Preparation, Characterization and Application of Vitamin-E fortified Nanocoatings on Fresh-cut Apples

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Abstract

Fresh-cut apples have a very low shelf life due to high vulnerability to oxidation in open air. The aim of the present work was to prepare enriched nanocoatings to increase not only the shelf life of the fruit, but to add to it nutritive values as well. Hence, four different edible coatings were prepared containing vitamin-E nanoemulsion. Vitamin-E nanoemulsion was successfully prepared by EPI (Emulsion Phase Inversion) method and the particles size distribution was checked by DLS (Dynamic Light Scattering) method. Potassium Sorbate and Calcium Chloride were added, respectively, as antimicrobial and antioxidant agents. In one coating, fresh lemon juice was used in place of ascorbic acid for comparison. To reduce respiration and water vapor permeability, methyl cellulose and stearic acid were added in different ratios to all the four coatings. Prepared coatings were then applied on freshly-cut apple pieces using dip method. Various characterization parameters were performed to analyze the quality of vitamin-E fortified edible nanocoatings such as weight loss, titrateable acidity, total soluble solids, and total phenolics for two weeks. In addition, antimicrobial activity of the prepared edible coatings was done using LBA (Luria Bertani Agar) culture media. All the coatings showed good results but the coating containing fresh lemon juice gave comparatively better results.

Keywords: Nanoemulsion, Fresh-cut apples, Fortification, Edible coatings, Antimicrobial agents, Fresh lemon juice

1. Introduction

Apple is an essential part of a healthy diet and contains a variety of important macro and micro nutrients such as sugars, proteins, vitamins and minerals (Campeanu*et al.*, 2009). Skin of the fruit is an important source of various important natural products such as phloridzin, catechins, anthocyanins and derivatives of hydroxycinnamic acid i.e. chlorogenic acid (; Lancaster and Dougall 1992; Nicolas *et al.*, 1994 and Awad*et al.*, 2000).

However, apples lack the recommended daily intake amount of certain essential elements such as vitamin E. In addition, the shelf life of a fresh- cut apple is very short due to its vulnerability to oxidation i.e. Millard reaction which results in rapid loss of water and enhanced microbial attack as compared to the uncut apple. Apple naturally contains on average 12mg of ascorbic acid, an antioxidant, which is inadequate to stop the enzymatic browning in it. Fresh-cut apple also offers the best suitable media for the growth of bacteria, molds and yeasts thereby further decreasing its shelf life.

Various methods have been applied to avoid the microbial growth in the fruits such as the washing of fresh cut fruit with solutions containing calcium ascorbate (Wang *et al.*, 2007) or to use a blend in mixture of sodium benzoate, potassium sorbate and cinnamon (Ceylan*et al.*, 2004). Frequent use of citric acid and ascorbic acid to decrease the microbial growth is also common in increasing the shelf life of food.

In recent years, edible coatings are being investigated to increase the life of fresh-cut fruits and vegetables. Main purpose of these coatings is to avoid direct contact of air with the open surfaces of fruits or vegetables thereby avoiding the oxidation reactions and rapid loss of water content. These coatings act as a water resistant barrier such as fish cod liver oil is kept in gelatin capsules (made up of hard water resistant coating) to avoid rancidity. Gelatin is an example of edible coating material and thus also adds to the sensory parameters of the food (Raybaudi- Massilia*et al.*, 2008). In addition to gelatin, various other edible materials have been successfully utilized to minimize the water loss in fresh-cut fruits and vegetables. For example, methyl cellulose-stearic acid was used as a hydrophobic edible barrier (Olivas *et al.*, 2003) in the fresh cut pears. However, these coatings did not add any nutritive value to the food being coated.

The aim of the present research work was to prepare not only hydrophobic edible coatings but to prepare a fortified one, which would also add to the nutritive value of the fresh-cut fruit. Apple, like some other fruits and vegetables, has some essential minerals, vitamins and dietary compounds in less concentration such as calcium, zinc and α-tocopherol (vitamin e) etc. 100 g of raw apple (including skin) contains almost 6 mg calcium, 0.18 mg vitamin E and about 0.04 mg zinc. According to a research, edible food materials provide only a portion of the required daily intake of certain nutrients. For example, only 8 percent of the recommended values for α-tocopherol are obtained from the edible food material (Maras *et al.*, 2004). This deficiency is usually compensated by using α-tocopherol supplements (Traber, 2004). However, scientists recommend that the intake of α-tocopherol from the naturally fortified food is far better than a corresponding encapsulated supplement (Leonard *et al.*, 2004).

Fortification of the food has become an important part of nutritive sciences in both developed and under developed regions of world to overcome the nutritive deficiencies. However, different eating habits and nutrient profiles, amount of the added constituent and compatible vehicle can vary (Gonzalez-Aguilar *et al.*, 2009).

