS was measured using a hand refractometer (Erma Inc. Tokyo, Japan) and expressed as brix^0. The titratable acidity was determined with 0.1 N NaOH using phenolphthalein as indicator. Guava pulp (3g) from fruit was homogenized using a mortar and pestle (grinder) and then centrifuged at 3500 rpm (Remi centrifuge, CE model, India) for 10 minutes; The supernatant phase was collected and analyzed to determine ascorbic acid content by 2,6-dichlorophenolindophenol titration (Sucharitha et al., 2018).

2.8. Total chlorophyll and total carotenoid content

Guava fruits are extracted for chlorophyll in a mortar pestle to a fine pulp with the addition of 20 ml of 80 % acetone. Absorbance was taken at 645 and 663 nm in UV-Spectrophotometer (Sudhakar et al., 2016**;** Arnon, 1949). Total carotenoid contents were evaluated according to Harborne JB, 1973, based on the absorbance values at 480 nm in UV-Spectrophotometer.

2.9. Sensorial properties

The acceptability of the samples was evaluated through the standard sensory evaluation techniques. The sensory attributes such as visual appearance, color, taste, texture, flavor and overall acceptability was carried out by selected panel of judges (10 Members) rated on a five-point hedonic scale (5-Excellent, 4-Very good, 3-Good, 2-Fair, 1-Poor).

2.10. Microbial growth rate

Total plate count and yeast & molds were determined by using pour plate method throughout the storage period. 10 g of sample was homogenized in 90 ml ringer's solution; other decimal dilutions were prepared from a 10^{-1} dilution. The total bacterial count was assessed by using plate count agar as culture media (HiMedia, M001). Petri plates were incubated at 35°C for 48 hrs. Similarly, the yeast and mold count were assessed by using dextrose agar as the culture media (HiMedia, M403). Petri plates were incubated at 30°C ± 2°C for 3 to 5 days (72 hrs). Samples were analyzed in duplicate from 0 to 16th day at an interval of 4 days and microbial counts were counted and expressed as log CFU/g (Chien et al, 2007).

2.11. Micro structure

The sample with 3×3 mm was fixed, dried and mounted on aluminium stubs and coated with gold at 5 mA and 1.5 kV using a sputter coater. The microstructures of coated and uncoated samples were analyzed using a scanning electron microscope (Carl Zeiss EVO MA15).

2.12. Statistical analysis

All the experiments were carried out in triplicates for the control and experimental samples. The data was subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for the average value of parameter among the five treatments and Tukey's test is used to compare the mean values between pair of samples. Differences were calculated to compare significant effects at $p \leq 0.05$ level using SPSS statistics 22 (IBM).

3. Results and discussion

3.1. Weight loss

Figure 1 shows the data for weight loss of whole fruits coated with different concentration of casein stored at ambient conditions (26± 1ºC) for a period of 16 days. Significant increase was noted in weight loss over the storage period in all the experimental samples. But the control sample shows higher weight loss (20.4%) at 12th day of storage when compared with the coated fruits. The results revealed that a lesser weight loss in S1 (16.56%) followed by S2 (16.58%), S4 (16.58%) and S3 (16.97%) samples. A small difference ($p<0.05$) was observed in weight loss within the coated samples at the end of storage period. The reason might be coated casein formed a layer like structure up on the fruit surface and acted like a barrier for water drips and helps to maintain/control the moisture content in fruits for long time. Similar results were observed in a study conducted by Perez-Gago et.al, 2019 on fresh cut apples coated with composite whey protein isolate and bees wax. Researchers found that reduced weight loss was in coated fruits compared with the control samples. According to the Valverde et al. (2005) grapes coated with *aloe vera* gel noted potential reduction in weight loss in comparison with control, indicating that any biopolymeric material can be formed a layer and acted like a shield to control the fruits moisture loss.

