Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2018, 7(3):20-41

Research Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

The Effect of Nano Materials On Edible Coating and Films' Improvement

Eman A. Abdel Khafar[1,3], Nahla S. Zidan [2,3], Hosam El Din Aboul-Anean[1*]

[1] Food Engineering and Packaging Dept., Food Technology Research Institute, Agricultural Research Center, Giza, Egypt,
[2] Home Economics Dept., Faculty of Specific Education, Kafr Elsheikh University, Kafr Elsheikh 33516, Egypt,
[3]Department of Nutrition and Science, Faculty of Home Economics, Unveristy of Tabuk 71491, Saudi Arabia
**E-Mail: hosam.ftri @ yahoo.com*

ABSTRACT

The aim of this study was to improve the edible coatings and films having a high potential to carry active ingredients such as nano materials as well as the quality of fruits and vegetables. The substances used in this experiment were Chitosan-Thyamol/(TPP)Tripolyphosphate(B), Gelatin-Coconut Fiber/titanium dioxide(TiO2) (C), Chitosan-Methyl Cellulose/Silica(SiO_2) (D), Gelatin–Chitosan/ (Ag/ZnO) (E), and Gelatin- Anthocyanin/kafirin (F) compared with edible coating non nano materials (A). The effects of edible coating on quality attributes of fruits including apples, red grapes, tomatoes and Sweet green pepper were studied during the storage at (0-2°C) and relative humidity of 90-95% for apples and red grapes, and at (8°C) and relative humidity of 90-95% for tomatoes and Sweet green pepper. Rheological properties of edible solution and suspensions were studied. Also mechanical properties, particle size distribution, zeta potentioal emulsion, X-R diffraction (XRD) films and scanning electron microscopy films were measured. The results demonstrated that the best samples were edible coating with nano suspensions (E) followed by samples (F, B, C and D) as compared with non nano coating (A) samples. Different analyses as well as microbiological tests were done on coated fruit and vegetables to observe weight loss, total soluble solids, firmness, total acidity, and ascorbic acid. The weight loss and microbial count in samples coated with (E) were reduced. On the other hand, it was observed that the nano coating apples, red grapes, tomatoes and sweet green pepper were kept with higher quality compared to non nano coating. In apples, red grapes, tomatoes and sweet green pepper dipped in solution of (E), the weight loss percentage was reduced, and the fruit quality was maintained for 63 days and 56 days of storage for apples and red grapes, respectively, and for 42 days and 35 days of storage for tomatoes and sweet green pepper, respectively.

Key words: *Edible Coating, Fruit and Vegetables, Nano Materials.*

INTRODUCTION

The active items such as anti-browning agents, colorants, flavours, nutrients, spices and antimicrobial compounds that can enhance products' shelf life and decrease the risk of pathogen growth on food surfaces are potentially contained by edible coatings [1]. Natural polysaccharides and proteins as raw materials for edible coatings and films have been taken into consideration because of offering an advanced food protection strategy, and fulfilling customers' demand for safe and healthy foods without synthetic agents. Films based on natural polysaccharides and proteins fulfill environmental concerns because of being completely biodegradable, and in many cases they can be used as an alternative to synthetic packaging [2]. Chitosan is a natural polysaccharide which is composed of a linear (1-4) linked 2- amino-2-deoxy-D glucan, can be chemically made from naturally existing chitin by treatment with alkali at the increased temperatures. Chitosan is nontoxic, biodegradable and biocompatible polymer. Chitosan extensively existing in the nature, has antibacterial effects, and film formability. Chitosan forms viscous solution in several organic acids. These viscous solutions have been applied to form functional films used for the development

and characterization of chitosan film containing morphology, physical, mechanical and degradation properties. Chitosan-based edible coatings showed very good results in deferring the decay and expanding the shelf life of several fruits and vegetables. Nanotechnology application of particular characteristics of nanoparticles can be a very useful technology in all sciences and industry branches. Recently, a lot of applications of nanotechnology in agricultural sciences have been introduced. Considering the extension of horticultural products' shelf life, nanotechnology can aid us in various fields, e.g. controlling growth and development of microorganisms, introducing as new generation of packaging coverage's (films) and controlling the influence of gases and the harmful rags [3]. Edible nanocoatings could be used on fruits and vegetables, and could provide a barrier to moisture and gas exchange, act as a vehicle to deliver colors, flavors, antioxidants, enzymes and antibrowning agents, and could also increase the shelf life of manufactured foods, even after the packaging has been opened [4]. Edible coatings and films, prepared from polysaccharides, and proteins can serve as a carrier for flavors, colorants, anti-browning agents and nutrients to improve food quality. Other active ingredients such as antimicrobial and antioxidant compounds for extending products' shelf life and reducing the risk of pathogen growth could also be achieved. The thickness of the coating layer adhered to the surface of fruit and vegetable depended mainly on the type of coating emulsion, and the variety of treated fruits and vegetables. Chitosan edible coatings extend the shelf life of the fruits and vegetables by minimizing the rate of respiration and reducing the water loss. [1]. According to Xiao *et al.* (2016), Kafirin is the prolamin protein in nanoparticles (100-300) with water over oil wetting preference [5]. Kafirin nanoparticles were found to stabilize oil-in-water type pickering emulsion (O/W2) stability. The films were printed with chitosan-tripolyphosphate (TPP) nanoparticles loaded with thymol. The films were prepared by casting high- viscosity chitosan solutions. Most procedures involve the addition of a TPP solution to a chitosan solution. A range of chitosan concentrations from 0.5 to 10 mg/ml in acetic acid solution has been studied. The TPP concentration used has ranged from o.25 to 1.0 mg/ml. The chitosan/ TPP mass ratio varied from 2 to 8. The phenomena of aggregation/particle fusion after preparation and the stability of the nanoparticle suspensions under different storage conditions have also been evaluated. These nanoparticles exhibit good loading capacity for different types of active ingredients [6]. The titanium dioxide (TiO_2) nanoparticles-chitosan composite has several advantages for interdisciplinary applications. For example, polluted waste water usually contains multiple hazardous materials such as pathogenic bacteria, heavy metals, *etc*. The TiO_2 nanoparticles have an excellent photocatalytic activity that is quite effective for the elimination of organic pollutants ; while chitosan possesses very impressing capability for heavy metal adsorption. Therefore, the integration of TiO_2 and chitosan can complement each other with their own advantages, and exert the best effectiveness for the treatment of wastewater pollutants by chemical grafting of antioxidant molecules (such as chitosan) directly onto the surface of TiO_2 nanoparticle. In literature, chitosan composite films with low Ag/TiO_2 nanoparticle have been reported to have anticancer and antibacterial effects. In addition, the mechanical properties of the TiO_2 nanoparticles-chitosan composite films can be effectively reinforced [7]. The antibacterial effects of silver (Ag) has been currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds. Silver nitrate solution causes argyria (staining of the skin) and a burning sensation on application. Ag ions and Ag-based compounds are highly toxic to microorganisms. Other forms of silver are available which do not have the disadvantages of the earlier solutions. Chemicals produce silver nanoparticles. Two other used methods included nanoparticle colloid and organic-inorganic composition film. Hybridation of Ag nanoparticles with amphiphilic hyperbranched macromolecules has been shown to exhibit good antimicrobial properties [8]. Films based on a mixture of oppositely charged polyelectrolyte natural polymers have been documented. Lately, it has been demonstrated that Layer-by-layer (LbL) electrostatic deposition of oppositely charged biopolymers can be used to provide advanced edible coatings leading to significant increase in the coated food quality Poverenov *et al*. (2014) [9]. Although, its physical characteristics like solubility, gelation, and adhesion abilities cannot be easily formulated while forming a standard edible coating. Combining chitosan with other hydrocolloids, particularly with proteins have been examined. Abugoch *et al.* (2011) and Hosseini *et al.* (2013) performed investigations of blended versus bilayered gelatin-chitosan films [10, 11]. Blended gelatin-chitosan formulation enriched with essential oils has also been applied as an edible coating in published studies on the implementation of the LbL gelatin-chitosan coating on food products. In the food industry, packaging is a critical process because it is central to food safety, quality, and product preservation. Mechanical properties and permeability of packaging films are essential to establish a good adhesion, ensure good barrier against bacteria transport, and allow a good stream of water vapor. Other required properties for packaging materials are durability, elasticity, flexibility and stress resistance. The natural nanostructured chitosan films have limited the

films to be developed as new flexible materials, therefore, they should be mixed with other polymers. Cellulose derivatives, such as methyl cellulose MC, can be chosen because it forms a continuous matrix with excellent film properties. Methyl cellulose is soluble in cold water where it forms a gel, and it has been used for different purposes for problems related to constipation, as an emulsifier and thickener in cosmetics, and as an additive (E 461) in food products because of its edible and nontoxic properties Turhan and Sahbaz (2004), and has different positive properties including good solubility and being the oxygen barrier Miller and Krochta (1997) [12, 13]. The coatings improve the products' aspects, reduce spoilage, and control gas exchanges (oxygen, carbon dioxide, and ethylene) between food components and the surrounding atmosphere. Different studies have analyzed edible films of methyl cellulose combined with lipids, polysaccharides, and polyethylene glycol, as edible packaging materials with good barrier properties and their effects on shelf-life of different foods [14].

Therefore, the purpose of this work was to study the effect of adding nano materials on edible coating chitosan/Tripolyphosphate (TPP), Gelatin-Fiber/titanium dioxide(TiO_2), chitosan-methyl cellulose/Silica(SiO_2), Gelatin–Chitosan/ (Ag/ ZnO), Gelatin/kafirin to quality attributes and prolonging the shelf life of fruits and vegetables**.**

MATERIALS AND METHODS:

Materials :

Apples (Anna malus variety) and Red grapes (Flame Seedless Varitey) were harvested at maturity storage in a private farm at " wadi elfaregh " at 65Km from Cairo –Alexandria while, vegetables, tomatoes (*Lycopersicon lycopersicum* cv. Suber Strain B', Variety) and sweet green pepper (*Capsicum annuum* L. cv. California WondeVariety) were obtained from the experimental station of Horticultural Research Institute (Elkalubia Governorate, Egypt). Fruits and vegetables were harvested on mid-june, 2017 when fully matured as followed in the commercial practice. At Central Lab of Agriculture Res. And Food Tech. Res Institute, Giza, Egypt the effects of different postharvest treatments on quality of fruit and vegetables were studied. The substances used in this experiment included : gelatin and chitosan which were obtained from Acros-organics Company New Jersey U.S.A; Citric acid, sodium citrate and sodium hydroxide which were obtained from (El Nasr Pharmaceutical chemicals company, Cairo, Egypt) ; and Glycerin and sodium hypochlorite which were obtained from (El-Gomhouria chemical company, Cairo, Egypt). Methylcellulose (MC) powder food grade was obtained from (El-Nour center for trading, Cairo, Egypt). Anthocyanin was obtained from Zhejiang Sliver Elephant Bio-Engineering Co., LTD, China. The essential oils (Thyamol) were obtained from (Across Organics, Belgium). Coconut fiber powder was obtained from Jenapharm, Germany. Lactic acid and Ethyl alcohol 95% produced by El- Gamhoria Company. Kafirin was obtained from Win Lab Company, (U.K). Titanium dioxide (TiO2), Tripolyphosphate (TPP), Silica (SiO_2) and (Ag / ZnO) were obtained from (Acmatic for Chemicals & Lab. Equipments Company, Cairo, Egypt).

Preparation of different treatments :

Fresh fruits and vegetables were immersed for one minute in the disinfectant solution of calcium hypochlorite (0.25 g\ L distilled water), then air dried. Fresh fruits and vegetables were divided into two groups : **The first group :** The cleaned fruits and vegetables were dipped in coating for 10 secs (the solution was kept constant in the beaker) and the samples were subsequently air dried.

The second group : The process was started with the other chosen coatings, then the coated and uncoated subsequently were air dried. The fruits and vegetables with different edible coatings were divided into two groups : **Group 1 :** Fruits including apples and red grapes were kept in refrigerator and stored at (0-2°C) and relative humidity of 90-95% RH. **Group** 2 : vegetables including tomatoes and sweet green pepper were kept in refrigerator and stored at (8 C°) and relative humidity of 90-95% RH. All the samples were wrapped with butter paper, then packaged in foam trays that the capacity of each was 6-8 fruits, and then they were stretched with sulfan (0.02mm thickness), but the red grapes were packed in foam trays having the capacity of 800 grams, and kept in carton boxes. All the samples were kept after packaging. The cooled storage was carried out in the post-harvest research department of Horticulture research institute, and Agriculture research center- Giza. During the storage period, the samples of investigated fruit and vegetables were periodically withdrawn for the analysis.