Nanotechnology has empowered the progress of nanofortified edible coatings upto the size of 5 nm. These nanocoatings can be applied to various food matrices ranging from fresh cut fruits and vegetables to sweets, cheese, bakery goods, fast food and meat etc. (Sekhon,

2010). Very little work has been done in the field of edible nanocoatings. Nanofortified edible coatings not only provide an effectual barrier to moisture and gas exchange increasing the shelf life of food but also add to its nutritive value (Haung*et al.*, 2009). These nanocoatings can also help to deliver various flavors, antioxidants, antibrowning agents and even edible colors in nano-scale level. Nanoscale fortifications of additives such as minerals, enzymes and vitamins can also improve the nutritional value of various food products (Azeredo*et al.*, 2009).

2. Material and Methods

2.1. *Preparation of vitamin-e nanoemulsion*

Vitamin-E nanoemulsion was prepared by emulsion phase inversion (EPI) method (Mayer *et al.*, 2013) in which orange oil extracted from dried orange peels was used as an organic phase. 10% wt. solution of orange oil and vitamin-E ester in 2:8 respectively was mixed up with a 10% wt. solution of Tween 40, a surfactant. Afterwards 80% water phase comprising of citric acid buffer, pH-3, was added into this mixture while the system was continually stirred using a magnetic stirrer, until the final volume of water content was touched.

2.2 Analysis of Prepared Vitamin-E Nanoemulsion

2.2.1. Electrical Conductivity Measurement

Inversion for the oil in water phase to water in oil phase was confirmed by performing conductivity measurements (Feng *et al.*, 2009). A mixture containing orange oil as organic oil, Tween 40 as a surfactant and citric acid buffer solution with pH-3 were mixed together at room temperature. The mixture was stirred continuously using magnetic stirrer. Electrical conductivity was measured at regular intervals using EC-215 conductivity meter.

2.2.2. Particles Size Determination

Dynamic light scattering, DLS method is very popular to determine the size of small particles which are in Brownian motion in a solution. Using this method a commercial dynamic light scattering instrument was used and size of the particles was determined.

2.3. Preparation of Edible Coatings

Four different edible coatings were prepared.

1. Coating 1

This type of coating contained a mixture of vitamin-C (Ascorbic acid), vitamin-E (α-Tocopherol), calcium chloride and potassium sorbate. 1% vitamin-C was added in calcium chloride and potassium sorbate of 0.25 and 0.1 percentage respectively. It was added to the mixture as an antimicrobial agent. 0.125% of vitamin-E nanoemulsion, as a fortified element and as an antioxidant was also added to the mixture.

2. Coating 2

This type of coating was composed of two parts. The first part was prepared in the same way as in type 1 coating. 1% vitamin-C, 0.25% Calcium chloride and 0.125% vitamin-E nanoemulsion were mixed together.

The next part of the coating was prepared by mixing a polysaccharide based coating of methyl cellulose containing potassium sorbate in it. It was prepared by dissolving powdered form of methyl cellulose in 200 ml mixture of water-ethyl alcohol containing three parts of water and one part of ethyl alcohol. The mixture was stirred for 10 minutes at 80oC. To obtain a shiny and transparent surface ethyl alcohol was added to the mixture. 2% solution of propylene glycol was also added as a plasticizer in the formulation.

3. Coating 3

This type of coating also composed of two parts. First part of coating was again same as it was in type 2 coating. It was prepared by mixing 1% ascorbic acid (vit.-C), 0.25% calcium chloride and 0.125% vitamin-E nanoemulsion. The other part of coating was composed of a coating of methyl cellulose-stearic acid, a polysaccharide coating, containing potassium sorbate in it.

4. Coating 4

This type of coating was also divided into two parts. In first part a mixture of 1% fresh lemon juice, 0.25% calcium chloride and 0.125% vitamin-E nanoemulsion was prepared. In the next part methyl cellulose containing potassium sorbate was prepared.

2.4. Collection of Apples

The 'Kalakulu' apples were purchased from the local market. Care was taken that all of them were of equal size and with no defect in any of them. 23 pieces of these apples were purchased and stored in controlled conditions of 4oC temperature and 80% relative humidity.

2.5. Application of Edible Coatings on Apples

Various methods to apply coatings on Apple were available. Method used in this research was 'Dip' method.

Following is the procedure performed to apply coatings on Apple:

1. Apple A

In this sample edible **coating1** was applied. With the help of sharp stainless steel knife apple was cut in small cubes and with the help of tooth picks they were dipped into the coating for 5-7 seconds. Afterwards coated apples were dried at room temperature for about 15 -20 minutes.

2. Apple B

Edible **coating 2** was applied to Apple B. Firstly cubic apples were dipped in first part of edible coating for about 5-7 seconds and were dried for 5 minutes and then second part of coating containing methyl cellulose was applied on apple. It was dipped for 5 seconds in this part. Afterwards coated apples were dried at room temperature for about 30 minutes.