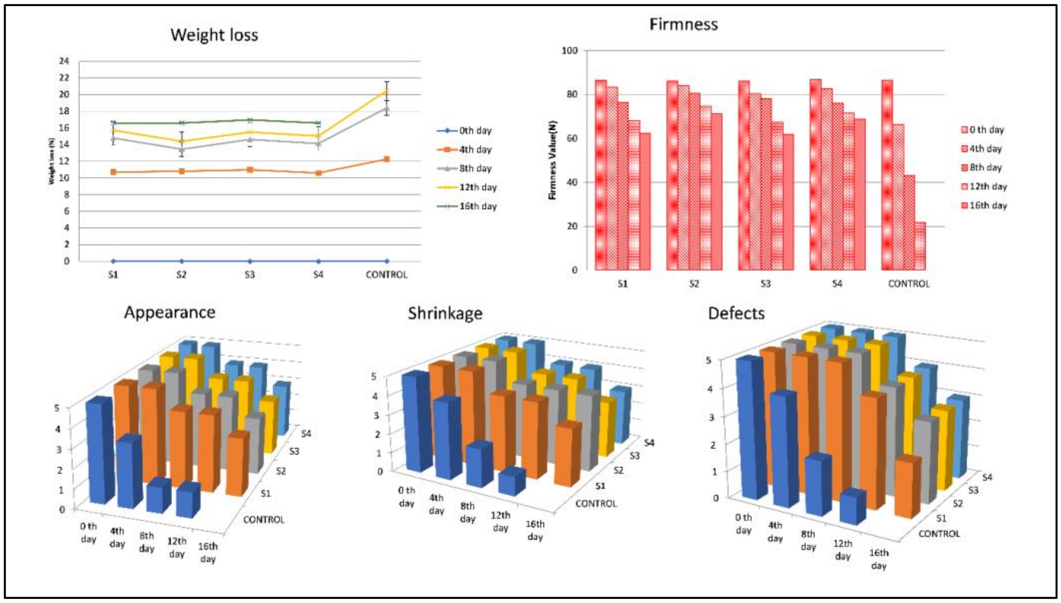

Figure 1. Weight loss, firmness, appearance, defects and shrinkage of casein coated whole guava fruits on storage at 26 ± 1^0 C.

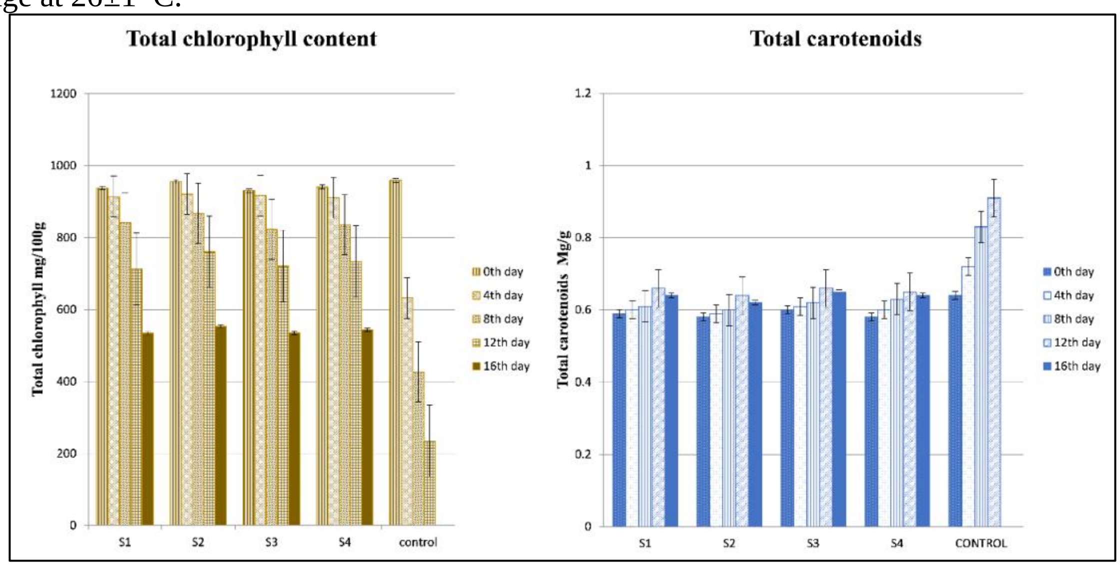

Figure 2. Total chlorophyll and carotenoid content of whole guava during the storage at 26 ± 1^0 C.

3.2. Firmness

The fruit firmness was measured based on the characteristic softening and dissolving of middle lamella in the cell wall. When fruit starts to ripening, the hemicellulose becomes soluble and leads to increased cell wall disruption causes decline in fruit firmness (Wills et al., 1980**;** Wang et al., 2013). In general, firmness of whole guava fruits decreased with the ripening process during the storage (Figure 1). Whereas, casein coated samples maintained stable firmness throughout the storage. During the initial days, control and casein coated samples resembles similar firmness patterns (86.11-86.90 N) in all experimental samples. However, up on storage/end of the storage a significant difference ($P<0.05$) between control and coated samples was observed, difference was also significant in between the different casein concentrations. A slow decreased firmness was noted for all casein coated samples ranges between 61.85 to 71.26 N at 16th day of storage. But, when coming to the control sample, firmness was drastically decreased to 21.82 N at 12th day of storage which is a un accountable firmness to measure fruit quality. 10% casein (S2) was identified as most effective coating to retain higher value of firmness among the all concentrations. The reason might be the physical shielding of fruits from the external environment give more pleasure to maintain internal biochemical reactions and delayed the production of ethylene and CO_2 which accelerates the ethylene production and prevents the shrinkage of fruits mesocarp and stops the sharp firmness decrease in fruits. Coating layer can prevent the water loss leads to the ionic balance in cell walls, and controlled gaseous exchange would help the maintaining the structure and integrity of cell wall components. The results were in accordance with Forato et al., 2015, worked on shelf life of guava fruits coated with cashew gum and methoxy cellulose. Their research study reveals edible coatings can successfully maintained fruits firmness by delaying the ripening process up on storage. In another study, Sánchez et al. (2015) observed that 'Rocha' pear slices coated with high concentrated chitosan resulted higher firmness value indicating the role of edible coatings in preserving textural profile of the fruits.

3.3. Visual quality of coated fruits

Visual quality of the casein coated guava fruits were presented in Figure 1 and it shows the optical determination of fruits quality coated with casein at different concentrations and stored at ambient temperature.