The substances used in this experiment on edible coatings and films were divided into six groups :

(A) Edible coating non nano materials

(B) Chitosan - Thyamol / (TPP)Tripolyphosphate nanoparticales

(C) Gelatin- Coconut fiber powder / titanium dioxide (TiO2) nanoparticales
(D) Chitosan-Methyl Cellulose / Silica(SiO_2) nanoparticales.
(E) Gelatin – Chitosan / (Ag / ZnO) nanoparticales, Layer-by-layer (LbL).
(F) Gelatin- Anthocyanin / kafirin nanoparticales

Preparation of Edible Coating and Film nanoparticales :

1. Preparatoin of gelatin/ chitosan solution : The solution contained (1.5 %, w/v) was prepared by dissolving gelatin in 100 ml of distilled water and 2 g glycerol, and stirred for 2 h while, chitosan solution (1.5 %, w/v) was prepared by dissolving chitosan in 100 ml of distilled water in acetic acid solution (1 %, v/v) and 1g glycerol. The, 200 ml gelatin and chitosan solutions were mixed in a beaker with 200 mL at 80 ^{0}C for 15 min [2].
2. A chitosan (0.3%, w/v) solution was dissolved in 0.1 M citric acid and stirred for 24h. 2% Tripolyphosphate (TPP) was prepared, and a final concentration of 1.0 mg/ml chitosan solution was added dropwise to the TPP solution (1.8ml/min) at a ratio of 2 :1 (v/v), TPP : chitosan with vigorous magnetic stirring at room temperature. The resulting suspension was centrifuged at 14°C for 30min**.** 1mlThyamol–loaded chitosan (NPs) nanoparticles, 0.1% (w/v) in citric acid was prepared and added to the previously prepared chitosan solution. The chitosan**/** Thyamol solution was added dropwise to the TPP solution [6].
3. 2g chitosan was dissolved in 2% acetic acid solution and titanium dioxide (TiO_2) $_{nanoparticales}$ (NPs) (0.05 g, dried powder) were mixed through constant magnetic stirring for10 minutes to obtain the Tio_2 (NPs) nanoparticles – 2N sodium hydroxids solution (20 wt %) was added to chitosan composite by a mixing homogenizer. The solution was blended degassed for 2 hrs, then, 2 grams of coconut fiber powder were added. The solution was stirred for 1 h well until it became a homogeneous solution, then it was heated and sonicated in a closed vessel at 72±2 °C for 20 min Keng-shiang *et al*. (2014) and Bhuvaneshwari *et al.* (2010) [7, 15].
4. **Film preparation of chitosan-methyl cellulose/silica(SiO_2), blended formulation :**
   Chitosan was dissolved in a solution of lactic acid in water (2% v/v) and stirred at room temperature for 4 h. Methylcellulose (1% w/v) was obtained by dissolving methylcellulose in a solution of EtOH/H2O 1 :1 (v/v) and stirred for 12 h. This hydro-alcoholic solution was used to improve the solubility of methylcellulose. As obtained solutions of chitosan and methylcellulose were mixed according to different proportions (chitosan : methylcellulose 50 :50) stirred for 10 min, 1% or 2% w/v silica (NPs) nanoparticles were added to the different solutions. SiO_2 NPs solutions were prepared in 2 ways : silica nano-powder in EtOH was dissolved until a final concentration of 1% w / was obtained. The final solutions (chitosan, methylcellulose and SiO_2 NPs) were stirred for an hour, then sonicated in a closed vessel for 20 min, and finally centrifuged at 4500 rpm for 10 min at 20 **º**C. To obtain the final films, 80 mL of each solution was poured on Teflon plate (size 20 × 20 cm) and left to dry in a ventilated oven at 60 **º**C overnight, and stored in aluminum foils [16].
5. **Film preparation of gelatin / chitosan** (Ag / ZnO), **Layer-by-layer (LbL**)
   The film-forming solutions were prepared in two stages : in the first stage, the layer of gelatin solution (1.5 %, w/v) was prepared by dissolving gelatin in 100 ml of distilled water. The solution was heated to 50±2 °C and stirred for 2 h. The second chitosan solution (1.5 %, w/v) was prepared by dissolving chitosan in 100 ml of distilled water in an acetic acid solution (1 %, v/v). The solution was heated to 50±2 °C and stirred for 2 h. After achieving homogeneity, gelatin and chitosan solution was added to 5 ml (5%w/v) of Ag and 5ml (5%w/v) of ZnO (NPs) nanoparticles, then 5 grams (Ag and ZnO) in 100 ml of distilled water was added, and then 5 ml of nano solution was added to the previously prepared. The final solutions (gelatin and chitosan (Ag / ZnO) were stirred for an hour, then sonicated in a closed vessel for 20 min. Gelatin/chitosan (LbL) portions of the film-forming solutions were poured into Teflon, and dried at 23±2 °C overnight [2, 17].
6. **Film preparation emulsions stabilized by utilizing Kafirin nanoparticles :**
   Edible colloid particles' emulsions were stabilized by utilizing Kafirin of inner water phase to oil phase. Soybean oil containing 1 %wt solution was prepared by dissolving 1.5 wt% gelatin and 0.05 wt % anthocyanin in 0.01 % sodium citrate – citric acid buffer (pH3.4). The external water phase was consisted of 1.5% kafirin (NPs) nanoparticales 1%wt. A two-step emulsification method was then added to prepared gelatin, soybean oil and water emulsions. The emulsion was prepared by a mixing homogenizer [18].

Physical and mechanical and Rheological properties :

1. **Rheological measurements:** Rheological parameters (shear rate and shear stress) of the selected edible nano coating and films were measured using a Brookfield Engineering labs DV- III Rheometer at 30°C. The samples were placed in a small sample adapter, and a constant temperature water bath was used to maintain the desired temperature. The viscometer was operated between 10 and 60 rpm. The sc4-25 spindle was selected for the measurement.
2. **Film thickness:** The thickness of the prepared edible film from nano materials was measured using a digital micrometer (mitutoyo digimatic indicator corporation, model: pk-1012 E, Japan). The film strips were placed between the micrometer jaws, and the gaps were slowly reduced until the first contact was noted [19].
3. **Loss of film weight in water:** The films specimens were first dried in a desiccator containing dry calcium chlorides. Dry film sample of 500mg were immersed in beakers containing 50ml of distilled water at room temperature during 24h with a periodical gentle shaker incubator. The films were removed from the water and placed back in the desiccator until constant weight. The weight loss in water was reported as a percentage of weight loss in water on the dry film basis as follow:
   % weight loss= initial dry weight -final dry weight ×100/ initial dry weight according to [20].
4. **Mechanical properties of selected nano materials' films:** The tensile properties (Tensile strength, elongation) were measured by a texture analyzer CT3. The films were cut into strips of 3×5cm. These were gripped at each end by a jaw, and then the jaws were moved at the controlled speed until the modulus was automatically recorded according to [21].
5. **Zeta sizer nano Company's name:** Malvern, UK. Model**:** Zeta sizer nano series (Nano ZS). Size range (nm):0.6:6000 nm Zeta potential range (mV**):** (-200:200mV) and XR- Diffraction. Model**:** XPERT-PRO-PANalytical-Netherland.
6. **Measurement of prepared edible films using scanning electron microscopy was done by:**
   INSPECT S- SEM schematic overview – TM 1999-2007 Bwilddate, FEL company Euld number D 8571 Machine type inspect S [22].
7. **Determination of Water vapor permeability (WVP)**
   The water vapor transmission rate [g/(s.m^2)] and water vapor permeability (WVP) through films were determined gravimetrically using the ASTM Method E96-95. A circular test cup was used to determine the WVP of the film. The film was first cut into circular shape that was larger than the inner diameter of the cup, the cup was filled with 50% distilled water and the film was sealed at the top using Paraffin oil, then the cups were placed in a desiccators containing calcium chloride. The weights of the cups were recorded every hour during 10 hours, and the specimens of each film were tested. Linear regression was used to estimate the slope of this line in g/h. The water vapor transmission rate (WVTR) and water vapor permeability was determined using the following :

   $$\text{WVPR}=\frac{\Delta m}{\Delta t A} \qquad \text{WVP=WVPR. L/}\Delta RH$$

   Where,$\Delta m/\Delta t$ is the moisture gain weight per time (g/s), A is the surface area of the film m2, L is the film thickness (mm) and ΔRH is the difference in relative humidity (ASTM E96–95).
8. **Measurement of gas Permeability**
   Gas (O2 and CO2) permeability at 30°C was measured in a designed stainless cell using a gas testing instrument. The model Witt Oxybaby headspace gas analyzer (O2/CO2) was used following the method described by Garcia et al. (2000) [23]. The gas permeability (P) was calculated according to the following equation :

   $$P=Q.X /A.t.\Delta p$$

   Where, P is the permeability of gas, (m^3/m. day. MmHg), Q is the quantity of gas diffused m3, X is the thickness of the film, A is the area of the film, m2, t is the time in day, and Δp is the pressure difference across the films.
9. **Physical and chemical properties:**

1. **Weight loss :** The weight of the coated fresh fruits and vegetables during cold storage was measured by monitoring the weight changes in the periods of storage, the weight loss was calculated as the percentage loss of initial weight as reported by [24] using the following equation : - **Weight loss%= g/wb×100**
   Where : **g :** fruits and vegetables weight. **Wb** : initial fruits and vegetables weight.
2. **Total soluble solids (TSS %) :** Total soluble solids were determined by the refractometric method at room temperature using an Abbe refractometer (carlzeiss jena) in juice obtained from a sample of slices, according to [25].
3. **Firmness :** The texture was determined by a universal testing machine (cometech, B type, Taiwan) in Food Technology Research Institute, Giza, Egypt, provided with the software. An aluminum 25 mm diameter cylindrical probe was used in a "Texture Profile Analysis" (TPA). Double compression test was done to penetrate to 50% depth, at 1 mm/s speed. The firmness (N) was measured according to Bourne (2003) [26].
4. **Total titratable acidity :** Total titratable acidity was measured for fresh samples as mentioned in the official method of the A.O.A.C. (2005) [27]. It was expressed as citric acid using sodium hydroxide N/10 and phynol phythaline as the indictor**.**
5. **Ascorbic acid :** Ascorbic acid content was estimated in fresh fruits and vegetables of tested materials according to AOAC, (2005) using 2,6 dichlorophenol-indophenols by titratable method [27]. The results were expressed as mg ascorbic acid per 100gm samples.
6. **Microbial analysis :**

Total microbiological count was determined according to Marshall, (1992) All the microbiological counts were carried out in duplicates [28].

1-Total plate count : The total colonies of bacteria were estimated using plate count agar medium. The Plates were incubated at 37ºC for 48 hours.

2- Pychrophilic bacterial : The total colonies of bacteria were estimated using plate count agar medium. The plates were incubated at 4ºC for 5 days.

3-Moulds and yeasts count : The moulds and yeasts were determined using the methods for the microbiological examination of foods described by American Bublic Health association (A.P.H.A, 1976) [29]. The malt extract agar medium was used. The plates were incubated at 25ºC for 5 days.

10. **Statistical Analysis:**
    The obtained data were subjected to the proper statistical analysis using the MSTAT statistical software. The mean values were compared using LSD method at 5% level. The data were tabulated and statistically analyzed using factorial analyses according to the completely randomized design [30].

RESULTS AND DISCUSSION

Evaluation of the effects of the nanomaterials on edible coatings and films :

Rheological properties of different nano materials - based film and coating emulsions :

The relation between shear rate and shear stress of nano materials are shown in table (1) and figure (1). The results indicated that the samples exhibited non-Newtonian pseudoplastic behavior and fitted well to the following equation $\tau=k\gamma^n \rightarrow$ **(1) Where :**

τ : shear stress, pa **γ:** shear rate 1/sec**, k:** consistency index**, n:** flow behavior index

As shear rate increased, the shear stress increased at different treatments (A, B, C, D, E and F), while viscosity decreased as shear rate increased, as shown in figure (1). Table (1) shows the relation between (k), (n) at different particle sizes. The results demonstrated that (k) decreased with different treatments and (n) did not give a trend. The results indicated that the behavior of the liquid solution and the type and size of particles, as well as the presence of ionized substances and electrolytes are related. Also, the consistency affected by changes in concentrate, and the interaction effect was performed with the average value of (k) and (n) for each treatment level, indicating that(n) and(k) might be correlated, and absolutely correlated parameters should be eliminated by expressing it as a function of others in order to create a one-parameter model to investigate this possibility Suisui **Jiang (2016),** [31, 32]. The best forming solution for the applications of edible films and coatings on fruits and vegetables was observed by [33].