3. Apple C

Edible **coating 3** was applied to this sample. Cubic apples were dipped in first part of coating for about 5-7 seconds and after 5 minutes of drying they were dipped in second part

of coating containing stearic acid for about 5 seconds. Apples were dried at room temperature for about 30 minutes.

4. Apple D

For this sample edible **coating 4** was used and the apples were dipped in first part of coating for about 5-7 seconds and then dried for 5 minutes. After drying they were dipped in second part of coating for about 5 seconds and then were dried at room temperature for 30 minutes.

After coating of apples they were placed in disposable plates and were covered with clink foil so that controlled conditions are retained. Then they were stored in refrigerator at 4^{o}C.

2.6. Analysis of Coated Samples

2.6.1. FTIR Analysis

To check that chemicals used in coatings are still risk free and can be consumed, FTIR analysis of the coated samples was performed using FT/IR-4100 type A model instrument with serial number B032961016.

2.6.2. Weight loss

The weight of every coated apple piece was measured for two weeks regularly on every two days. Weight was measured on day 1, 3, 5, 7, 9, 11 and 13th. Analysis was performed in duplicate.

2.6.3. Total Phenolic Contents

Using Gallic acid curve method total phenolic contents were analyzed in nano-coated apple samples at 24 hour after coating. Experiment was performed in duplicate using a 721G visible spectrophotometer.

2.6.4. Quantification of Fruit Acids by Titration

Apple malic acid content was examined every 4 days for two weeks in duplicate. Amount of acid in apple was determined by titrateable acidity. Titrateable acidity of apple fruit extract was quantified using method of titration.

2.6.5. Quantification of Total Soluble Solids (TSS) Using Refractometer

Soluble solid content of apple juice of different samples were determined every 4 days for two weeks using a Refractometer ATAG0 DR-A1, calibrated at 20oC using a sucrose scale.

2.6.6. Antimicrobial Analysis of Nanocoated Apples

Five apples were washed with distilled water and dried. Then each apple was cut into 6 equal pieces and all were coated with one type of coating. After drying of coated apple pieces, juice was extracted with the help of sterilized juicer. In the same way all 5 apples were coated with all different prepared nanocoatings. Preliminary screening of crude extract of Apple fruit for antibacterial activity was determined by well method on LBA media (kaushik*et al.*, 2009). Bacterial inoculums 50 µl in concentration were spread on prepared plates with the help of spreader which was sterilized.

A well was made in agar plated with the help of sterile cork borer with 0.8 cm diameter. 100 µl of prepared extract was poured in inoculated plates. After that those plates were incubated for 24 hours at 37 ºC and antibacterial assay was determined by measuring the

inhibition zone (IZ) in cm, if any was present around the diffused well. Whole bacterial antibacterial activity was done under aseptic environment. Each measurement was performed three times and repeated twice a time against all four bacterial species.

3. Results and Discussion

3.2. Conductivity Measurements

Electrical Conductivity of prepared nanoemulsion was measured in regular intervals. As the amount of added buffer solution increased, conductivity also showed increase.