3.3.1. Appearance

For good marketability of any fresh produces like fruits and vegetables, appearance is an important visual determinant quality factor decides consumer perception. In present study the guava fruits were evaluated for quality by their physical appearance. Figure 1 show that the mean score for appearance began to fall from 8th day of storage. During the 4th day, the uncoated fruit samples scored significantly lower ($p< 0.05$) than the four casein coated samples and continued up to 12th day of storage. It was found that the S1, S2, S3, S4 coatings continued better scores at the end of the storage period. This shows that the casein coatings were more compatible to preserve the freshness of the guava fruits.

3.3.2. Defects

Defects include microbial spoilage, discoloration; pitting and softening of the fruits were evaluated and depicted in Figure 1. According to the picture, higher mean score indicates the lesser the defects and the same was used to determine the degree of defects. No decay was recorded in coated fruits until 11th day of storage but, coated fruit samples began to change color from 12th day and it is early in the case of control samples. The decaying process was attained maximum in control sample at 12th day where coated samples were not observed decaying. There is a significant difference was observed in all coated samples in comparison with control up to 12th day. S2, S3, S4 scored better results than the S1, though the means are not statistically different at 16th day and it is clear that there are no signs of microbial spoilage till the end of the storage period in casein coated fruits.

3.3.3. Shrinkage

Shrinkage is a physical and optical determinant that decides the physical appearance and freshness of the fruits. Statistical means for shrinkage of whole fruits treated with different concentration of casein with and without fortification of ascorbic acid were presented in the Figure 1. Increased shrinkage was observed during the storage in control samples while coated samples showed without any shrinkage during the initial days. However, shrinking starts from 8th day onwards in all coated fruits, moreover little difference was noted in between coated fruits. Because, concentration and the composition of the casein coatings seems to be influenced the control of shrinkage process in the fruits. Up to the 12th day, it was noted that all coated samples were significantly different when compared to control. However, coated samples also started to shrink during the later stage of storage and it is evident that casein coatings successfully controlled the shrinkage of fruits by creating low relative humidity and preventing moisture and gaseous escape from the fruit tissues during the storage. Due to the physical properties and the plasticizer of thick coatings/films shows wrinkling during storage (Miranda et al., 2004). However, all coated samples did not differ significantly within the storage period. But the S2 casein coated sample scored low when compare with other coated samples.

3.3.4. pH of the fruits

The results from the Table 1 concluded that increased storage period increases the pH from 0 to 16 days with respect to all coated and control fruit samples. However, when compared to control sample, there is a significant difference (P<0.05) was noted between the four coated samples from 4th to 16th day of storage. Control sample which was not coated with casein have been completely spoiled on 13th day of storage. Above observations may be concluded that casein coatings showed a significant effect on pH control in whole guava fruits. Coatings can lower the increase in pH during storage period because, it creates an internal modified atmosphere that helps in delay of fruit ripening by arresting various metabolic reactions leads to the control effect on pH of coated fruits. Similar results were reported by Togrul and Arslan. (2004), Maftoonazad et al. (2008), Wani et al. (2014), results shows that edible coatings can controlled the pH levels in both cut and whole fruits.

Table 1. Effect of casein coatings and control samples in storage period on p^{H}, Total soluble solids (TSS), titratable acidity (TA), Vitamin C (Ascorbic acid) of whole guava

Parameters	Storage periods (days)	Samples				
		Control	S1	S2	S3	S4
pH	0	3.71±0.04^{a}	3.60±0.05^{a}	3.67±0.00^{a}	3.59±0.02^{a}	3.68±0.01^{a}
	4	4.23±0.03^{a}	3.93±0.01^{bA}	3.85±0.02^{CA}	3.94±0.01^{dA}	3.86±0.01^{eA}
	8	5.12±0.01^{a}	4.90±0.00^{bA}	4.79±0.00^{CBA}	4.92±0.01^{dAB}	4.79±0.01^{eCA}
	12	5.92±0.02^{a}	5.72±0.02^{bA}	5.60±0.00^{cBC}	5.71±0.00^{dADE}	5.59±0.00^{eCCE}
	16	*	5.92±0.00^{a}	5.81±0.01^{bA}	5.91±0.01^{cBA}	5.82±0.01^{dAB}
TSS (%)	0	10.30±0.34^{a}	10.30±0.28^{a}	10.10±0.34^{a}	10.30±0.34^{a}	10.0±0.15^{a}
	4	12.80±0.34^{a}	10.50±0.34^{bA}	10.30±0.28^{cA}	10.50±0.28^{dA}	10.40±0.28^{eA}
	8	15.31±0.28^{a}	12.47±0.28^{bA}	12.31±0.28^{cA}	12.54±0.28^{dA}	12.46±0.34^{eA}
	12	17.44±0.28^{a}	14.30±0.23^{bA}	14.0±0.34^{cA}	14.40±0.28^{dA}	14.20±0.28^{eA}
	16	*	15.31±0.28^{a}	15.29±0.34^{a}	15.34±0.28^{a}	15.32±0.34^{a}
	0	1.15±0.34^{a}	1.17±0.34^{a}	1.19±0.28^{a}	1.18±0.34^{a}	1.15±0.28^{a}