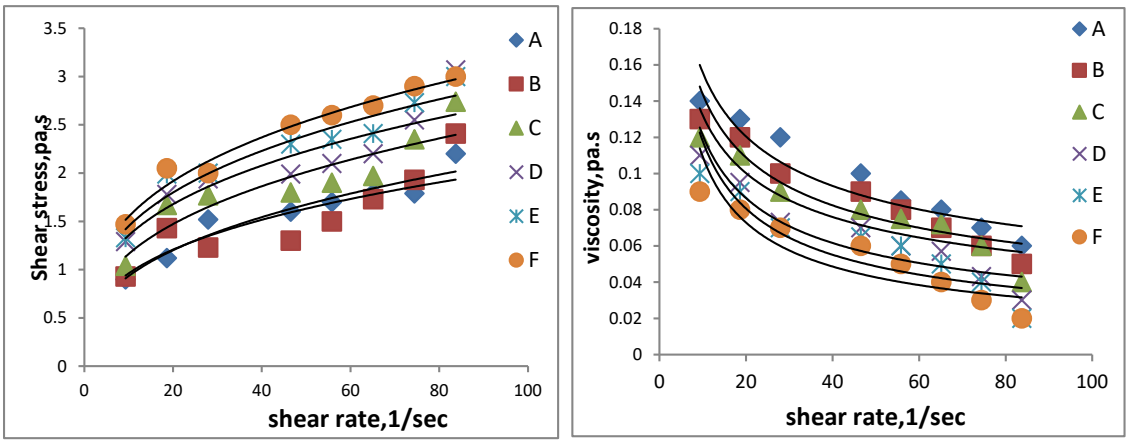


Figure 1 : Relation between Shear rate and of (Shear Stress and viscosity) at different nano materials on emulsion produced films

Table 1 : Relation between consistency index (k) and flow behavior index (n) of nano materials on emulsion produced films :

Treatments	K	N	R^2
A	0.765	0.306	0.967
B	0.710	0.310	0.925
C	0.666	0.308	0.866
D	0.531	0.342	0.854
E	0.406	0.362	0.947
F	0.451	0.329	0.745

Physical and mechanical properties of prepared edible films by adding nano materials :

The results are shown in table (2), it can be noticed that the non nano materials coated treatment, had the highest thickness value of 53,44,40,42,37and 39 um respectively in films prepared from A, B, C, D, E and F. The thickness of film added to nano- composites edible coating (E) was lower than that nano- composites edible coating (F). From the results, tensile strength and elongation percentage was investigated using texture analyzer C3, it can be observed that the highest values of tensile strength (84.22 N/M^2), elongation (47.50%), (Oxygen 17.0 $M^3.M/M^2$ X^{10-7}), (CO2 14.46 $M^3.M/M^2$ X^{10-8}), water vapors permeability 0.027 [$g/(s.m^2)$] and solubility (2.6**%**) were recorded for (E). Also, (B, C, D and F) respectively showed higher tensile strength (25.88,29.40,28.19 and 70.63 N/M^2), elongation (37.75,35.00,17.50 and 38.75%), (Oxygen32.5,24.2,23.0 and 19.9 $M^3.M/M^2$ X^{10-7}), (CO 2 13.18, 13.29, 17.45 and 16.00 $M^3.M/M^2$ X^{10-8}), Water vapors permeability [0.85,0.34,0.45 and 0.029 $g/(s.m^2)$] and solubility (11.6,8.4,9.0 and 7.5**%**) than those of non nano coated film (A). High film elongation is always a desirable characteristic if the film is to be used for food applications [34]. Similar results were reported by Nelson caro *et al.* (2016) [6].

Table 2 : The thickness and mechanical properties of the selected nano material films :

Treatments	Thickness um	Tensile strength (N/M^2)	Elongation (%)	Oxygen (O2) Permeability $M^3.M/M^2 \times 10^{-7}$ day.mmHg	CO2 Permeability $M^3.M/M^2 \times 10^{-8}$ day.mmHg	Water vapors Permeability [g/m^2.24hr]	"% Solubility" loss in weight after dipping in water and drying
A	53	17.84	19.86	36.9	57.58	0.36	12.8
B	44	25.88	37.75	32.5	13.18	0.085	11.6
C	40	29.40	35.00	24.2	13.29	0.034	8.4
D	42	28.19	17.50	23.0	17.45	0.045	9.0
E	37	84.22	47.50	17.0	14.46	0.027	2.6
F	39	70.63	38.75	19.9	16.00	0.029	7.5

Determination of particle size distribution and Zeta potential emulsion of produced films and X-R diffraction (XRD) films of tested nano materials on based film and coating emulsions formed from them :

1- partical size distribution .

Table (3) and Figure(2), show the addition of nano materials on the properties of the dispersion of film and coating emulsions evaluated based on the change in size (z-average) and the z-potential of the nanoparticles relative to the film and coating emulsions sample, poly dispersity index (PdI) in the peak was 0.381, 0.463 , 0.457, 0.690 , 0.602 and 0.653 for (A), (B) , (C) , (D) , (E) and (F) respectively as well as the hydrodynamic diameter of the particle size in the peak was 345.6 , 337.1 , 160.1, 159.4 , 23.35 and 122.8 for (A), (B) ,(C) ,(D) ,(E) and (F) respectively . The results were similar to the findings of Jie Xiao *et al*.,(2016) who found that the amounts of nano materials with chitosan, polysaccharides, proteins - kafirin and waxy maize starch were, 240nm ,360nm , 340 and 342-825nm, respectively [35]. These results were in agreement with Nelson caro *et al.* (2016) who found that the suspensions containing starch nanocrystals range from 200-300nm [6].

Table 3 : The Effect of partical size distribution and zeta potential emulsion produced films and X-R diffraction(XRD)films of tested nano materials on based films and coating emulsions formed from them :

Treatments	partical size distribution(nm)		Zeta potentioal(mv)		X-R diffraction(XRD) [°2Theta] [%]	
	poly dispersity index (PdI)	hydrodynamic diameter (nm)	z- potentioal	z- deviation	below	above
A	0.381	345.6	3.21	33.7	19.95	34.90
B	0.463	337.1	0.813	12.6	18.97	37.16
C	0.457	160.1	7.06	3.60	8.98	44.10
D	0.690	159.4	-1.60	3.69	21.52	35.92
E	0.602	23.35	18.7	3.25	11.20	50.02
F	0.653	122.8	2.98	2.95	8.28	59.93

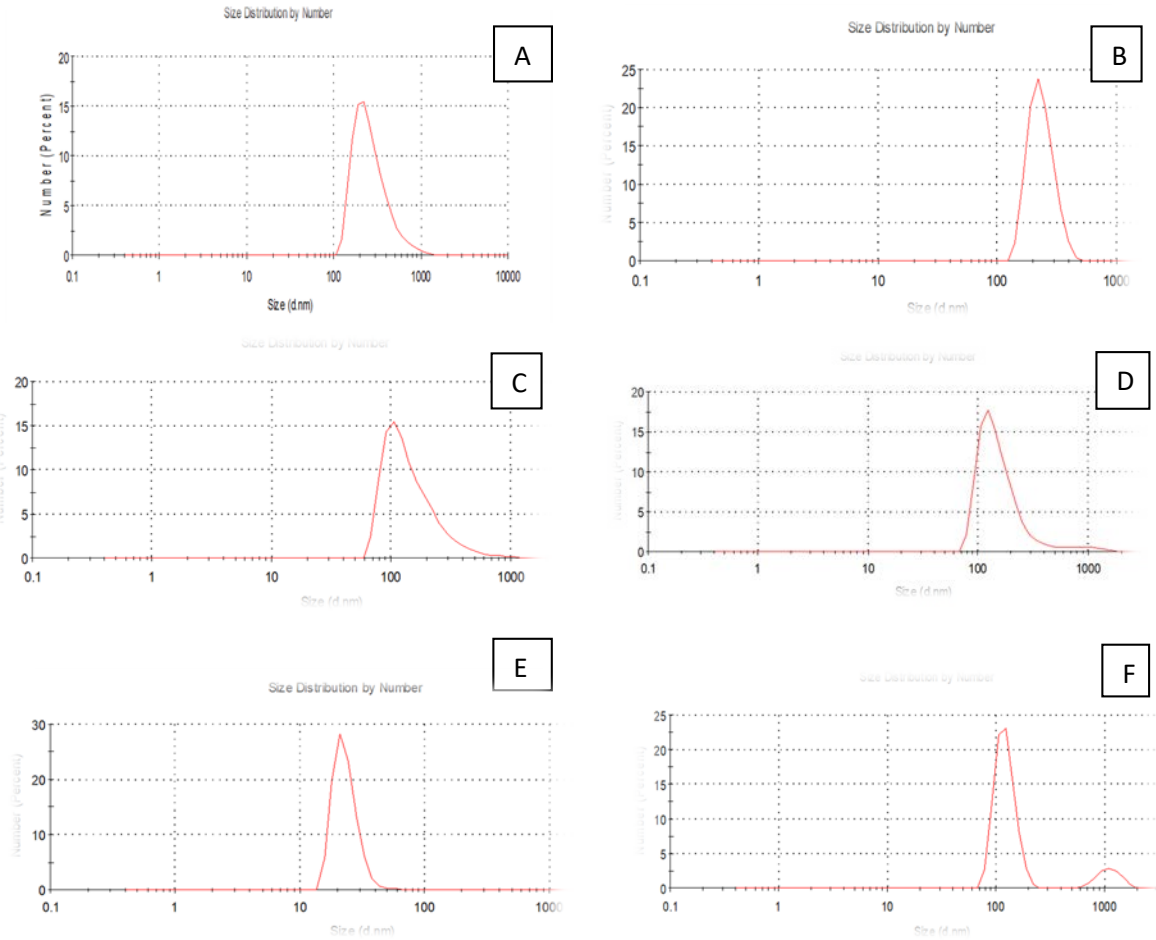


Figure 2 : (A, B, C, D, E, and F) : particle size emulsion of produced films

2- zeta potential

As can be seen in table (3) and figure (3), Zeta potential was measured to determine the stability of nano particles, it depends on the particle size, solution, pH and electrical properties. The results showed (A) Zeta potential(mV): 3.21 and Zeta Deviation (mV): 33.7 , as found in the chracterization of the two peaks of zeta potential in the first peak -18.8 (mV) , the area below the curve was 58.4 % and the second peak was 35.7 (mV) , the area below the curve was 41.6 % . However, it was found from the chracterization of one peak of zeta potential distribution and Zeta Deviation, the area below the curve was 100 % that the contents of Zeta potentioal in the peak of different tretments B , C , D , E and F had zeta potentioal of 0.813, 18.7, -1.60, 7.06, 2.98, respectively, as well the contents of zeta deviation in the peak of different treatments (B) , (C) , (D) , (E) and (F) had zeta deviation of 12.6, 3.25, 3.69, 3.60 and 2.95, respectively .Suisui Jiang *et al*., (2016) [36] and Nelson caro *et al.* (2016) [6].

