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Biopolymer Product from pullulan Material (Polysaccharide) used for Natural Film & Coatings in Food Preservation

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Abstract

Pullulan is a non-ionic polysaccharide obtained from fermentation of black yeast like Aureobasidium pullulans and in order to preserve the film from oxidation and microbial contamination maintain the stability of the film and extend the shelf life of guava, as this substance inhibits microbial growth and oxidation and improves the quality of the film. Acceptable sensory characteristics appropriate barrier properties (CO2, O2 and water) microbial, biochemical and physicochemical stability public health safety effective carrier for antioxidant, flavor, color, nutritional or anti-microbial additives it could be indicate that these findings may be of application benefit in the field of food industry and Simple technology for production Low cost this study was to produce an pullulan natural edible film and coatings from polysaccharides and the theological, mechanical properties, permeability and scanning electron microscopy (SEM) of the prepared films were determined. The thickness, tensile strength, elongation, % solubility of produced by pullulan natural edible film with concentration of antimicrobials action of extract Luria leaves/acetic acid was the highest, followed by that of pullulan natural edible film with tripolyphosphate (TPP). Therefore, the addition of concentration of antimicrobials action of extract Luria leaves/acetic acid and (TPP) to pullulan natural edible film have the potential to provide a safe edible films decreased microbial growth and consequently prolonged the shelf life of guava fruit, As well as improved the physiochemical changes properties of the guava fruit. The substances used in this experiment were, film with glycerin A(0%), film with pullulan B(20%), film with pullulan C (40%) and film with pullulan D (60%) on quality attributes and prolong shelf- life of guava fruit during storage. All products were stored at (8 ± 1°C) for 35 days and the quality parameters such as weight loss, total soluble solids, firmness, total acidity, color and microbiological test were determined. Samples coated with (B) reduced the weight loss and total soluble solids. The results observed that treatment pullulan B(20%) was the best treatment in terms of reduction of microbial load followed by treatment pullulan C(40%) followed by treatment pullulan D (60%) until 25 days of storage as compared to A(0%).The levels of decrease in chemical and microbial load in the samples from both of the pullulan natural edible film were related good quality guava fruit. As well as on the same physiochemical tests. It is clear that the pullulan natural edible film of the aromatic concentration of antimicrobials action of extract Luria leaves/acetic acid glacial from Luria Leaves and the (TPP) have kept the Quality of guava fruit up to 25 days of storage.

Key words: *Pullulan; Edible natural films; Rheological and mechanical properties; Scanning electron microscopy films and coated guava*

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Introduction

Pullulan is a non-ionic polysaccharide obtained from fermentation of black yeast like Aureobasidium pullulans and is currently exploited in food and pharmaceutical industries. It is non-toxic, non-immunogenic, non-carcinogenic, non-mutagenic, pullulan is being explored for various biomedical applications . Pullulan is rapidly emerging as an industrially important source of polymeric materials which are gradually becoming economically competing with natural gums made from marine algae and other plants. Hydrocolloids gums are polysaccharides used to increase viscosity and gelatinization and have many applications in the food industry as thickeners, emulsifiers, and stabilizers. Hydrocolloids slow starch digestion potentially due to the formation of a physical barrier that limits the effect of digestive enzymes. Dietary intervention can help to prevent and manage the disease. Food hydrocolloids have been shown to have favorable properties in relation to glycaemic regulation. Hydrocolloids can reduce or restrict contact between enzymes and substrates, reduce mass transfer in the gut (e.g., by increasing viscosity or gelling) and inhibit enzyme activity Deepak Kumar et al (2012). Pullulan is extracellular polysaccharyde produced by Aureobasidium pullulans.