Titrable acidity(mg /100g)	4	0.98±0.23[a]	1.11±0.34[a]	1.12±0.28[a]	1.13±0.34[a]	1.09±0.28[a]
	8	0.81±0.46[a]	0.96±0.28[a]	0.98±0.23[a]	0.97±0.23[a]	0.99±0.23[a]
	12	0.69±0.23[a]	0.74±0.28[a]	0.76±0.34[a]	0.75±0.34[a]	0.74±0.28[a]
	16	*	0.52±0.28[a]	0.59±0.28[a]	0.56±0.34[a]	0.54±0.17[a]
Vitamin C (mg/100g)	0	238±0.34[a]	234±0.40[bA]	237±0.46[aB]	464±0.40[cC]	472±0.40[dD]
	4	208±0.28[a]	200±0.28[b]	204±0.23[c]	412±0.28[d]	418±0.34[e]
	8	168±0.28[a]	189±0.34[b]	196±0.34[c]	382±0.46[d]	390±0.23[e]
	12	118±0.40[a]	152±0.46[b]	158±0.23[c]	314±0.34[d]	316±0.46[e]
	16	*	113±0.46[a]	126±0.28[b]	290±0.28[c]	301±0.23[d]

Mean values± standard error. Means with the same letters within a period of storage (rows) are not significant ($p<0.05$), *sample deteriorated

Note: - S1 (casein 5%) and S2 (casein 10%), S3 (casein-5% fortified with ASA) and S4 (casein-10% fortified with ASA) samples.

3.4. Total soluble solids (TSS)

Mean values for total soluble solids of whole guava fruits coated with different concentration of casein with and without fortification of ascorbic acid were shown in Table 1, a gradual increase in all samples throughout the storage period was noted. The highest value for TSS was noted in control sample (17. 44^0 brix) in which general ripening process was continued without any hurdles. TSS values for casein coated samples at the end of the storage were likely 15.31 and 15.29° brix for S1 (casein 5%) and S2 (casein 10%) samples, and it is 15.34, 15.32° brix for S3 (casein-5% fortified with ASA) and S4 (casein-10% fortified with ASA) samples. According to the results, a significant difference was observed in all coated samples when compared to control samples from 4^{th} to 12^{th} day of storage. A research study carried out by Keqian Hong et al. (2012) stated that the edible coated guava fruits resulted lower TSS value than uncoated samples at the end of the storage. The reason may be controlled ripening process was achieved in coated fruits by inhibiting/slow down the production of ethylene, abscisic acid and other growth hormones with in the fruit tissues (Bashir and Abu-Goukh, 2003; Krishna and Rao, 2014). According to the literature, it is well reported that edible coatings can slow down the gaseous exchange and create a modified atmosphere inside the coating further leads to the unavailability of optimum level of O_2 and CO_2 required for ripening process (Dang et al., 2008; Taşdelen and Bayindirli, 1998). However, a quite suitable edible coatings can slow down the ripening process when compared to uncoated fruits but not completely, and defined phenomena was quite sufficient for fresh whole guava fruits for retailing.

3.5. Titratable acidity

Table 1 shows the mean values for titratable acidity (%) of casein coated and uncoated guava fruits stored at ambient storage condition (26±1°C) upto16 days. The results revealed that there is a significant decline in acidity of fruits up on storage. The maximum value for acidity was observed in S2 (casein 10%) as 0.59% followed by S3 (casein-5 % fortified with ASA) 0.56%. However, recorded values for the S4 (casein-10% fortified with ASA) and S1 (casein 5%) were 0.54 and 0.52% which were little lower than S2 and S3 indicated a little decline in acidity for casein coated samples. The maximum drop was found in control sample (0.69%) at 12^{th} day of storage was an evidence for the acidity regulation of casein coating by delaying senescence of the fruits. In general, acidity was drastically increased up on ripening of fruits which was slow down by delaying various growth factors related to the senescence. Han et al. (2004) reported the faster reduction in acidity gives rise to a faster senescence which leads to the rapid quality deterioration of the fruits. However, the different concentrations of casein can effective to maintaining and controlling the acidity in guava fruits. However, the acidity changes were not significantly differing in coated samples but when compared to the control, drastic acidity changes were noted. Above observations stated that casein edible coatings can be delayed the maturation and ripening as much as possible required in maintaining fruits quality during the storage.

3.6. Vitamin C

Ascorbic acid is a well-known organic antioxidant compound named as vitamin C which is an essential vitamin required for the human body. Fruits and vegetables are general and natural source for the vitamin C required for daily intake. Ascorbic acid content in fruits gradually decreases during senescence (Lentheric et al., 1999) and made them unavailable for some times as its heat sensitivity and photolytic nature. So, to maintain stable ascorbic acid levels in whole fruits, casein edible coatings were fortified with different concentrations of Vitamin C. According to the experimental data, vitamin C content was slowly decreased in all experimental samples during the storage. Decreasing trend was little accelerated in control when compared to the coated samples and the difference was significant at $p<0.05$. Changes in ascorbic acid content for the casein coated and control fruits during the storage period were shown in Table 1. The results show gradual decline during storage. The initial ascorbic acid content for the experimental samples was ranged from 234-472 mg/100g. Compared with the control, the casein coating significantly increased the amount of ascorbic acid in the guavas. After 16 days of storage, the ascorbic acid retention for the guavas treated with casein (S1, S2) was 113 and 126 mg/100g. The vitamin C content of fortified ascorbic and casein treatment (S3, S4) was 290 and 301mg/100g, respectively, whereas the control samples maintained 118 mg/100g of the ascorbic content at 12th day of storage period. The addition of ascorbic (10% w/v) to the casein based edible coatings helped to preserve the natural ascorbic acid content in fresh guavas, thus helps to maintain its nutritional quality throughout storage. Same results were identified in a study conducted by Tapia et al. (2008). This concludes that the casein coating creates a modified atmosphere and slowed down the loss of vitamin C during ambient storage temperature. Similar results were reported by Mathooko. (2003) and Ayranci and Tunc. (2004), according to their study edible coatings formed a shield like structure on fruit surface and controlled the vitamin C loss during the storages.