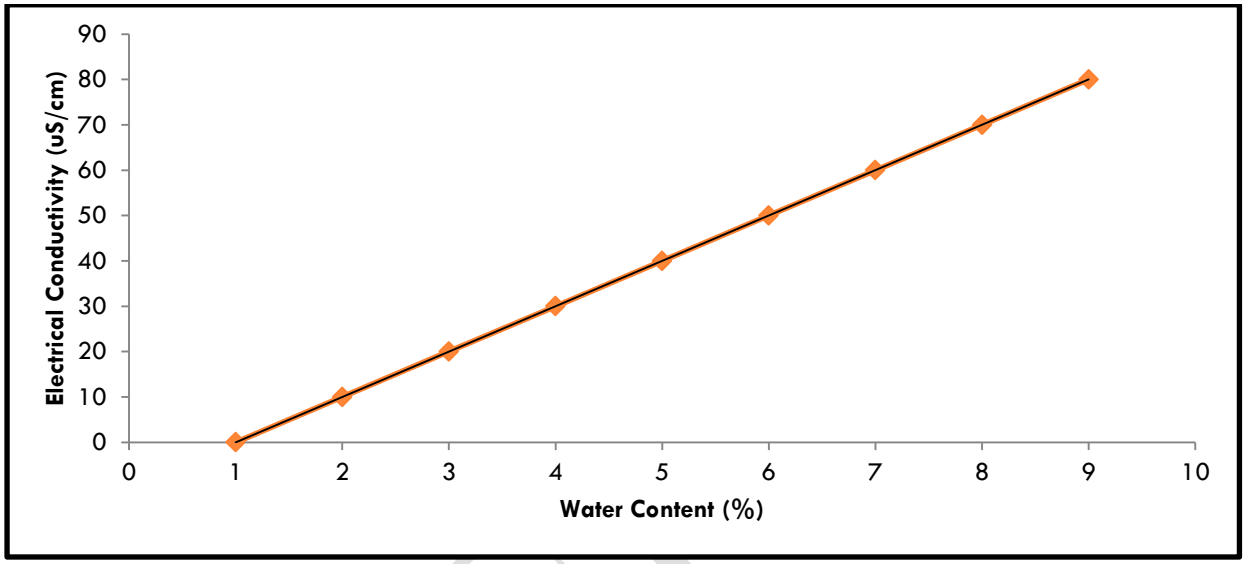

Fig.1. Electrical conductivity of the nanoemulsion is increasing with the increase in the water content.

3.3. Particles Size Determination

Using dynamic light scattering (DLS) method, particles size distribution of prepared nanocoating was determined. Tween 40 surfactant used in this nanoemulsion is useful to obtain a narrow range particles size distribution (Mayer *et al.*, 2013). Average particle diameter (Z-Average) obtained in this prepared polydisperse nanoemulsion was 759.7 nm, PDI = 0.798

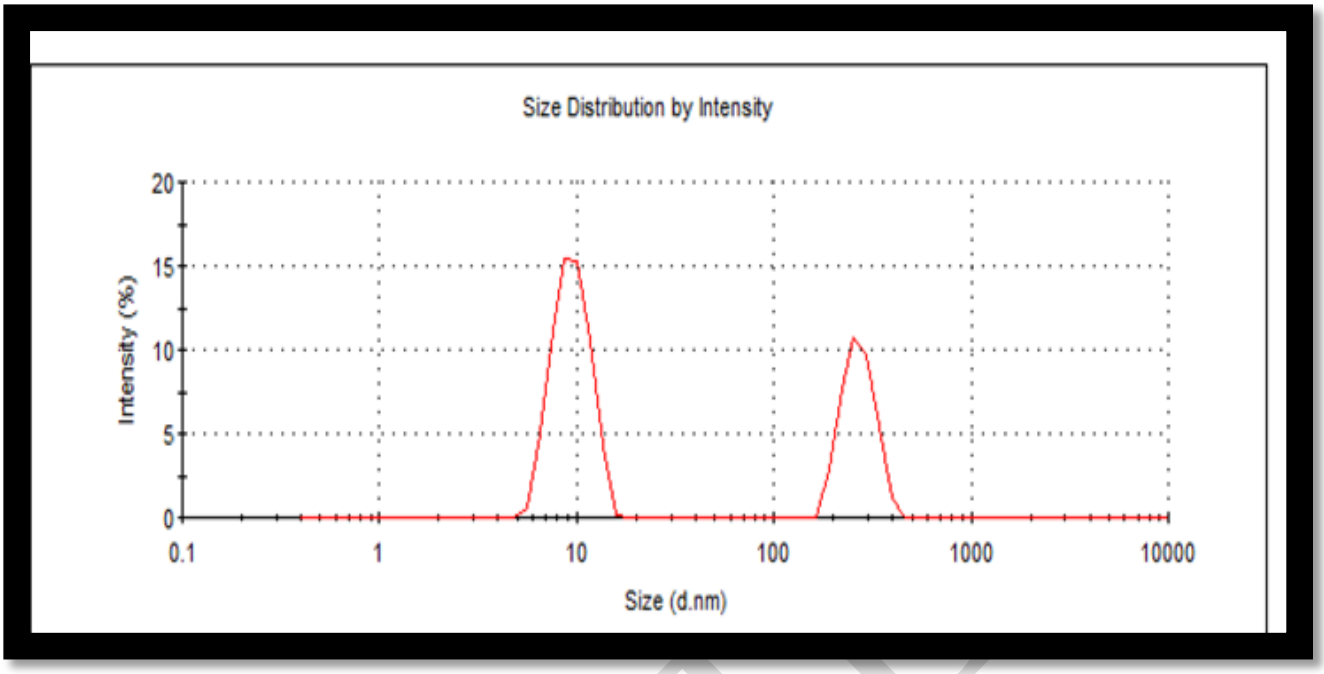

Fig. 2. Particles Size Distribution was measured by dynamic light scattering method. Peak 1 having 9.488 nm mean diameter with 62.4 % intensity while peak 2 relatively a small intensity peak with 270.1 nm mean diameter and 37.6 % intensity were obtained.

3.4. FTIR Analysis

FTIR analysis of the coated samples was performed and obtained spectrums showed a regular pattern for all nano-coated apple samples. Confirming that there were no ambiguous compounds present in the coatings and can be consumed.