It has got the permissin to be applied as a food additive [U.S. Food., 2002, European., 2004]. Pullulan is an extracellular homo-polysaccharide produced by Aureobasidium pullulan, which is one of good candidates for the production of edible films (Gounga and others 2008). Pullulan is a linear mix of α-D-glucan consisting mainly of maltotriose repeating units interconnected by α-(1→6) linkages. The regular alternation of α-(1→4) and α-(1→6) bonds enhances water solubility and results in a distinctive structural flexibility Chao Zhang.,et al (2013) Pullulan is an extracellular microbial polysaccharide, which mainly consists of maltotriose units interlinked each other by an a 1-6 glycosidic bonds, is synthesised from starch by the ubiquitous fungus Aureobasidium pullulans (Shih, Daigle, & Champagne,2011). Pullulan is highly water soluble in nature and possesses distinctive film-forming properties compared to other polysaccharides (Wu, Zhong, Li, Shoemaker, & Xia, 2013).

Pure forms of pullulan films are colourless, transparent, odourless, tasteless, highly water permeable, and show lower oil and oxygen permeability (Singh, Saini, & Kennedy, 2008). Despite its many advantages, its high cost has limited the wide usage of pullulan and its films for various industrial applications (Xiao, Lim, & Tong, 2012a). Many researchers have tried to overcome this, simply by blending pullulan with other biopolymers,in order to produce films with modified physicochemical and mechanical properties. Recent studies have focused on blending of pullulan with other polysaccharides, such as alginate, chitosan,cellulose derivatives, and starch (Wu et al., 2013; Xiao et al., 2012a). Among those polysaccharides, starch is cost-effective,renewable and easily biodegradable. It has long been used in the development of various edible and biodegradable films (Ghanbarzadeh et al., 2010). Consumer demand for safe and natural food packaging materials has spurred the research and development of films, coatings and packaging that are derived from food grade polysaccharides, proteins and lipids. Food based packaging materials are also sustainable, biodegradable and environmentally friendly. 1–4 Due to excellent biodegradability and unique gas barrier properties, pullulan and chitosan are potentially useful film and packaging materials.Improved mechanical properties of films have been reported when pullulan is combined with proteins or polysaccharides. 10–13 Chitosan forms more transparent and flexible films.

The properties of composite films of chitosan and pullulan blended film-forming solutions at different pH. They reported that the glass transition temperature and molecular interactions that affect physical properties of pullulan–chitosan blended films were highly sensitive to the water content. 14 The properties of the films with different ratios of pullulan to chitosan prepared in our laboratory have been previously studied, and the composite films formed with a 1 : 1 ratio of pullulan and chitosan from a 2% acetic acid solution were found to have the best properties Yue Li,et al (2015). Essential oils are frequently used to control the growth of pathogenic bacteria and prevent degradation in foods (Zivanovic et al., 2005). In edible film systems, an antimicrobial agent slowly passes from the film layer to the food and thus a high concentration of antimicrobial agent remains in the film and at the surface of the food, providing a longer effect against microorganisms (Coma et al., 2002). Production of pullulan natural edible film and coatings from polysaccharides extracted from polysaccharides in order to preserve the film from oxidation and microbial contamination maintain the stability of the film and extend the shelf life of guava, as this substance inhibits microbial growth and oxidation and improves the quality of the film. Acceptable sensory characteristics appropriate barrier properties (CO2, O2, water, oil) microbial, biochemical and physicochemical stability public health safety effective carrier for antioxidant, flavor, color, nutritional or anti-microbial additives it could be indicate that these findings may be of application benefit in the field of food industry Simple technology for production and Low cost (FDA., 1998), Hechman and heller, 1986).

The objective of this work is biopolymer product from pullulan to produce edible natural films. And study rheological and mechanical properties, permeability, scanning electron microscopy and coated guava to select the best edible nature films estimated by the previous tests.