3.7. Total chlorophyll and carotenoids

Figure 2 shows the values for Chlorophyll and carotenoid content of whole guavas coated with different concentration of casein edible coatings, In the present study the Chlorophyll content decreased while carotenoid content increased during ripening in guava fruits.

Total chlorophyll value in control sample was reduced rapidly from 959.5 mg/100g to 235mg/100g. Casein coated fruits shows slow decrease in total chlorophyll content from 955 ± 0.34 mg/100 g, fresh weight on initial day to 544.3 ±0.34 mg/g, on 16th day of storage period. The coated fruit samples (S1, S2, S3, S4) significantly ($p<0.05$) delayed the reduction in chlorophyll pigment at end of the storage period in guava fruits. The reduction of chlorophyll content in the guava fruit treated with casein is similar with the result of Keqian Hong et al. (2012) and Soares et al. (2007). The decline in chlorophyll is due to increase in chlorophyll degrading enzymes (chlorophyllase, chlorophyll oxidase, and peroxidase) during ripening. There was a significant difference of carotenoid content in all coated and control sample. In the present study carotenoid content of 0.65 mg/g was observed for control fruit on initial day whereas similar value of 0.65 was observed in coated fruit on 16th day of storage period. There was no significant difference in carotenoid contents among the coated samples at the end of the storage period.

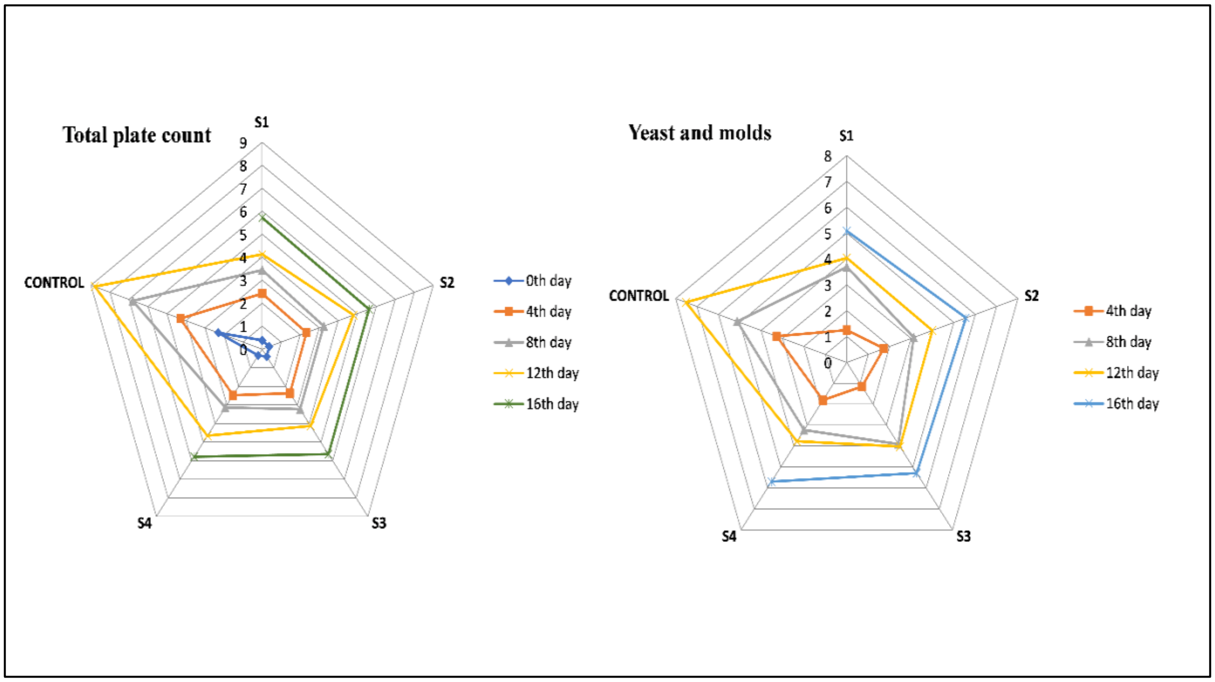

Figure 3. TPC and yeast & Molds of casein coated guava fruits during the storage

3.6. Sensory quality of casein coated guava fruits on storage

3.6.1. Appearance

Table 2. Effect of different casein coatings and control sample on sensory scores of whole guava fruits during 16 days of storage period.