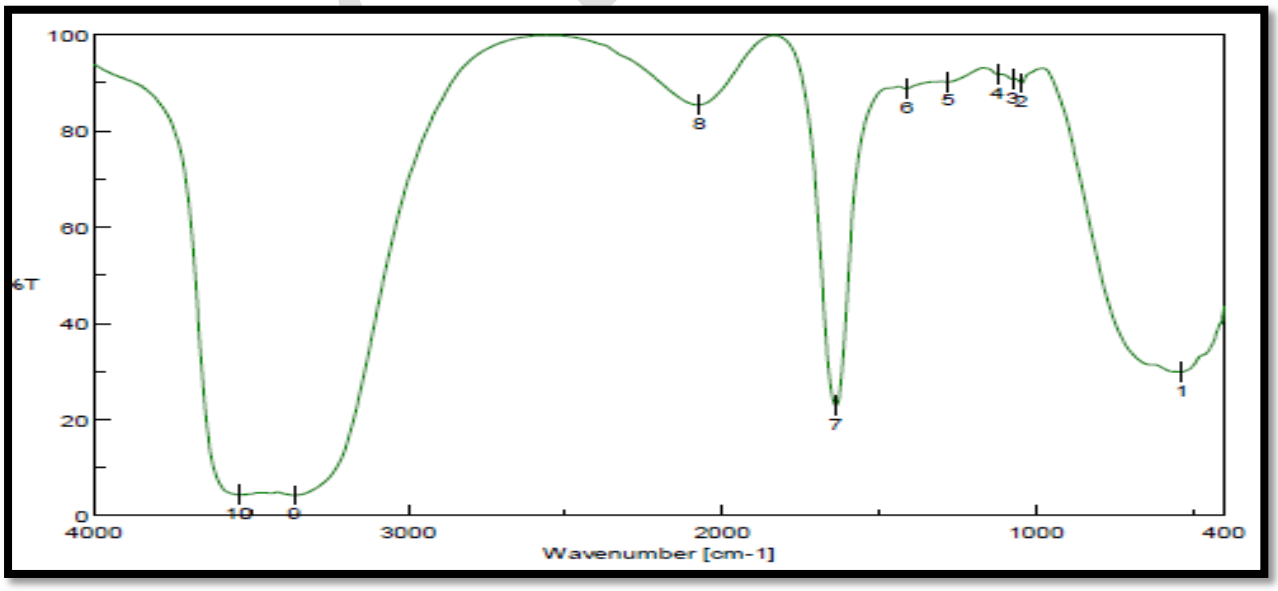

Fig. 3.Infra red spectrum shown by sample D containing fresh lemon juice, potassium sorbate, calcium chloride, methyl cellulose. Most of the peaks are shown by C-O, carbonyl bonds ranging from 1300-1000 cm^{-1}. Another peak ranging from 1600-1450 cm^{-1} is possibly due to presence of aromatic compounds.

3.5. Weight Loss

An important physical analysis in post-harvest life of fresh-cut fruit is weight loss. When fruits are cut into slices, their internal skinless part is exposed to air and chance of weight loss increases manifold (Olivas *et al.*, 2003). The main reason behind this water loss is attributed to the respiration process and transpiration process which are important metabolic processes. Gaseous exchange along with water loss on fruits surface is controlled by stomata present on outer layer of fruit (Abbasi*et al.*, 2011).