Materials and Methods

Materials

Luria leaves were obtained from the local market. At Central Lab of Agriculture Res. Food Engineering and Packaging Dept. The materials used in this experiment were: Ethyl alcohol 95%, Chloroform and Methanol produced by were obtained from chemical comp., UK. NaCl, HCl, saccharose, yeast extract and glycerin (P05650, were obtained from (El-Gomhouria chemical company. Glycerol and sodium hydroxide was obtained from (Acmatic For Chemicals & Lab. Equipment Company, Cairo, Egypt). Acetic acid glacial and sorbitol was obtained from (Across Organics, Belgium). Whatman No.1 filter paper and decanter 50 mesh was obtained from across-organics Company New Jersey U.S.A. chitosan powder was obtained from Win Lab Company, (U.K). Tripolyphosphate (TPP) elephant Bio-Engineering Co., LTD, China. K_2HPO_4, $(NH_4)_2SO_4$, $MgSO_4$ was obtained from Jenapharm, Germany.

Strains: Batch fermentation kinetics of pullulan from Aureobasidium pullulans using low cost substrates. The name "pullulan" was the first to describe the formation of this extracellular polysaccharide by Aureobasidium pullulans (syn. Pullularia pullulans) In order to obtain pullulan the Aureobasidium pullulans B-1 strain was used. It was taken from the Microbiological Resources center (Cario MIRCEN) cultivate the fungus and get pullulan, the liquid base of the standard cultivation medium containing the following composition was used (g/L): saccharose – 60.0, K_2HPO_4 – 7.5, NaCl – 1.5, $(NH_4)_2SO_4$ – 0.72, $MgSO_4 \bullet H_2O$ – 0.4, yeast extract – 0.4. The medium pH was 6.0 and was adjusted with 1 mol/L HCl and distilled water 1L before sterilization [Gniewosz et al. 1999]. The medium was autoclaved for 15 min at 121°C, cooled and the initial pH was adjusted to 7.0. A loop – full of culture from slant was transferred to 250mL conical flask containing 50mLculture medium. The flask was incubated at 28°C for 48 hours in a rotary shaker incubator (200 rpm). These cultures (5%v/v) were used to inoculate the production medium for all the fermentation studies. 1 ml cultures were then transferred into the fresh medium and cultivated in the same conditions as above for 96h. The fungus biomass was centrifuged (11 000 rpm, 20 min at 4°C) in order to separate cellular biomass from supernatant liquid. Pullulan was precipitated from the supernatant with 96% ethanol and the precipitate was centrifuged (11 000 rpm for 20 min). Then pullulan was purified following Thirumavalavar et al (2008) and Roukas and Biliaderis procedure [1995]. After the purifying process pullulan was dried up in 80°C. The dry pullulan was then grinded in mill.

Preparation of pullulan/chitosan

Solutions of chitosan (2g/1% Tripolyphosphate(TPP) were prepared separately by dissolving each in 100 mL of deionised water while stirring with a magnetic stirrer at room temperature. Subsequently, pullulan and different chitosan solutions were mixed in the ratios of A0% 100:0, B20% 80:20, C40% 60:40 and D60% 40:600 (v/v) using a mixed 2000 rpm and homogenized 4000 rpm at 95°C. Following this, all the blended solutions were autoclaved at 121°C for 15 min and allowed to cool at room temperature. The mixtures were then centrifuged at 3000 rpm for 2 min, in order to remove air bubbles from the solutions. Into these mixture solutions according to

Paulraj Kanmani, Seung Taik Lim (2013).

Extraction of phenolic compounds from Luria Leaves

The air-dried ground (80 mesh) plant material (20g for each sample) was extracted with solvents ratio (ethanol: water, 80:20 v/v) and aqueous methanol (methanol: water, 80:20 v/v) (200 ml) for 6 hours at room temperature in an orbital shaker in a water bath in separate experiments. The extracts were separated from the residues by filtering through what man No.1 filter paper. The residues were extracted twice with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 45°C, using a rotary evaporator. The dried crude concentrated extracts were weighed to calculate the yield and stored in a refrigerator (-4°C) according to (Bushra et al., 2009). In this part of investigation, we used crude phenolic compounds extracts (as antioxidants) are prepared by adding (0.5 to 4.5%) acetic acid glacial adhesion the described film formation solution mentioned above was modified. Acetic acid gives effect to total extracted as the highest antioxidant activity, which were acidified with 1-5% acetic acid. Maceration was conducted for 1h at ambient temperature in a dark place according to Nyi Mekar Saptarini1and Irma Erika Herawati (2018), Olafsson., et al (1993) and Nelson et al., (2016).