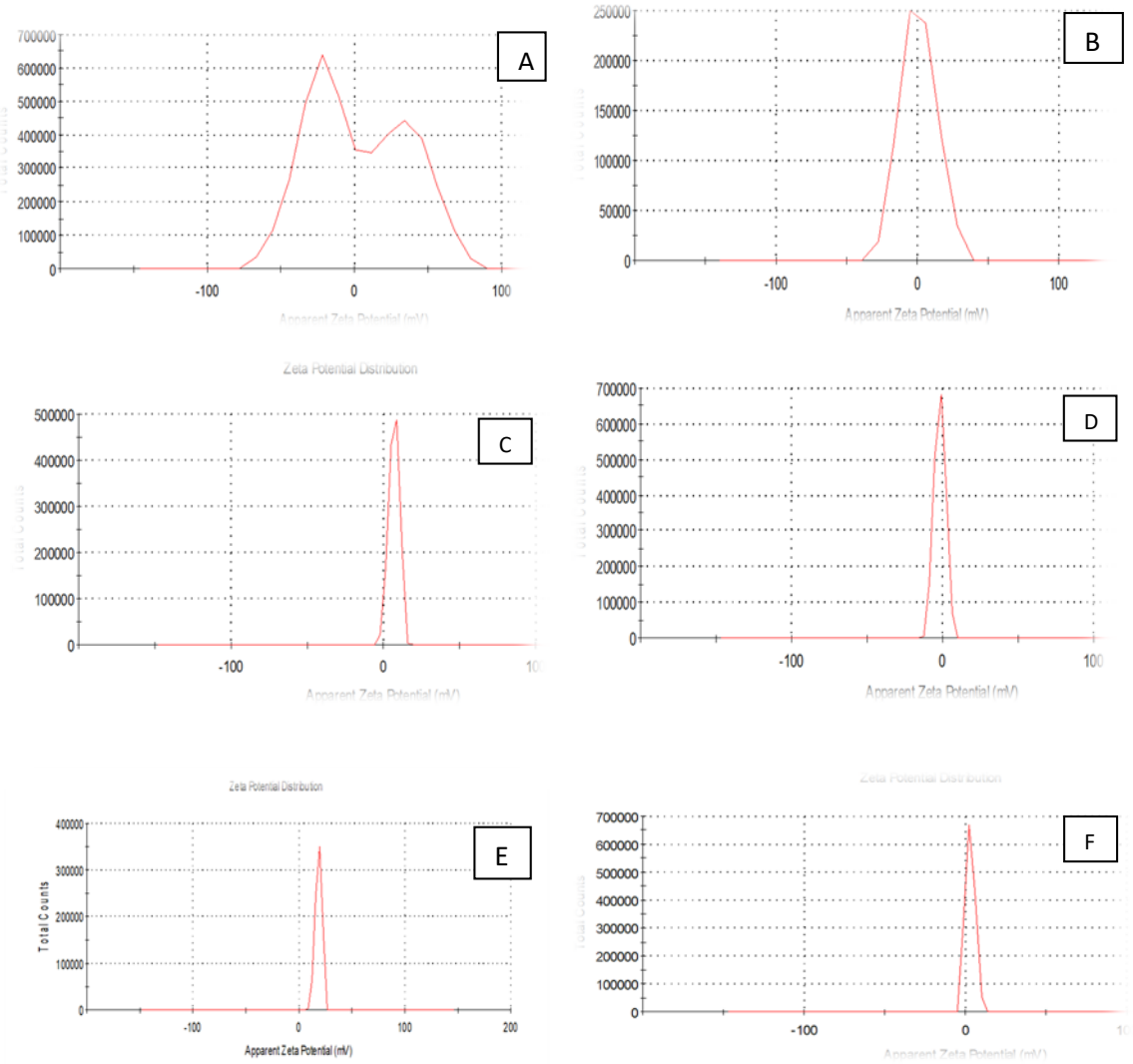


Figure 3 : (A, B, C, D, E, and F) : zeta potential emulsion produced films

3- X-R diffraction(XRD) films

Table (3) and fig (4), show the X-R diffraction(XRD) Position [°2Theta] [%], pattern of samples A, B, C, D, E and F. For samples A and D, the diffractogram exhibits four distinct diffraction peaks A (19.95,21.69,31.03and 34.90) which match D (21.52,27.19,29.34 and 35.92), sample B exhibits five distinct diffraction peaks (18.97, 20.92, 24.80, 30,64, and 37.16) which match the diffraction peaks of C (8.98,12.86,20.50,22.72,25.36,27.72 and 44.10), samples E, F exhibit eight distinct diffraction peaks. E exhibits (11.20, 21.89, 27.30, 32.4, 36.75, 40.08, 45.49 and 50.02), which match distinct diffraction peaks of F (8.28, 13.00,20.36,21.16, 27.72, 30.19, 32.16, and 59.93). Similar results were found by Jie Xiao et al., (2016), Suisui Jiang *et al*., (2016) [35, 36]. These different peaks are associated with

surface deposits in both types of nano material films and the control. The XRD shows an increase in intensity of peak at 31.7°corresponding to nano materials [6].

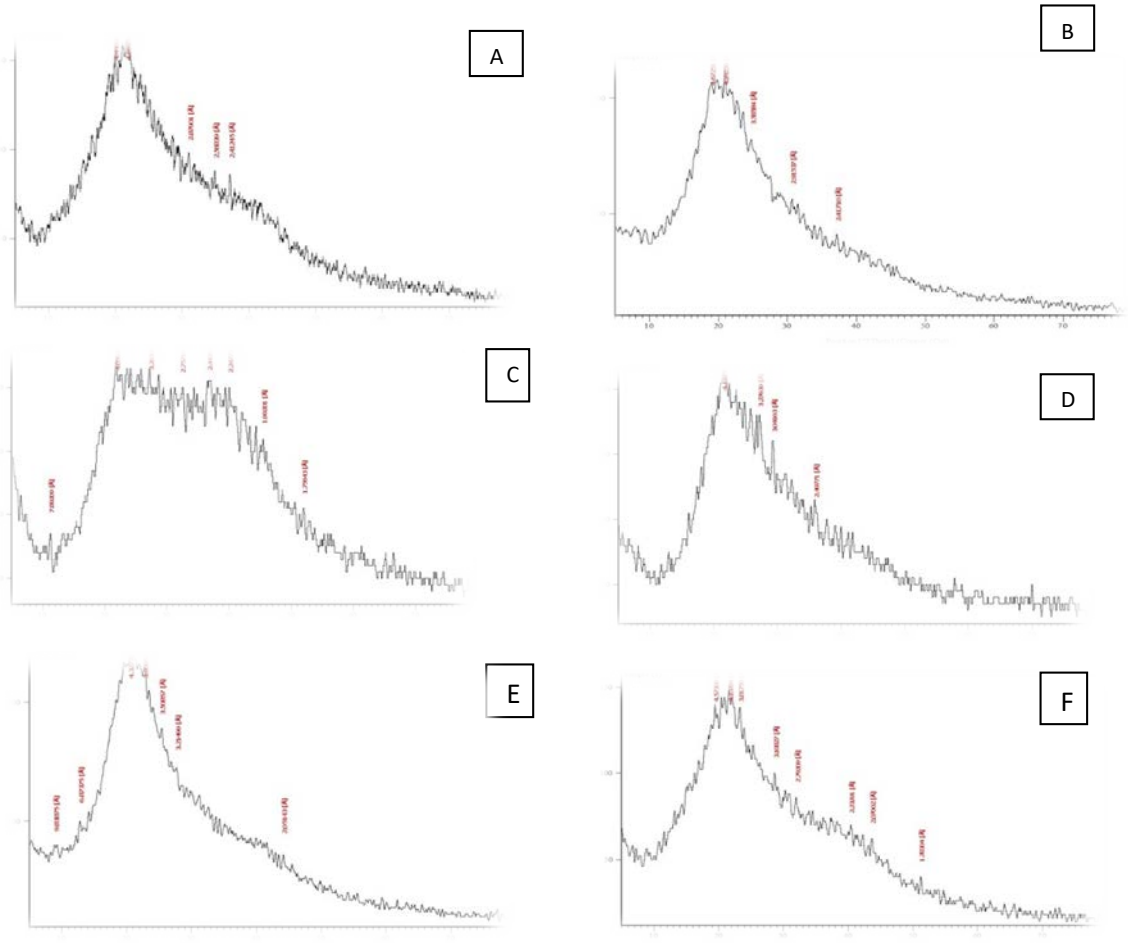


Figure 4 : (A, B, C, D, E, and F) : X-R diffraction films

Determination of the microstructure of produced natural films using scanning electron microscopy (SEM**)**
Technique :

The microscopic images of six nano coated and non nano coated samples are presented in figure (5). (A, B, C, D, E, and F). The addition of nano materials on edible coating **emulsion** gave films characterized with smooth surface and rough bottom. The film had a homogeneous structure with some micro granules embedded in a continuous matrix. However, the addition of nano materials on emulsion film as natural polymer improved the final product films. They were characterized with polygonal crystals, spherical morphology, smooth surface and rough hilly bottom fig (5). Also, the nano range required for the preparation of spherical particles of uniform size (≈200nm) has been found to be rather narrow, with several other kinds of poly-disperse aggregates and anisotropic structures being formed above or below the optimum nano. The results also indicated that the particle size had a wide range of shapes and sizes. SEM micrographs: The best nano coated treatment image characterization was the average droplet size ranged from (1.07-20 um**)** and the spherical morphology followed by F showed the average droplet size ranged from (1.24-20 um**),** the spherical morphology followed by B showed the average droplet size ranged from (1.42- 40um) of polygonal crystals, the average droplet size of C ranged from (1.84- 40um**)** polygonal and ellipsoidal , the average droplet size of D ranged from (2.19- 50 um) of polygonal, as compared with non nano coated A image characterization average droplet size which ranged from (2.17-50um) polygonal crystals. When a section was taken to characterize the nano-sphere, the image was (E 320nm) followed by (F375nm), (B500nm), (C470nm), (D 450nm), as compared with non nano coated (A was 728nm). Generally, the scanning microscopy study may be useful for recognizing the microstructure and morphology of the produced films which can help in choosing the proper film formulas for coating and packaging purposes. Also, the color appearance of produced films may be of importance because it could affect consumers' acceptance of coated items. Nelson caro *et al.* (2016) found that the

starch nanocrystals were observed to be spherical in shop, with particle size primarily within the range of 200-300nm [36].

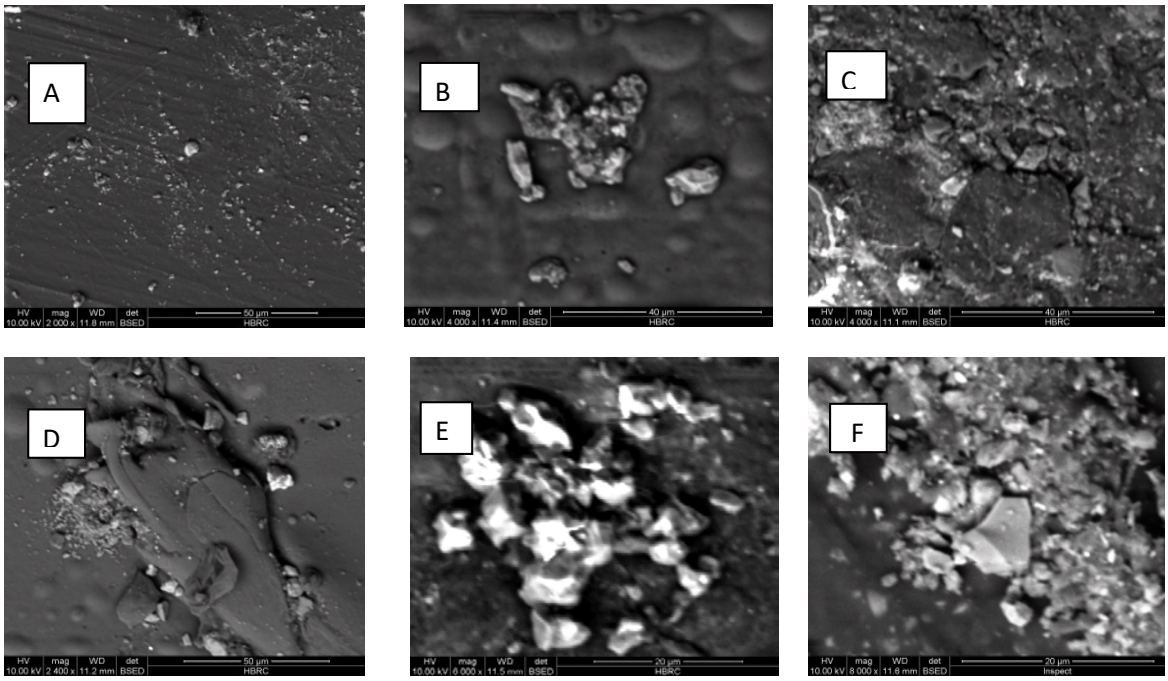


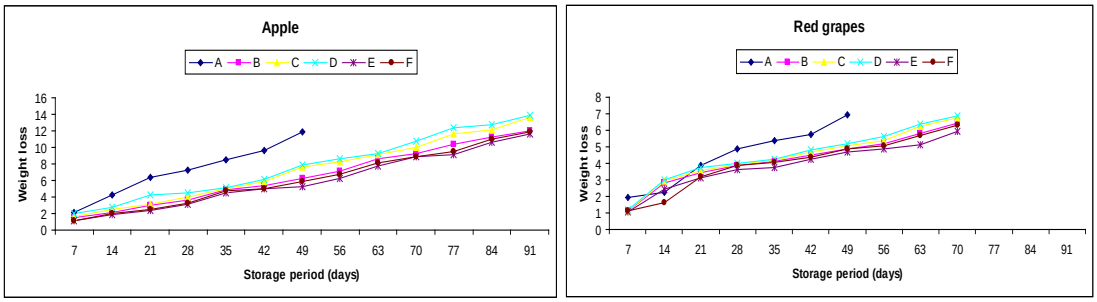
Figure 5 : (A,B,C,D,E, and F) SEM Films

Application of chosen proper edible coatings to Fresh fruit and vegetables :

Changes occurred in physico-chemical and microbiological of coated fruit and vegetables during the storage period :

Weight loss percentage :

The results obtained are presented in Figure (6). In this figure, it can be observed that the weight loss increased with increasing the storage period at cold temperature for treatments packaged in nano coated and non-nano coated forms. In non-nano coated treatment, a higher weight loss was indicated comparing to the nano coated treatments. The results of the study on nano coated treatments showed that the best treatment was E followed by F followed by B, C, D, as compared with non-nano coated A which was the least .The apples, red grapes , tomatoes and sweet green pepper were kept as follows: 91, 70, 63 and 63 days, as compared to control, the results were 49, 49, 35 and 35 days, respectively.These results might be obtained due to the nanoparticles which have been found as zigzag in the new film, preventing the penetration of oxygens. In other words, the oxygen for entering into the film should pass a longer route [4]. Therefore, with limited gas exchange due to the coating barrier, the enzymatic activity, and the metabolism involving respiration can be thus affected resulting in lower weight loss [37]. Most likely, edible coatings and films' nano-particles caused reduction of moisture condensation on the fruit surface [38]. Normally, the weight loss occurs during the fruit storage due to its respiratory process, the transference of humidity, and some processes of oxidation [39].