Sensory evaluation attributes	Storage periods (days)	Samples				
		Control	S1	S2	S3	S4
Appearance	0	5±0.46^{a}	5±0.28^{a}	5±0.34^{a}	5±0.40^{a}	5±0.23^{a}
	4	3±0.46^{a}	4±0.28^{a}	4±0.34^{a}	4±0.40^{a}	4±0.23^{a}
	8	2±0.46^{a}	4±0.34^{bA}	4±0.23^{cA}	4±0.40^{dA}	4±0.28^{eA}
	12	1±0.11^{a}	3±0.28^{bA}	3±0.34^{cA}	3±0.40^{dA}	3±0.23^{eA}
Colour	0	5±0.28^{a}	5±0.28^{a}	5±0.34^{a}	5±0.40^{a}	5±0.23^{a}
	4	3±0.46^{a}	4±0.34^{a}	4±0.40^{a}	4±0.23^{a}	4±0.28^{a}
	8	2±0.28^{a}	4±0.46^{bA}	4±0.34^{cA}	4±0.28^{dA}	4±0.40^{eA}
	12	1±0.11^{a}	3±0.40^{bA}	3±0.34^{cA}	3±0.28^{dA}	3±0.46^{eA}
Taste	0	5±0.46^{a}	5±0.34^{a}	5±0.28^{a}	5±0.40^{a}	5±0.23^{a}
	4	3±0.28^{a}	4±0.34^{a}	4±0.28^{a}	4±0.23^{a}	4±0.40^{a}
	8	2±0.28^{a}	4±0.23^{bA}	4±0.46^{cA}	4±0.34 ^{dA}	4±0.40 ^{eA}
	12	1±0.11^{a}	3±0.46^{bA}	3±0.28^{cA}	3±0.34 ^{dA}	3±0.40^{eA}
Texture	0	5±0.46^{a}	5±0.17^{a}	5±0.28^{a}	5±0.40^{a}	5±0.23^{a}
	4	3±0.28 ^{a}	4±0.28^{a}	4±0.23^{a}	4±0.40^{a}	4±0.34^{a}
	8	2±0.35 ^{aA}	4±0.^{23aA}	4±0.46^{aA}	4±0.40^{aA}	4±0.34^{aA}
	12	1±0.11^{a}	3±0.40^{b}	3±0.46^{c}	3±0.28^{d}	3±0.34^{e}

Flavour	0	5±0.28[a]	5±0.17[a]	5±0.40[a]	5±0.46[a]	5±0.23[a]
	4	3±0.34[a]	4±0.23[a]	4±0.40[a]	4±0.34[a]	4±0.46[a]
	8	2.6±0.35[a]	4±0.40[aA]	4±0.46[aA]	4±0.23[aA]	4±0.34[aA]
	12	1±0.11[a]	3±0.34[bA]	3±0.28[cA]	3±0.17[aA]	3±0.28[eA]
Overall acceptability	0	5±0.28[a]	5±0.23[a]	5±0.34[a]	5±0.40[a]	5±0.46[a]
	4	3±0.34[a]	4±0.46[a]	4±0.40[a]	4±0.23[a]	4±0.34[a]
	8	2±0.28[a]	4±0.34[bA]	4±0.40[cA]	4±0.23[dA]	4±0.28[eA]
	12	1±0.11[a]	3±0.34[bA]	3±0.28[cA]	3±0.34[dA]	3±0.34[eA]

Mean values± standard error. Means with the same letters within a period of storage(row) are not significant ($p<0.05$), *sample deteriorated
Note: - S1 (casein 5%) and S2 (casein 10%), S3 (casein-5% fortified with ASA) and S4 (casein-10% fortified with ASA) samples.

Means regarding appearance of coated and control samples of guavas kept at ambient storage conditions are given in Table 2. The mean values for S1 and S2 were 5±0.28 and 5±0.34 at 0 day that decreases to 3±0.28 and 3±0.34 at 12th day, respectively. However, the mean values for S3 and S4 were 5±0.40 and 5±0.23 at 0 day that decreases to 3±0.40 and 3±0.23 at the same storage intervals. Maximum decline in appearance was found in control sample as values are ranging from 5±0.46 to 1±0.11 from initial to 12th day of storage period respectively, control sample maintained its shelf life up to 12 days.

3.6.2. Colour

Mean values of casein coated guavas for attribute colour was given in Table 2. Maximum decline in colour values was found in S1 as described by values ranging from beginning (Score 5) to the end of the day (Score 4) respectively. All the treatment delayed the decline in sensory quality and extended the shelf life. However, after 12 days of storage the control sample became unacceptable for consumption. The casein coated samples retained the sensory quality. Edible coatings were proven in maintaining the moisture content and keep a fresh appearance (Alikhani, 2014). Coatings can act as the carriers of anti-browning agents and improves the water vapour properties by the addition of glycerol (Rojas-grau et al., 2008)

3.6.3. Taste

Mean values regarding taste of coated and control samples of guavas kept at ambient storage conditions are given in Table 2. The mean values for S1 and S2 were 5±0.34 and 5±0.28 at 0 day that decreases to 3±0.46 and 3±0.28 at 12th day, respectively. However, the mean values for S3 and S4 were 5±0.40 and 5±0.23 at 0 day that decreases to 3±0.23 and 3±0.40 at the same storage intervals. Maximum decline in taste was found in control sample as values are ranging from 5±0.46 to 1±0.11 from initial to 12th day of storage period respectively. The control sample maintained its shelf life up to 12 days of storage period.