Methods

Preparation of pullulan natural edible film and coatings solution from pullulan.

The substances used in this experiment edible film and coating was divided four groups and for the modification of matrix composition of studied edible film and coating solution:

In this trial, the described film formation solution mentioned above was modified by adding 20, 40 and 60% of pullulan b, c and d treatments loaded on chitosan films. Four pullulan natural edible films were prepared from chitosan by suspension solution consisting of 2% w/v in 100 ml of deionised distilled water according to Yue Li, et al (2015) film-forming pullulan with the incorporation to contain 3% v/v glycerin, 1% tripolyphosphate and (0.5/4.5%) crude phenolic compounds extracts (as antioxidants) luria leaves/acetic acid glacial . The edible films were formulated to (b, c and d treatments. Edible film mixtures were manually homogenized and their pH values were adjusted to 8 and kept in a water bath at 95 ± 2°C for 30 min, then an edible film solution was filtered and poured on Teflon which used as casting surface, after drying films were separated from plates. Table (1) shows the formulas of edible films.

Film code	Film components %			
	chitosan	20% pullulan	40% pullulan	60% pullulan
A0%	2%	-	-	-
B20%	2%	20%	-	-
C40%	2%	-	40%	-
D60%	2%	-	-	60%

A0 = Edible films + glycerin + Tripolyphosphate (TPP)

B20% = Edible films + 20%

C40% = Edible films+40% D60% = Edible films + 60

Storage treatments of studied guava fruit

Guava: Samples of guava (Psidium guajava) 'Lucknow- 49', used in this investigation, were grown in a local farm on loamy soil (Elkalubia Governorate, Egypt) and received the normal agriculture practice during the two successive seasons (2020). Uniform guava fruit, in size and free from physical damage and selected for their uniformity, maturity stage, size, color, external appearance and absence of damage and fungal infection, were harvested at ¾ surface color stage, packed in field box and transported to the lab. Guava was stored overnight at 8ºC. In the next day, the guava fruits were washed with tap water and then immersed for 2 min. in disinfectant solution of calcium hypochlorite (0.25g L−1). At Central Lab of Agriculture Res. and Food Tech. Res. Institute, Giza, Egypt to study the effect of different postharvest treatments on quality of fruit. The different coated groups were dipped for one minute in the edible film mixture. Coated guava were drained after dipping and packaged in plastic trays with approximately six fruit 500 g. After that, all boxes were cold stored at 8°C and 90-95% RH for 25 days, and kept in carton boxes all samples were kept after packaging .The cooled storage was carried out in the post-harvest research department, Horticulture research institute, Agriculture research center-Giza. During storage period samples of investigated strawberry were periodically with drown for analysis.

Physical and mechanical and rheological properties of prepared on edible films:

1- Rheological measurements: Rheological parameters (shear rate and shear stress) of the selected edible natural films were measured using a Brookfield Engineering labs DV- III Rheometer at room temperature of selected the most suitable solutions. 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- Measurement of prepared pullulan natural film and coatings using scanning electron microscopy by: INSPECT S- SEM schematic overview–TM 1999-2007 Bwilddate, FEL company Euld number D 8571 Machine type inspect S. (Sotelo-Boy et al., 2015).

3- Film thickness: The thickness of the prepared and coatings treatments A0, B20%, C40% and D60% was measured using a digital micrometer (mitutoyo digimatic indicator corporation, model: pk-1012 E, Japan). Film strips were placed between the micrometer jaws and gap and was slowly reduced until the first contact was noted. (Tien et al., 2000).