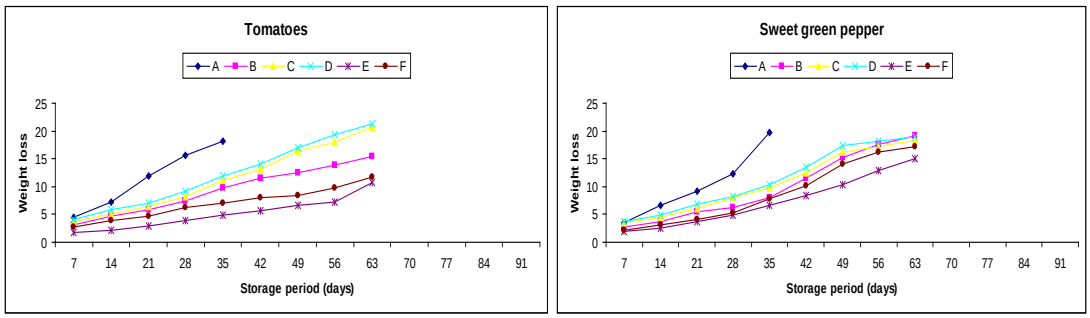


Figure 6 : The Effect of edible coating on weight loss in fresh fruits and vegetable. (a) Apple [$LSD_{0.05}$ = 0.1939], (b) Red grapes [$LSD_{0.05}$ = 0.1954], (c) Tomatoes [$LSD_{0.05}$ = 0.1976], (d) Sweet green pepper [$LSD_{0.05}$ = 0.1962]

Total Soluble solids (TSS) :

Changes occurring in TSS of edible coatings of fresh fruits and vegetables are shown in Figure (7), it could be noticed that TSS of nano coated and non nano coated fresh fruits and vegetables gradually increased with increasing the storage period at cold temperatures in both samples. The results of the study on nano-coated fruits and vegetables showed that the best treatment was E followed by F followed by B, C, D, as compared with non-coated A which was the worst. According to El sheikh *et al.,* (1997), the increase in total soluble solids in the fruit at the storage period might owe much to the higher rate of moisture loss through transpiration than the rate of dry matter loss through respiration [40]. According to [25], the results of the natural metabolic processes occurred in fruits during the storage as a result of moisture losses from the fruit and/or hydrolysis of starch (ripening) which led to the increase of the total soluble solids. Positive correlation was found between the decrease in fruit firmness and the increase in TSS as the fruits continued to lose their resistance to puncture, and TSS continued increasing. In a study done by Saleh *et al.,* (2005), the total soluble solids significantly ($p<0.05$) increased with storage time in all the treatments with the exception of the fruits covered with the bilayer films, which had no significant changes with time [41]. The foam tray wrapped with PVC film showed a small increase in total soluble solids [42], and had higher total soluble solids and slightly advanced starch breakdown. Soluble solid of the stored grapes increased during the storage. As it is known, when fruits are stored, the moisture of the stored products decreases during the storage period, and consequently the soluble solid amount of the stored product increases [43].

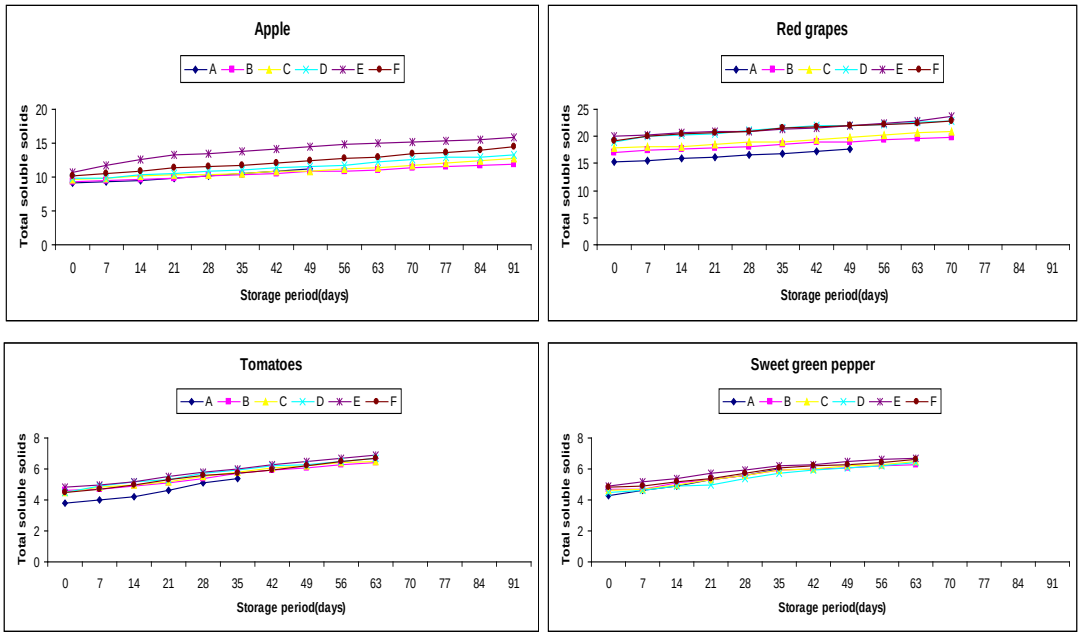


Figure 7. The Effect of edible coating on total soluble solids in fresh fruits and vegetables, (a) Apples [$LSD_{0.05}$ = 0.1342], (b) Red grapes [$LSD_{0.05}$ = 0.1331], (c) Tomatoes [$LSD_{0.05}$ = 0.1356], (d) Sweet green pepper [$LSD_{0.05}$ = 0.1365]

Color changes :

The changes in color of edible coatings of fruits and vegetables are shown in figure (8), it could be noticed that the color of nano coated and non-nano coated fresh apples, red grapes, tomatoes and sweet pepper gradually increased with increasing the storage period at cold temperatures in both samples. The results of the study on nano-coated films showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. Data in fig (8) showed that there was a marked increase in color change values of nano coated compared to the other treatments, for both damaged and non-damaged surfaces, the color differences determined on damaged and non-damaged non nano coated surfaces compared to the white standard color variation on damaged surfaces was, however, considerably higher than that of non-damaged ones. The color differences among nano-coated with different coatings were not significant ($p < 0.05$) and remained statistically unaltered throughout the storage period for non-damaged surfaces**.**

The results of the study showed that the best treatment was E followed by F followed by B, C, D as compared with the control A which was the least. The apples, red grapes, tomatoes and sweet green pepper were kept as follows: 91, 70, 63 and 63 days respectively, as compared to the control, the results were 49, 49, 35 and 35 days, respectively. Changes in the hue-angle (h^o) value of coated fruit with storage time were slight and only became significant at the end of the storage period. Chroma was reduced by about 30% for control and 10% for coated fruits. It has been observed that the uncoated fruits are significantly darker than the coated fruits throughout the storage period. The chitosan concentration of the coating solution gave rise to significant differences in the surface color of fruits by the end of the storage period [1]. The packaging and storage were good enough to protect the grapes, and the change in color of the bunches were acceptable [43].

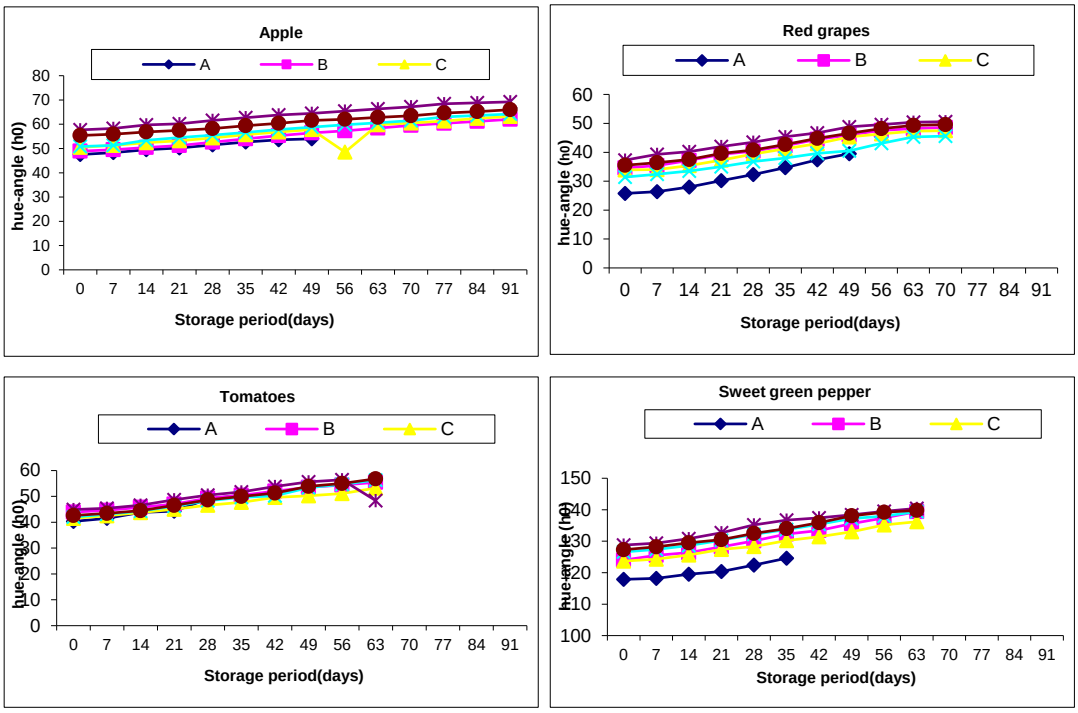


Figure 8 : The Effect of edible coatings on color (hue-angle (h^o) in fresh fruits and vegetables. (a) Apples [$LSD_{0.05}$ = 1.225], (b) Red grapes [$LSD_{0.05}$ = 1.341], (c) Tomatoes [$LSD_{0.05}$ = 1.211], (d) Sweet green pepper [$LSD_{0.05}$ = 1.223]

Changes in Firmness of the examined fruits and vegetables during storage :

The results of the measurements of fresh fruits and vegetables' firmness are shown in Fig (9), it could be noticed that the firmness of non-coated and coated fruits and vegetables (apples, red grapes, tomatoes and sweet pepper) was gradually decreased with increasing the storage period at cooled temperatures. The results of the study on nano-coateds showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. The apples, red grapes, tomatoes and sweet green pepper were kept as follows: 91, 70, 63 and 63 days respectively, as compared to non-nano coated ones in which the results were 49, 49, 35 and 35 days, respectively. The firmness of fresh fruits and vegetables as measured by mechanical methods was used to determine their maturity and ripeness ; as it is important in handling procedures and is a component of texture influencing

sensory perception of fruits and vegetables by consumers. Since, consumers regard texture as a positive quality attribute donating freshness of products and contributing to the enjoyment of eating [25]. [44, 45] found that increased respiration activated increasing water loss most likely decreased the potential texture depression. The decrease in fruits' firmness most likely occurred due to the gradually breakdown and depression of lower molecular weight fraction which were more soluble in water, and this was directly correlated with the rate of softening of the fruit [40, 43, 46, 47**]**.