3.6.4. Texture

Mean values of casein coated guavas for attribute texture was given in Table 2. Maximum decline in texture values was found in S1 as described by values ranging from beginning (Score 5) to the end of the day (Score 4) respectively. All the treatments delayed the decline in sensory quality and extended the shelf life. However, after 12 days of storage the control sample became unacceptable for consumption. The casein coated samples retained the sensory quality.

3.6.5. Flavour

Mean values of flavour of coated and control samples of guavas kept at ambient storage conditions are given in Table 2. The mean values for S1 and S2 were 5±0.17 and 5±0.40 at 0 day that decreases to 3±0.34 and 3±0.28 at 12th day, respectively. However, the mean values for S3 and S4 were 5±0.46 and 5±0.23 at 0 day that decreases to 3±0.17 and 3±0.28 at the same storage intervals. Maximum decline in flavour was found in control sample as values are ranging from 5±0.28 to 1±0.11 from initial to 12th day of storage period respectively, control sample maintained its shelf life up to 12 days.

3.6.6. Overall acceptability

Results of sensory evaluation of guavas coated with casein edible coatings were plotted in Table 2. All the coated samples delayed the decline in sensory quality and extended the shelf life. Maximum fruit overall acceptability score (3.00) was recorded in all coated samples. With irrespective of the control and coatings there was decrease in overall acceptability as the storage period advanced. There was a significant difference between the control and coated samples at the end of the storage period. The overall acceptability of casein coated guava fruits was best as compared to control. Guava fruits coated with casein edible coatings have greater retention of green colour than the uncoated fruits, shows delay in ripening in coated fruits. The control sample was spoiled on 12th day of storage period. During storage period the panel members found glossy appearance with acceptable flavor.

3.7. Microbial quality of casein coated fruits

Fruits are rich in nutrients and contains highest amount of moisture which creates a better environment for the growth of microorganism. The Figure 3 shows the growth curve of total bacterial count in fresh guavas with and without casein coatings, stored for 16 days. Results of the present study shows there was a significant increase ($p<0.05$) in the total plate counts (TPC) and yeast and mold counts (Y&M) during storage period for all samples. There was a significant difference between the TPC and Y&M for casein-based coating samples and uncoated control sample at all storage time. This shows that the incorporation of casein-based formulation into guavas significantly reduced the TPC and Y&M count of coated samples during storage period. However, the growth of microorganisms in the control sample (S0) was higher than the growth with coatings in the 8th day of storage reaching 6.80 log cfu/g for (TPC) and 7.52 log cfu/g for (Y&M) at 12th day of storage. According to the IFST (Institute of food science and technology), 10^6 cfu/g is considered as limit of acceptance of fruit-based products during the shelf-life period. (Bierhals et al, 2011). The casein coated samples reached 10^5 cfu/g at 16th day of storage. In the present study the incorporation of 5% and 10% casein without and with fortification decreased the microbial load and extended the shelf life of guava samples (S1, S2, S3, S4) to 16 days at ambient storage conditions.

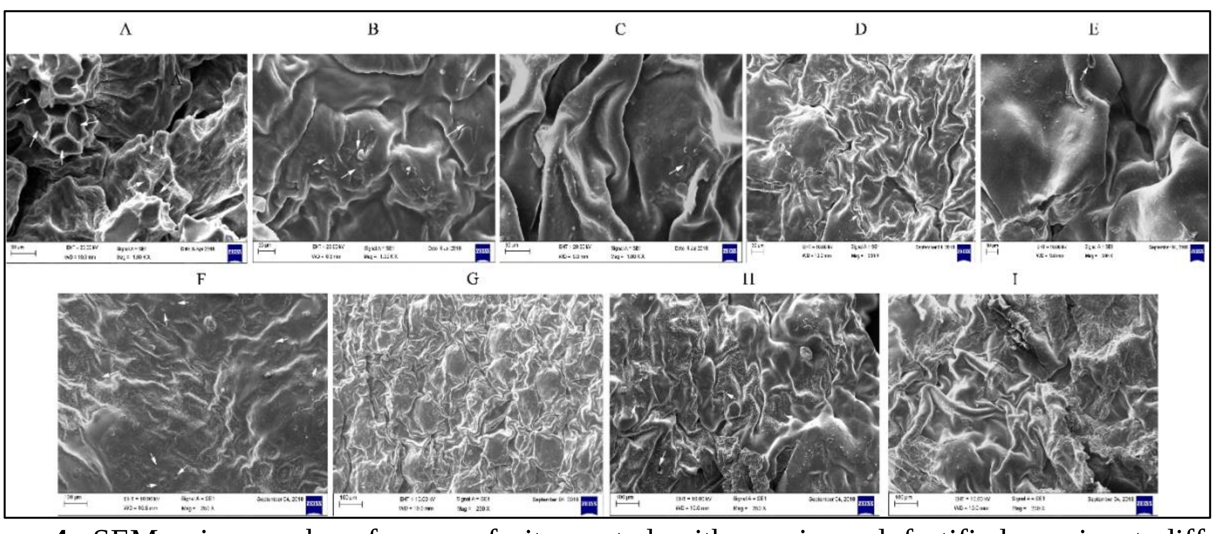

Figure 4. SEM micrographs of guava fruits coated with casein and fortified casein at different concentrations (A- control; B-5% casein initial; C-5% casein stored; D-10% casein initial; E-10% casein stored; F-5% casein fortified initial; G-5% casein fortified and stored; H- 10% casein fortified initial; I-10% casein fortified and stored).