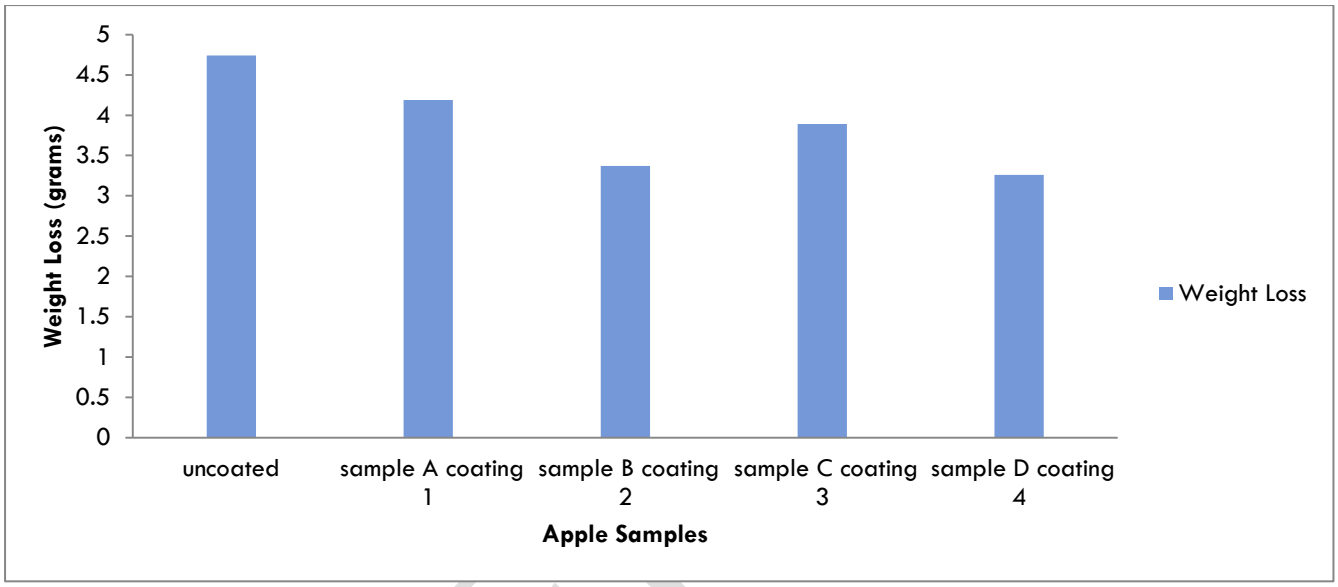

Fig. 4. Comparative Weight Loss changes in all prepared edible coatings during 2 weeks at 4◦C with uncoated sample. Minimum weight loss is shown by sample D having coating 4 on it which contains Fresh lemon juice in it.

3.6. Titrateable Acidity

Titrateable acidity tends to decrease as fruit becomes ripe and mature (Sadler and Murphy, 1998). As time passes carbohydrates present in fruits tends to breakdown and as a result glucose and sucrose content increases in fruit which results an increase in sugar content and decrease in acid content. Primary acid present in apple is malic acid. Amount of acid present in apple is measured in term of malic acid content. Multiplication factor for malic acid in apples is 0.0067. So, 1 ml of 0.1M NaOH is equivalent to 0.0067 g of malic acid.

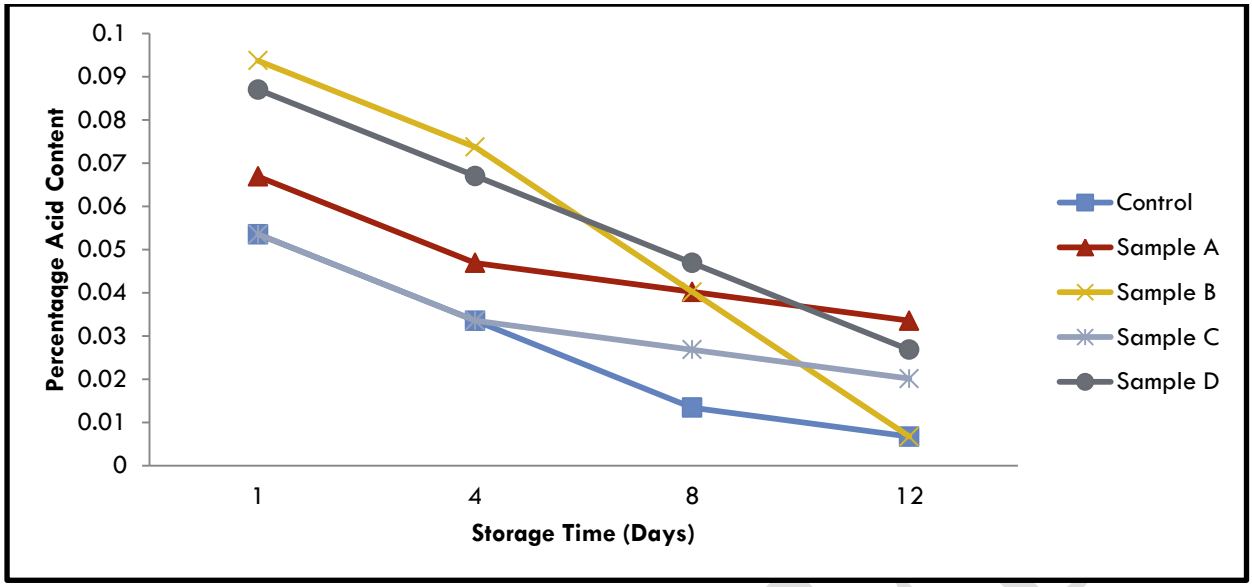

Fig. 5. Percentage Acid Content in all nano-coated apple samples comparative to control (uncoated) sample decreased as storage time of 12 days, increased. Initially acid content in coated samples is high as compare to uncoated sample because of externally added ascorbic acid in form of vitamin-C in coating emulsions.

3.7. Determination of Soluble Solids (TSS) or Sugar by Refractometer

Soluble solids are determined by the capacity of present sugar in sample to deviate light. Water content is reduced in storage time which results in concentrated juices of apple samples. These highly concentrated juices will give high value of sugar content or soluble solids. Due to these reasons amount of soluble solids in apple samples increases during storage time.

Conversion of complex polysaccharides into simple structures of sugars is also responsible for increase in soluble solids during storage time (Ben and Gaweda, 1985).