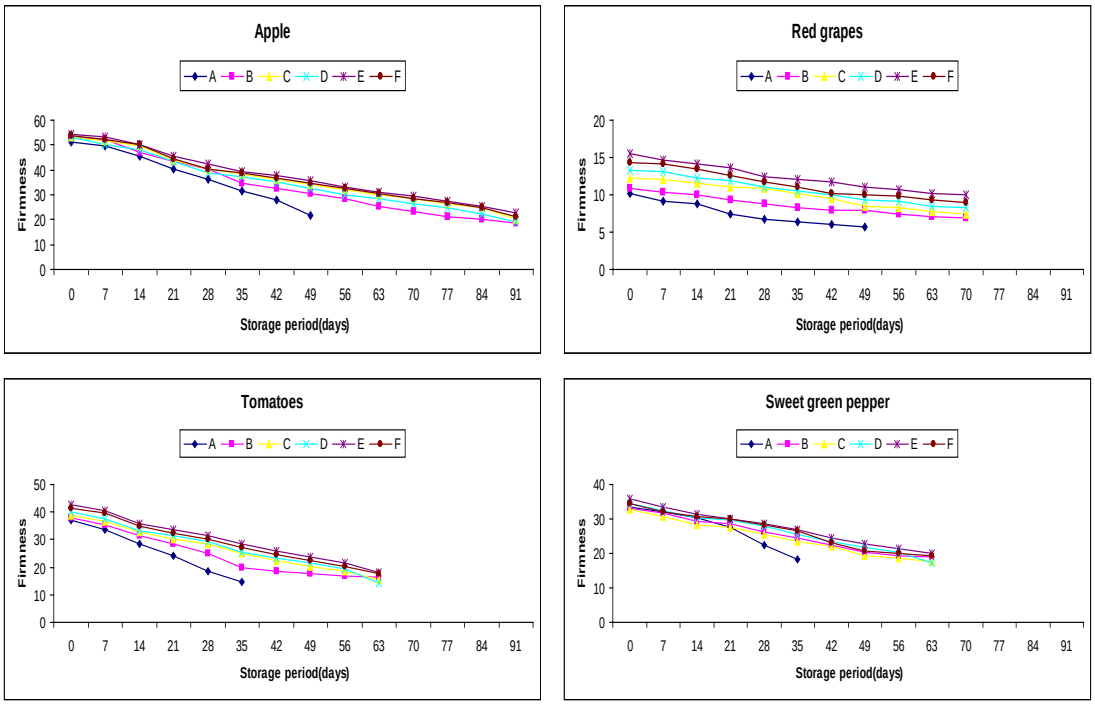


Figure 9 : The Effect of edible coatings on firmness in fresh fruits and vegetables. (a) Apples [$LSD_{0.05}$ = 0.435], (b) Red grapes [$LSD_{0.05}$ = 0.351], (c) Tomatoes [$LSD_{0.05}$ = 0.234], (d) Sweet green pepper [$LSD_{0.05}$ = 0. 564]

Total acidity :

The changes in the total acidity of fresh fruits and vegetables were determined during storage period. The obtained results are recorded in fig (10). The results indicated that the total acidity were gradually decreased with increasing the storage period for apples, red grapes, tomatoes and sweet green pepper. The results of the study on nano-coated ones showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. The results of this study have been in agreement with those obtained by [44] and [25] who found that the titratable acidity of apples was gradually decreased with increasing the storage time. Also, the titratable acidity decreased along with increased storage time in both uncoated and coated fruits [24]. However, the decrease of acidity during the storage demonstrated fruit senescence. The same authors outlined that coatings may be slowed down by the changes in titratable acidity and the fruit senescence can be effectively delayed. This was probably because the semi-permeability of coating films formed on the surface of the fruit might have modified the internal atmosphere i.e. the endogenous CO2 and O2 concentration of the fruit, thus retarding ripening process. [48] found that the reduction of organic acid concentration with decreasing acidity during the period of storage demonstrated fruit ripening and senescence. This may be caused by the respiration activities in tomatoes. Artes et al (2000) found that the pH value of peppers increased with increasing the storage period to 12 weeks at 5ºC. While, the titratable [49].

Acidity tended to decrease during the storage in all the treatments. The total acidity of the stored grapes increased during the storage. As it is known, when fruits are stored, the moisture of the stored products decreases during the storage period and consequently the total acidity amount of the stored product. Increases [43].

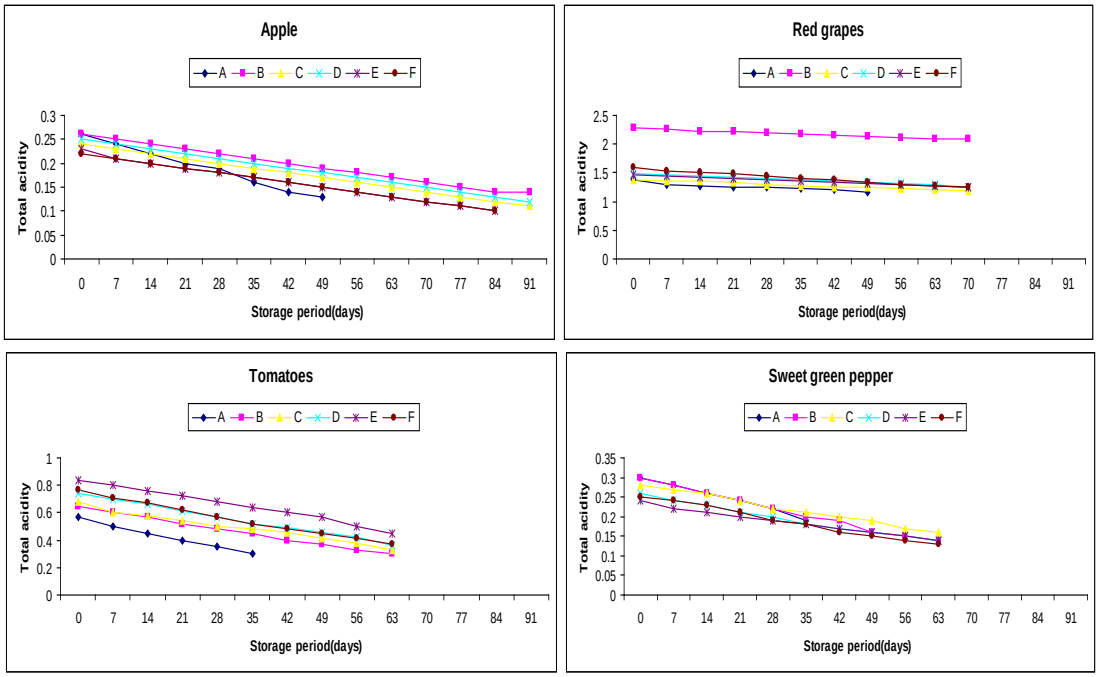
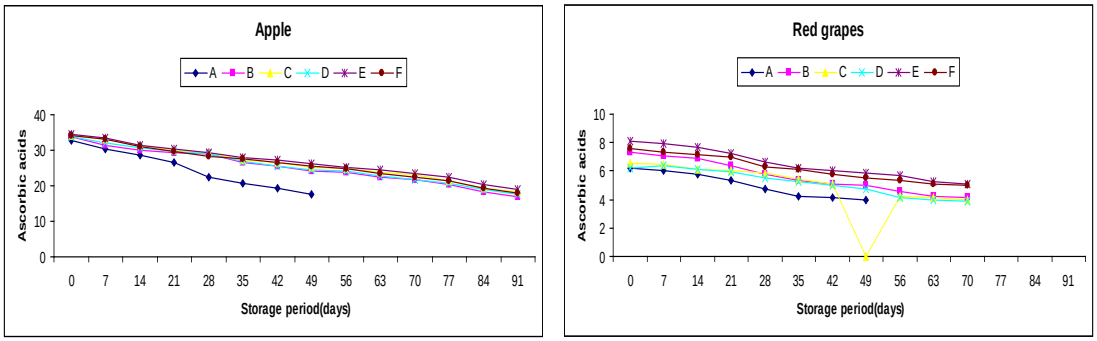


Figure 10 : The effect of edible coating on total acidity in fresh fruits and vegetables (a) Apples [$LSD_{0.05}$ = 0.153], (b) Red grapes [$LSD_{0.05}$ = 0164], (c) Tomatoes [$LSD_{0.05}$ = 0184], (d) Sweet green pepper [$LSD_{0.05}$ =0.142]

Ascorbic acids :

The obtained results are recorded in fig (11), the results indicated that the ascorbic acid decreased with increasing the storage period for fruits and vegetables. The results of the study on nano coated ones showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. The decrease in ascorbic acid during the storage may be due to the conversion of ascorbic acid to dehydroascorbic acid because of the action of ascorbic acid oxidase [50]. According to Greierson and Kader (1986) and El-sheikh (1998), the reduction in vitamin C during the period of storage might be due to the higher rate of vitamin C loss through the respiration than the water through transpiration [51, 52]. Tomatoes are a rich source of ascorbic acid (vitamin C). On the basis of their fresh weight, the average content of vitamin C was about 25 mg/100 g [53] ; however, the values varied with the cultivars. The effect of the light on the ascorbic acid content during the growth was well reviewed by Hobson and Davis (1971) [54]. It could be concluded that the coating process protected ascorbic acid of vegetables and fruits from oxidation and reduced depression attributes of ascorbic acid of pepper. [55] outlined that the vitamin C content of fruits was decreased progressively from the harvest to the end of 30 days' storage period at room temperature. According to [56, 57] there was a significant decrease in vitamin C over 10 days of storage period of unpacked and packaged vegetables including fresh green pepper. The amount of decrease was about 11% after 10 days of storage. Also, they found that the packages' type did not have any significant effects on ascorbic acid content of stored pepper.



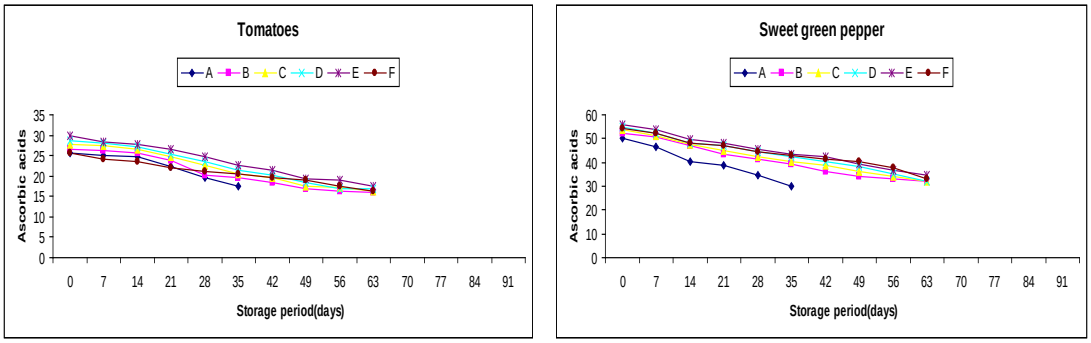


Figure 11 : The effect of edible coatings on ascorbic acids in fresh fruits and vegetables (a) Apples [$LSD_{0.05}$ = 0.243], (b) Red grapes [$LSD_{0.05}$ = 0.253], (c) Tomatoes [$LSD_{0.05}$ = 0.273], (d) Sweet green pepper [$LSD_{0.05}$ = 0.321]

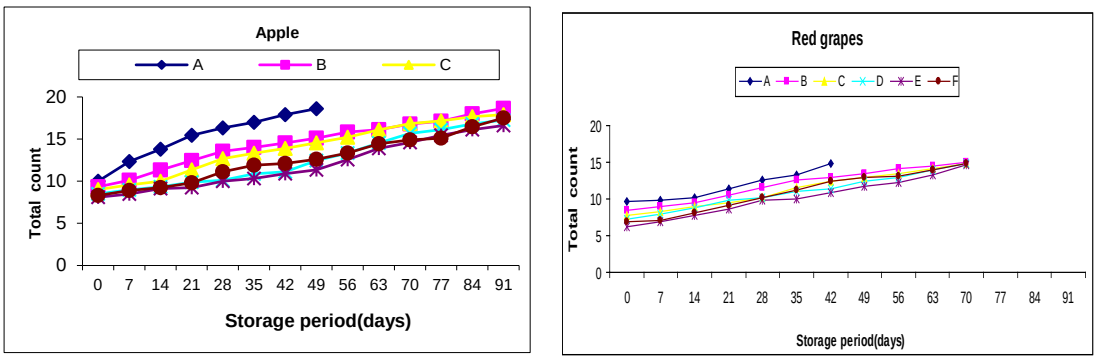
Microbial evaluation of edible coatings on fresh fruits and vegetables

The changes in total counts, psychrophilic bacteria count, and mould and yeast counts of edible coating and film nano-particles in fresh fruits and vegetable were determined during the cold storage period.

Chitosan edible coatings prolonged the shelf life of the fruits and vegetables by minimizing the rate of respiration and decreasing the water loss. Chitosan coating provides a defensive barrier against bacterial contamination and loss of moisture from the surface of food products, therefore expanding their shelf life. With a slight increase in the concentration of chitosan coating, the beneficial effects of chitosan on postharvest life and quality of the food were intensified. [1]

1- Total count :

The obtained results are recorded in Fig (12). The results indicated that the total plate counts gradually increased with increasing the storage period at cold temperature in the coated and noncoated forms. Non-coated treatments, indicated higher counts than the coated ones. The results of the study on nano coated ones showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. The addition of nano-particle materials as antimicrobial agent to coating emulsion improved the fruit microbial quality and decreased the microbial counts [58-60] found that the coating treatment of fruits and vegetables allowed a limited gases exchange and respiration, additionally, prevented the occurrence of fermentation process and minimized the microbial count. Also, they added that high microbial counts appeared after three weeks in the control samples which were stored at room temperature. In addition, it was remarked that the samples treated with non nano coating showed higher counts after storage than those treated with nano coating. This may be due to relatively high thickness of the wax which prevented the respiration and led to anaerobic conditions and fruit degradation. In addition, it could be reported that the edible coating and film nanoparticle materials have remarkable effects on the rate of microbial counts during the storage at cooled temperature. Moreover, the addition of nisin as antimicrobial agent to coating emulsion improved the vegetables' microbial quality, and decreased the microbial counts. The results were in agreement with [47, 60].