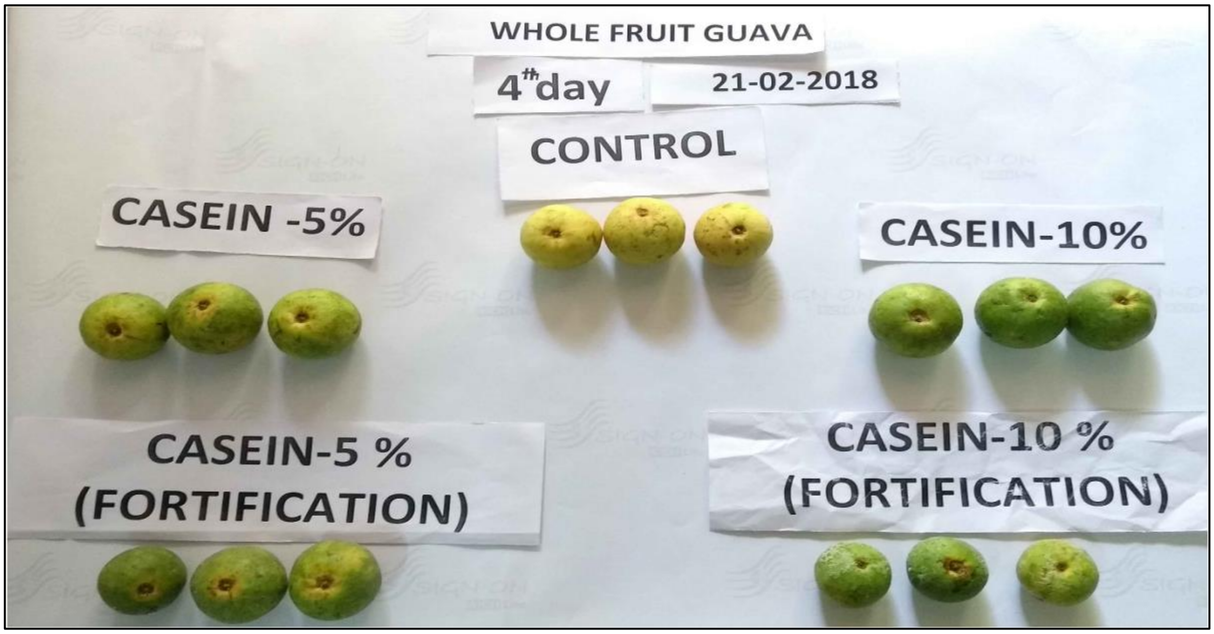

Figure 5. Appearance and physical state of casein coated guava fruits at various concentrations during the storage at ambient temperature.

3.8. Micro structure and morphology of casein coated fruits

The arrows in Figure 4 indicate micropores and stomatal aperture on guava skin in control, coated and fortified 5% and 10% initial and stored casein coated fruit. The results of scanning electron microscopy clearly show several micropores, rough and disordered surface with cavities on the guava fruit skin surface (S0) which helps in transpiration (Figure 4 (A)). While the guavas coated with 5% (S1) and 10% (S2) casein on the initial day (Figure 4 (B and D)) showed tightly compacted, less pores but few stomatal apertures are still open. After storing for sixteen days the surface of the guava (Figure 4 (C and E)) showed the stomata was entirely sealed and covered with casein coating on outer surface of the guava. The casein coating on the fruit surface produces a more uniform covering which appeared to be smoother, rigid and more ordered structure without cavities. Pereira-Kechinski et al. (2012) proposed that if the natural openings were covered by coating, this could physically prevent the invasion of the pathogen, and therefore reduce disease incidence.

The arrows in Figure 4 (F and H) indicate stomatal aperture on guava fruit surface in casein 5% and 10% fortified (S3) and (S4) initial samples. After storing for sixteen days Figure 4(G and I) the casein coatings on the fruit surface produces a more uniform covering which appeared to be smoother without opening of stomatal aperture. It resembles formation of casein coatings was homogeneously distributed on the surface of guava fruit.

4. Conclusions

Casein based coatings with the addition of plasticizer like glycerol and fortifying agent as ascorbic acid was applied as coating for fresh whole guava fruits. All casein coatings act as barrier in retention of fruit quality and helps in loss of vitamin C through fortification. The milk based edible coating is most emerging technique in preserving and extending the shelf life of guavas upto 16 days at ambient storage conditions (26± 1ºC) and also helpful for commercial storage without affecting the nutritional quality during preservation and storage process.

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