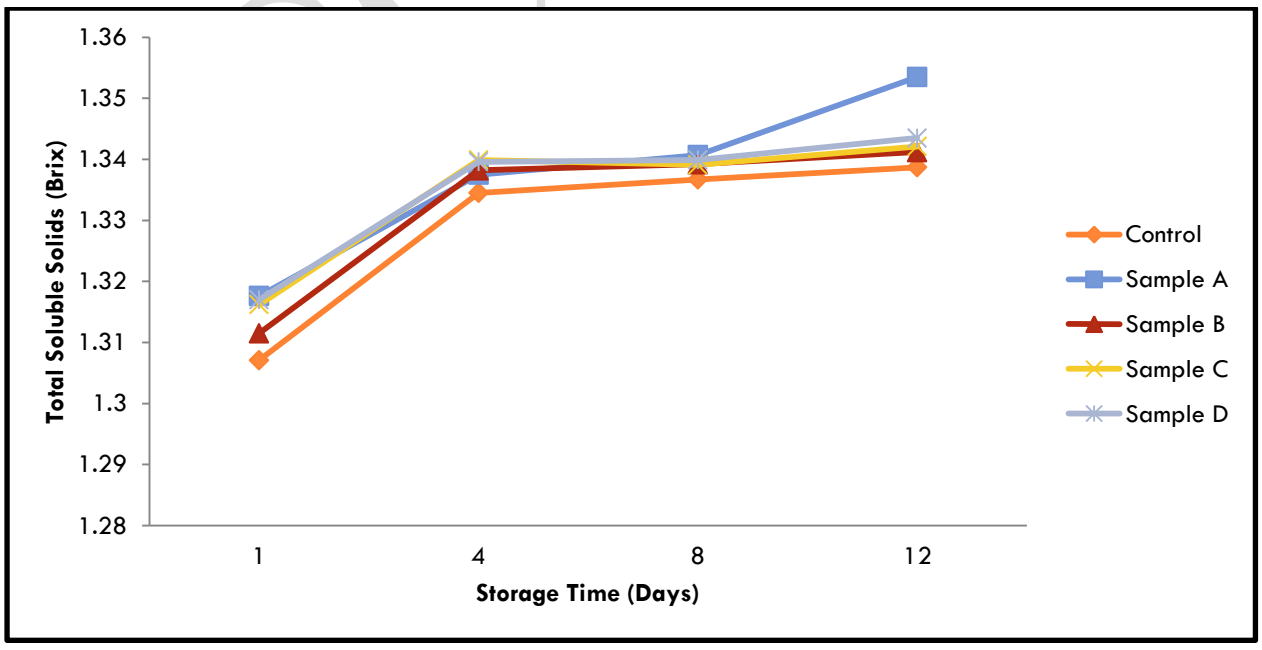

Fig. 6. Total Soluble Solids in all nano-coated apple samples and uncoated control sample shows a prominent increase with increase in storage time period. Maximum amount of

soluble solids obtained was 1.3535oBrix on last day of reaction with Sample A having coating type 1 coated on it. While minimum amount of soluble solids was 1.3071oBrix which was obtained on first day of reaction with control sample.

3.8. Total phenolic Contents

Phenolic contents were checked after 24 hour of coating using visible spectrophotometer and following Gallic acid curve method.