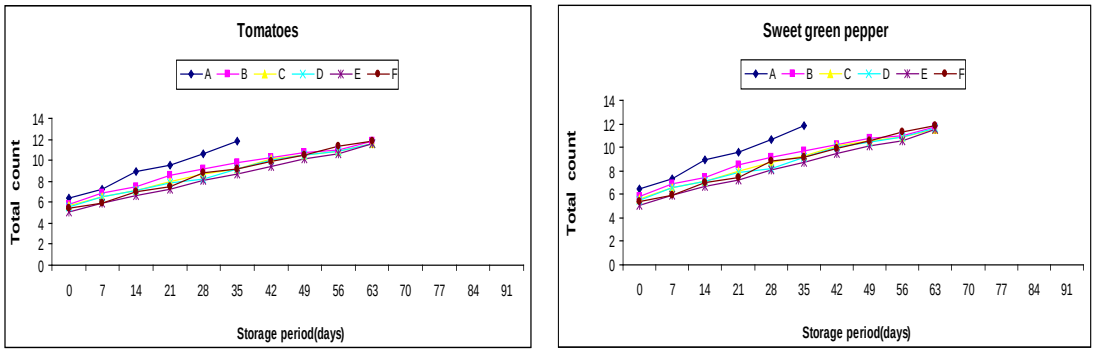


Figure 12 : The effect of edible coating on total count (CFU x 10^2/ g) in fresh fruits and vegetables (a) Apples [$LSD_{0.05}$ = 0.432], (b) Red grapes [$LSD_{0.05}$ = 0.456], (c), [$LSD_{0.05}$ = 0.467], (d) Sweet green pepper [$LSD_{0.05}$ = 0.475]

2- Psychrophilic bacterial :

The obtained results are shown in Fig. (13), and indicated that the psychrophilic bacteria count gradually increased with increasing storage period at cool temperatures. The results of the study on nano coated showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. In addition, it could be reported that the coating of apples, red grapes, tomatoes and sweet green pepper with nano coated and non nano coated had remarkable effects on the rate of psychrophilic bacteria counts during the storage at cool temperatures. Moreover, the addition of nano-particle materials as an antimicrobial agent to coating emulsion improved the fruit microbial quality and decreased the microbial psychrophilic bacteria counts. [61] and [59] found that the coating of apples increased the period of storage and delayed ripening depending on surrounding media in combination with cool temperature (4 ºC) and RH (85%) management which exhibited continuous improvement of fruit life. [61] found that the increased cooled storage period of tomatoes may cause delayed ripening and psychrophilic bacterial counts, depending on the surrounding media which exhibit continuous improvement of the fruit life, in combination with the cooled temperature (4 ºC) and RH (85%) management. [62] and [47] found that the coating of pepper increased storage period with delayed ripening through conditions surrounding the media for the improvement of the fruits' life, in combination with the cool temperature (4ºC) and RH (85%) management.

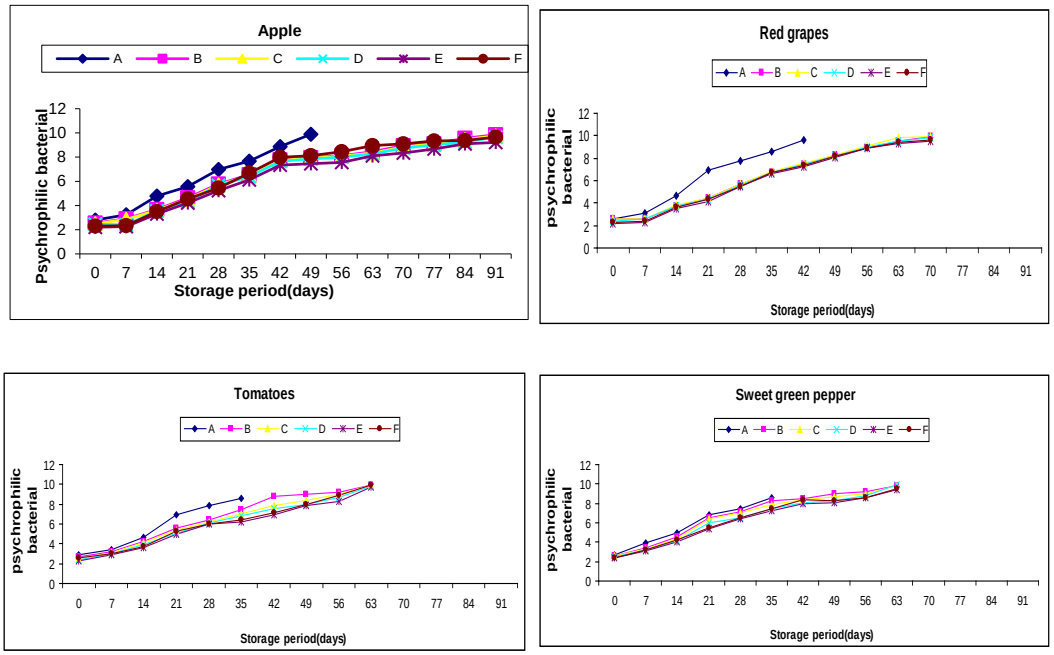


Figure 13 : The effect of edible coating on psychrophilic bacterial (CFU x 10^2/ g) in fresh fruits and vegetables (a) Apples [$LSD_{0.05}$ = 0.412], (b) Red grapes [$LSD_{0.05}$ = 0.411], (c) Tomatoes [$LSD_{0.05}$ =0.432], (d) Sweet green pepper [$LSD_{0.05}$ = 0.423]

3- Moulds and yeasts :

The obtained results are shown in Fig (14), and indicated that the mould and yeast counts gradually increased with increasing the storage period at cool temperature. The results of the study on nano coated showed that the best treatment was E followed by F followed by B, C, D, as compared with non nano coated A which was the least. The apples, red grapes, tomatoes and sweet green pepper were kept as follows: 91, 70, 63 and 63 days respectively, as compared to non-nano coated in which the results were 49, 49, 35 and 35 days, respectively. The addition of nano-particle materials as antimicrobial agent to coating solution improved the fruit microbial quality and decreased the microbial mould and yeast counts. This might be due to the increasing of RH in refrigerating chamber and the suitability of the refrigerator temperature for yeast growth according to [62]. This might be due to the improvement of the post-harvest life, in combination with temperature and relative humidity management [63].

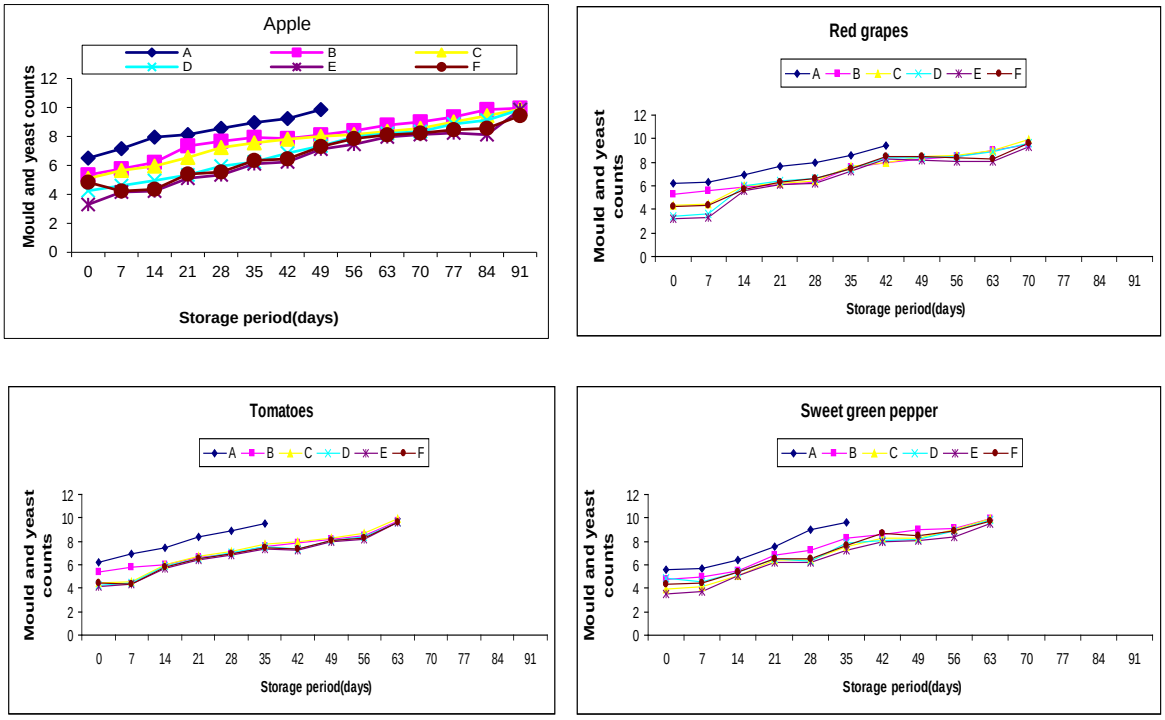


Figure 14 : The effect of edible coating on mould and yeast counts (CFU x 10^2/ g) in fresh fruits and vegetables (a) Apples [$LSD_{0.05}$ = 0.421], (b) Red grapes [$LSD_{0.05}$ = 0.417], (c) Tomatoes [$LSD_{0.05}$ = 0.422], (d) Sweet green pepper [$LSD_{0.05}$ = 0.435]

CONCLUSION

The edible coatings as having a high potential to carry active ingredients such as nano-materials maintained weight loss, total soluble solids, firmness, total acidity, ascorbic acid, total count, psychrophilic bacteria count and mould & yeast quality attributes during the storage at (0-2°C) of fruits including apples and red grapes and at (8±1°C) of vegetables including tomatoes and sweet green pepper at relative humidity of 90-95%. The results indicated that the best nano coating treatments were (E) followed by (F) followed by other coefficients (B, C, D) as compared with non nano coating A which was the least. The nano coated apples, red grapes, tomatoes and sweet green pepper were kept as follows: 91, 70, 63 and 63 days respectively, as compared to non nano coated ones for which the results were 49, 49, 35 and 35 days, respectively.

Other Physical and chemical properties were studied e.g. rheological properties and particle size distribution emulsion, zeta potential emulsion, X-R diffraction(XRD) films, and scanning electron microscopy films. Adding nano materials on edible coating to prolong the shelf life of products reduced the risk of pathogen growth and improved the quality of fruits and vegetables.

The results of the analysis of edible coatings and films of nano materials indicated that the best nano coating treatment was in (E) followed by (F) followed by other coefficients (B, C, D) as compared with non nano coating A

which was the least. In apples, red grapes, tomatoes and Sweet green pepper fruits dipped in solution of (E), the weight loss percentage was reduced, and the fruit quality was maintained for 63 days and 56 days of storage for apples and red grapes, respectively ; and 42 and 35 days of storage for tomatoes and sweet green pepper, respectively.

ACKNOWLEDGEMENTS

The project was funded by the Deanship of Scientific Research (DSR) University of Tabuk, Kingdom of Saudi Arabia (KSA) under the grant No. (S-0170- 1438). The authors, therefore, acknowledge the thanks to DSR technical and financial support.