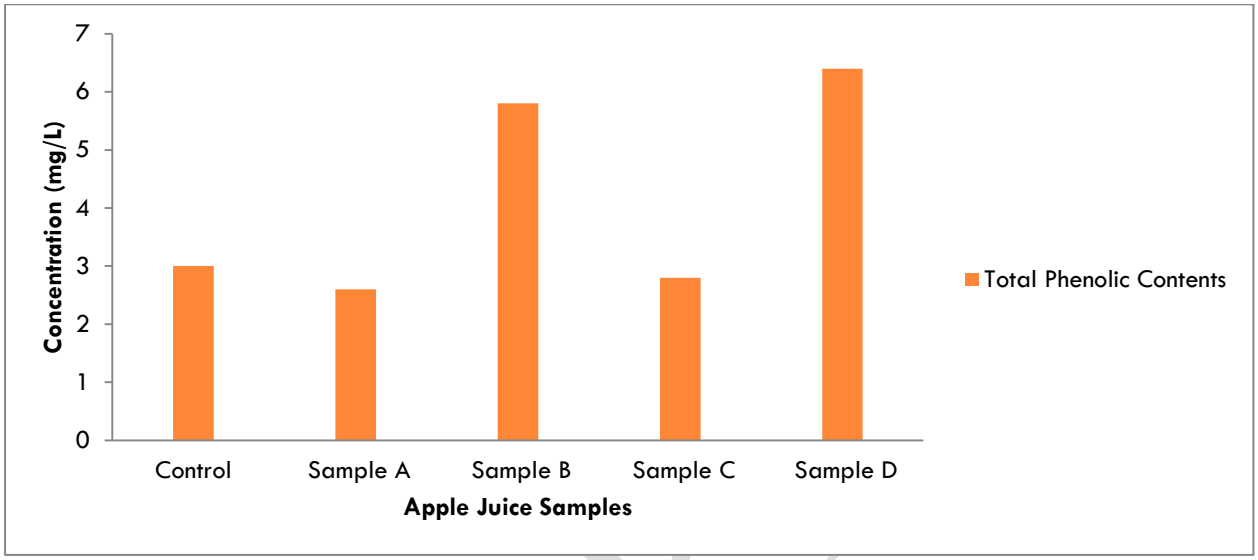

Fig. 7. Total Phenolics in all nano-coated apple samples and uncoated control sample shows a total phenolics loss in 24 hour coating time. Maximum loss was shown by Sample A, 2.6 mg/L and minimum loss was shown by sample D, 6.4 mg/L. Coating 4 on sample D was efficient to retain volatile components in it as compared to the rest of the coated samples which have lost their volatiles to the surrounding environment.

3.9. Antimicrobial Analysis

Antibacterial assay for nano-coated apple samples was performed using well-diffusion method using Luria Bertani Agar medium.

Sr. No.	Name of Bacterial species	Source	FCBP Acc. No.
01	*Xanthomonascampestris*	*Lycopersiconesculantum*fruit, Lahore	FCBP: **003**
02	*Pseudomonas syringae*	Cherry fruit, Lahore	FCBP: **009**
03	*Burkholderiapseudomallei*	Sugarcane stem, Hugra Shah Muqim	FCBP: **036**
04	*Salmonella gallinarum*	Sugarcane stem, Hugra Shah Muqim	FCBP: **038**
05	*Acinetobacterbaumannii*	Psidium guajava fruit, Lahore	FCBP: **124**

Table 1. Five different types of bacterial strains obtained from first culture bank of Pakistan with their Acc. numbers

Inhibition zones (IZ) were measured for all prepared sample media. Results obtained for all samples are discussed below:

JUICE SAMPLE	BACTERIAL SPECIES				
	XANTHOMONAS CAMPESTRIS (A)	PSEUDOMONAS SYRINGAE (B)	BURKHOLDERIA PSEUDOMALLEI (C)	SALMONELLA GALLINARUM (D)	ACINETOBACTER BAUMANNII (E)
A	2.23	2.26	1.63	2.56	2.36
	2.10	2.46	2.06	2.86	2.56
	2.90	2.66	2.30	2.26	2.80
Mean	2.41 cm	2.46 cm	1.99 cm	2.56 cm	2.57 cm
B	3.86	3.16	2.0	2.20	2.06
	4.13	2.96	1.23	2.56	2.73
	3.93	3.36	2.06	2.40	2.70
Mean	3.97 cm	3.16 cm	1.76 cm	2.38 cm	2.49 cm
C	3.83	2.46	2.46	1.16	2.96
	3.80	3.30	2.30	2.33	2.76
	3.83	3.20	2.30	2.23	2.73
Mean	3.82 cm	2.98 cm	2.35 cm	1.90 cm	2.82 cm
D	4.43	3.13	2.96	2.86	2.73
	3.60	3.40	2.63	2.86	2.76
	4.06	2.86	2.33	2.76	3.20
Mean	4.03 cm	3.13 cm	2.64 cm	2.82 cm	2.90 cm
E	2.46	2.96	1.70	1.30	2.90
(Control)	2.90	2.93	1.73	2.43	2.40
	2.60	2.40	1.96	1.46	2.56
Mean	2.65 cm	2.76 cm	1.63 cm	1.73 cm	2.62 cm

Table 1.Inhibition Zones in centimeters, shown by all apple samples including coated and uncoated for five different species of bacteria.

Fig. 8. Maximum Inhibition Zone Shown by Sample D for *Xanthomonascampestris* Bacterial Specie

Fig. 9. Minimum Inhibition Zone Shown by Sample B for *Burkholderiapseudomallei* Bacterial Specie.

4. Conclusions

Vitamin-E (α-Tocopherol) edible nano-coatings in amalgamation with antimicrobial agent and fresh lemon juice as an antioxidant efficiently increased the shelf-life of fresh-cut apple pieces by 12 days at 4^oC storage temperature and enhanced its nutritional value. Coating 4 containing fresh lemon juice in a mixture of potassium sorbate and methyl cellulose showed least weight loss and maximum phenolics. Amount of total soluble solids increased overall in all coated samples as the storage time increased while amount of acid content in all coated sample decreased as the storage time increased. Coating 4 inhibited the growth of *Xanthomonas Campestris* bacterial specie greatly.

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