REFERENCES

1. Rayees Ahmad Shiekh, Maqsood Ahmad Malik, Shaeel Ahmed Al-Thabaiti and Muneer Ahmad Shiekh (2012) Chitosan as a Novel Edible Coating for Fresh Fruits Food Sci. Technol. Res., 19 (2), 139 – 155, 2013.
2. Elena Poverenov, Roi Rutenberg, Shani Danino and Batia Horev (2014) Gelatin-Chitosan Composite Films and Edible Coatings to Enhance the Quality of Food Products : Layer-by-Layer vs. Blended Formulations. Food Bioprocess Technology, 7(5), 1333-1337.
3. Yadollahi, A. ; K. Aarzani and H. Khoshghalb (2010). The role of nanotechnology in horticultural crops postharvest management. Acta Hort. 875 : 49-56
4. Azeredo, H..; L.H.Mattoso;T.G.Williams; R.D.Avenc-Bustillos and T .H .Me ugh (2009) Nanocomposite edible films from mango pure reinforced with cellulose nanofibers.J .Food .Sci.,74(5):31-35
5. Xiao, J, wang, X, A., Gonzalez, A, J, P., Xia, Q (2016) Kafirin nanoparticales- stabilized pickering emulsion : microstructure and rheological behavior. Food Hydrocolloids 54, 30 -39
6. Nelson Caro, Estefania Edina, Mario Diaz-Dosque, Luis Lopez, Lilian Abugoch, ristian Tapia (2016). Novel active packaging based on films of chitosan – tripolyphosphate- thymol nanoparticales via thermal ink-jet printing. Food Hydrocolloids 52, 520 - 532
7. Keng-shiang, Alexandr Mihai Grmezesc, Ching-Hui Yang and Chihyu wang., (2014) Immbilization and stabilization f Tio2 Nanoparticales in Alkaline – Solidificated chitosan Spheres Without Cross-Lining Agent. International journal of Latest Research in science and technology, Volume 3, Issue 2: page No.174 -178.
8. Honary S, Ghajar K, Khazaeli P and Shalchian P. (2011). Preparation, Characterization and Antibacterial Properties of Silver-Chitosan Nanocomposites Using Different Molecular Weight Grades of Chitosan. Tropical Journal of Pharmaceutical Research February ; 10 (1) : 69-74
9. Poverenov, E., Danino, S., Horev, B., Granit, R., Vinokur, Y., & Rodov, V. (2014) Layer-by-layer electrostatic deposition of edible coating on fresh cut melon model : anticipated and unexpected effects of alginate-chitosan combination. Food and Bioprocess Technology,7(5), 1424–1432
10. Abugoch, L. E., Tapia, C., Villaman, M. C., Yazdani-Pedram, M., & Diaz- Dosque, M. (2011). Characterization of quinoa proteinchitosan blend edible films. Food Hydrocolloids, 25(5), 879–886.
11. Hosseini, S. F., Rezaei, M., Zandi, M., & Ghavi, F. F. (2013). Preparation and functional properties of fish gelatin-chitosan blend edible films. Food Chemistry, 136(3–4), 1490–1495.
12. Turhan KN, Sahbaz F (2004) Water vapor permeability, tensile properties and solubility of ethylcellulose-based edible films. J Food Eng 61 :459–466.
13. Miller KS, Krochta JM. (1997) Oxygen and aroma barrier properties of edible films : a review. Trends in Food Sci Technol 8 :228–37.
14. Cai Z, Kim J (2008) Characterization and electromechanical performance of cellulose–chitosan blend electro-active paper. Smart Materials and Structures 17 :1–8.
15. Bhuvaneshwari, S., Srthi, D., Sivasubramanian, Niranjana Kalyani and Sgnabai et al (2010). Development and characterization of chitosan film. International journal of engineering Research and Applications, vol1, Issue 2, pp. 292- 299.

16. ASTM. (1993). Standard practice for conditioning plastics and electrical insulating materials for testing : D618-61 (reapproved 1990). In : Annual book of American Standard TestingMethods, Vol. 8.01. Philadelphia, Pa. : ASTM. P 146–8.
17. Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards - A. Physics and Chemistry, 81(1), 89–96.
18. Jie xiao, Xuanxuan Lu and Qingrong Huang., (2017). Double emulsion derived from kafirin nanoparticales stabilized pickering emulsion : Fabrication, microstructure, stability and vitro digestion profile. Food Hydrocolloids62, 230 - 238
19. Tien, C. L. ; Letendre, M. ; Ispas-Szabo, P. ; Mateescu, M. A. ; Delmas-Patterson ; Yu, H. L. and Lacroix, M. (2000). Development of biodegradable films from whey proteins by cross-linking and entrapment in cellulose. J. Agric. Food Chem., 48 :5566.
20. Munoz, P. H. ; Lopez-Rubio, A. ; Del-Valle, V. ; Almenar, E. and Gavara, R. (2004). Mechanical and water barrier properties of glutenin films influenced by storage time. J. Agric. Food Chem.,52 :79-83.
21. Hernandez-Mun, P., Villalobos, R. and Chiralt, A., (2004). Effect of cross-linking using aldehydes on properties of glutenin-rich films. Food Hydro., 18 :403
22. Sotelo-Boy M.E. Sotelo-Boy, G. Valverde-Aguilar, M. Plascencia-Jatomea, Z.N. Correa-Pacheco, A. Jim´enez-Aparicio, J. Solorza-Feria, L. Barrera-Necha, S. Bautista-Ba˜nos (2015) Characterization Of Chitosan Nanoparticles Added With Essential Oils. In Vitro Effect On Pectobacterium Carotovorum Caracterizacio´ N De Nanoparti´Culas De Quitosano Adicionadas Con Aceites Esenciales. Efecto In Vitro En Pectobacterium Carotovorum. Vol. 14, No. 3 (2015) 589-599.
23. Garcia M.A., Martino M.N., NE (2000) Zaritzky, Lipid addition to improve barrier properties of edible starch-based films and coating, J. Food Sci. 65941947
24. Han, C., Y. Zhao, Leonard S.W and Traber M.G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria x ananassa) and raspberries (Rubus ideaus). Postharvest Biology and Technol., 33 : 67-78
25. Konopacka, D. and Plocharski, W. J. (2004). Effect of storage conditions on the relationship between apple firmness and texure acceptability, Postharvest Biology and Technology, 32 :205-211
26. Bourne, M.C., (2003). Food texture and viscosity : concept and measurement. Elsevier Press, New York/London.
27. A.O.A.C. Association of Official Analytical Chemists. (2005). Official Methods of Analysis 18th ed., A.O.A.C, USA Washington. D.C.
28. Marshall, S., (1992). Standard methods for examination of dairy products American public Health Association (ABHA). Washington DC, USA.
29. A.P.H.A., (1976). American Public Health Association.Compendium of methods the microbiological examination Foods, Washington.U.S.A.
30. Snedecor, G.W. and W.G. Cochran (1989). Statistical methods.7th Ed. The Iowa State Univ., Press Amer, lowa – USA, pp. 50.
31. Dail, R. V. and Steffe, J. F. (1990). Rheological characterization of cross-linked waxy maize starch solutions under low acid aseptic processing conditions using tube viscometry techniques. J. Food Science, 55(6) :1660-1665.
32. Hagenimana, A. ; Ding, X. and Gu, W, Y. (2007). Steady state flow behaviours of extruded blend of rice flour and soy protein concentrate. Food Chemistry,101 :241-247
33. Ottone, M, L ; Perirotti, M, B and Deiber, J. A (2005). Modeling the rheology of gelatin gels for finite deformations part2. Viscoelastic solid Model Polymer, 4938-4949.
34. Chen, H. (1995). Functional properties and applications of edible films made of milk proteins. J. Dairy Sci., 78 :2563
35. Jie xiao, Yunqi Li, Qingrong Huang (2016). Recent advances on food-grade particles stabilized pickering emulsion : Fabrication, characterization and research trends. Trends in Food Science &Technology 55, 48-60
36. Suisui Jiang, Chenzhen Liu, Zhongjie Han, Liu Xiong, Qingjie Sun (2016) Evaluation of rheological behavior of starch nanocrystals by acid hydrolysis and starch nanoparticles by self-assembly : A comparative study. * Food Hydrocolloids 52 ,914-922

37. Raymond, L.V., M. Zhang and S.M. R. Azam (2012) Effect of chitosan coating on physical and microbial characteristics of fresh –cut green peppers (capsicum annum L.,) Pakistan. Jurnal of Nutritin ,11(10) :904-909.
38. Zhu, X. ; Wang, Q.M. ; Cao, J. K. and Jiang, W. B. (2008). Effects of chitosan coating on postharvest quality of mango (Mangifera indica L.CV. Tainong) fruits. J. Food Process.Preserv., 32 : 770–784.
39. Ayranci, E., S. Tunc, (2003). A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. Food Chem., 80, 423-431.
40. El-Sheikh, T. M. ; AbdEl-Rahman, S. Z. and Safia, M. H. (1997). Effect of calcium chloride and hydrated lime on keeping quality of sweet pepper and cucumber fruits. Annals Agric. Sci., Moshtohor, 35(4) :2371-2389.
41. Saleh, M. M. ; Kabeel, M. A. and Emam, M. S. (2005). Influence of soil mulching and plant shading on growth, yield, fruit quality and storage ability of sweet pepper. J. Agric. Sci. Mansoura Univ., 30(5) :2775-2794.
42. Wand, S. J. E. ; Theron, K. I. ; Ackerman, J. and Marais, S. J. S. (2006). Harvest and post-harvest apple fruit quality following applications of kaolin particle film in south African orchards Scientia Horticulture, 107 :27-276.
43. Selcuk Arin and Serap Akdemir (2004) Quality properties Changing of Grape During Storage Period. J of Biological Sciences 4(2) : 253-257
44. Bai, J. ; Alleyne, V. ; Hagenmair, R. D. ; Mattheis, J. P. ; Baldwin, E. A. (2003). Formulation of zein co Bai, j. ; Hagenmaier, R. D. and Baldwin, E. A. (2003). Coating selection for 'Delicious' and other apples. Postharvest Biology Technology, 28 :381-390.ating for apples (malus domestica bark). Postharvest Biology and Technology, 28 :259-268.
45. Patricia, S. ; Palmu, T ; Carlos, R. and Grosso, F. (2005). Effect of edible wheat gluten-based films and coatings on refrigerated strawberry quality. Postharvest Biology Technology, 36 :199-208.
46. Brody, A. L. and Marsh, K. S. (1997). The Wiley Encyclopedia of Packaging Technology second edition. A Wiley –interscience publication, New York
47. Lerdthanagkul, S. and Krochta, J. M. (1996). Edible coating effects on postharvest quality of green bell peppers. J. Food Sci., 61(1) :176- 179.
48. Djedidi, M. ; Gerasopoulos and Maloupa, E. (2003). The effect of different substrates on the quality of F. carmello tomatoes grown under protection in a hydroponic system. Cahier Options Mediterraneennes, 31 :379-383.
49. Artes, F. ; Villaescusa, R. and Tudela, J. A. (2000). Modified atmosphere packaging of pomegranate. J. Food Sci., 65(7) :1112- 1116.
50. Mapson, C.W. (1970). Vitamins in Fruits : Stability of l-Ascorbic acid in Biochemistry of Fruits and their Products.Academic Press, London, pp. 376–387.
51. Greierson, D. and Kader, A. A. (1986). Tomato crop : A scientific basis for improvement. Text book chapter (6) New York.
52. El-sheikh, T. M. (1998). Physiological studies on yield quality and storage ability of tomato fruits. Annals of Agric. Sci., Moshtohor, 36(1) :513-526.
53. Olliver, M. (1967). Occurrence in foods. In : The Vitamins, W.H. Sebrell and R.S. Harris, eds., Vot. 1, pp. 359-367, Academic Press, New York.
54. Hobson, G.E. and Davies, 3.N. (1971). The tomato. In : The Biochemistry of Fruits and their Products, A.C. Hulme, ed., pp. 437-482, Academic Press, New York.
55. D'aquino, S. ; Piga, A. ; Agabbio, M. and Mccollum, T. G. (1998). Film wrapping delays ageing of Minneola tangelos under shelf-life conditions. Postharvest Biology and Technology, 14 :107-116.
56. Hussein, A. ; Odumeru, J. A. ; Ayanbadejo, T. ; Faulkner, H. ; McNabw, B. ; Hager, H. and Szijarto, L. (2000). Effects of processing and packaging on vitamin c and β-carotene content of ready- to – use (RTU) vegetables. Food Research International, 33 :131-136.
57. Saleh, M. M. ; Kabeel, M. A. and Emam, M. S. (2005). Influence of soil mulching and plant shading on growth, yield, fruit quality and storage ability of sweet pepper. J. Agric. Sci. Mansoura Univ.,30(5) :2775-2794.

58. Petracek, P. D. ; Huating, D. and Poa, S. (1998). The influence of applied waxes on postharvest physiological behavior and pitting of grapefruit. Postharvest Biology and Technology, 14 :99-106.
59. Chen, M. J. ; Weng, Y. M. and Chen, W. (1999). Edible coating as preservative carriers to inhibit yeast on Taiwanese-style fruit preserves. J. Food Safety, 19 :89-96.
60. Lee, H. C. ; An. S. D ; Lee, C. S. ; Park, H. J. and Lee, S. D. (2004). A coating for use as an antimicrobial and antioxidative packaging 284 material incorporationg nisin and α- tocopherol. J. of Food Engineering, 62 :323-329.
61. Gil, M. I. ; Conesa, M. A. and Artes, F. (2002). Quality changes in fresh cut tomato as affected by modified atmosphere packaging Postharvest Biology and Technology, 25 :199-207.
62. Koide, S. and Shi, J. (2006). Microbial and Quality evaluation of green peppers stored in biodegradable film packaging. Food Control, 18 :1121-1125.
63. Koseki, T., Okuda, M., Yonehara, Y., Hachida, K., Iwata, H., Aramaki, I. and Hashizume, K. 2004. Effects of high temperature during grain-filling on characteristics of rice for sake brewing. J. Brew. Soc. Jpn. 99 : 591-596.