4- Percentage of Solubility in water: The films specimens were first dried in a desiccator containing dry calcium chlorides. A dry film sample of 500 mg was immersed in beakers containing 50 ml of distilled water at room temperature during 24 hours with periodical gentle shaker incubator. Films were removed from the water and placed back in the desiccator until constant weight. Loss of weight in water was reported as a percentage of weight loss in water on the dry film basis according to Munoz et al. (2004).

$$\% \text{ weight loss} = \frac{\text{Initial dry weight -final dry weight}}{\text{Initial dry weight}} \times 100$$

5-Mechanical properties of prepared on pullulan natural film and coatings: Measurement of treated A0, B20%, C40% and D60% films, the tensile properties (Tensile strength, elongation) were measured by a texture analyzer CT3. The films at different treatments A0, B20%, C40% and D60%were cut into strips 3×5cm. These were gripped at each end by a jaw and then the jaws were moved a part at the controlled speed until and young's modulus was automatically recorded according (ASTM). (2003) and (Hernandez, 2004).

6- Measurements of Water vapor permeability (WVP): The water vapor transmission rate [g/(s.m2)] and (WVP) through films was determined gravimetrically using the)ASTM Method E96-95(. A circular test cup was used to determine the WVP of the films at different treatments A0, B20%, C40% and D60%. A circular test cup was used to determine the WVP of the film. The film was first cut into a 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 desiccator containing calcium chloride. The weights of the cups were recorded every hour during 10 hours and two specimens of each film were tested. Linear regression was used to estimate the slope of this line in g/h. The (WVTR) and water vapor permeability was determined using the following equation:

$$WVPR = \frac{\Delta m}{\Delta tA} \quad WVP == \frac{WVTR.\ L}{/\Delta RH}$$

Where,Δm/Δt 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.

7- Measurement of gas Permeability: Gas (O2 and CO2) permeability at 30°C was measured in a designed stainless cell using a gas testing instrument, model Witt Oxybaby headspace gas analyser (O2/CO2) following the method described by García et al. (2000). The gas permeability (P) was calculated according to the following equation:

$$P = \frac{Q.X}{A.t.\Delta p}$$

Where, P is the permeability of gas, (m3/m. Day. Mm Hg), Q is the quantity of the gas diffused m3, X is the thickness of the film, A area of the film, m2, t is the time, day and Δp is the pressure difference across the films at different treatments A0, B20%, C40% and D60%

Physico-chemical and microbiological properties

Weight loss, total soluble solids (TSS %) and acidity: were determined according to the methods of AOAC (2010).

Firmness: Texture was determined by a universal testing machine (cometech, B type, Taiwan) In Food Technology Research Institute, Giza, Egypt. Provided with software. An aluminum 25 mm diameter cylindrical probe was used in a "Texture Profile Analysis" (TPA) double compression test to penetrate to 50% depth, at 1 mm/s speed test. Firmness (N), (Bourne, 2003).

Color measurement: Internal color measurements L and values of guava fruits were measured by using Minolta Chroma Meter, Model CR – 200. Calibration was done by a white plate before use. Color changes were quantified for L value which refers to the lightness, and a value which refers to yellow tonality (Barbagallo et al. 2012).

Microbial analysis: Total microbiological count was determined according to Marshall, (1992) all the microbiological counts were carried out in duplicates.

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

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

Results and Discussion

Rheological properties of pullulan natural film and coatings

Rheological properties (viscosity, shear stress) of samples were measured at different treatment's A0%, B20%, C40% and D60% and different shear rates (9.30-70, 93 1/s). Figure, (1-2) and table (2) Shows the relation between shearrate and apparent viscosity for different samples .The results indicated that as shear rate increased, apparent viscosity decreased so all samples behaves as pseudoplastic behavior on the other hand K (consistency index) for treatment B20%, C40% and D60% increased with increasing concentration of pullulan compared with A(0%) which had consistency index (0.1958). The flow behavior index (n) increased with increasing concentration of pullulan from 20 to 60% compared with samples A(0%) which had blow behavior (0.637). this may be due to consistency affected by changes in concentrate and interaction effect was performed with the average value of (k) and (n) for each treatment level and absolute correlated one of the parameters should be eliminated by expressing it as a function of another creating a one parameter model as reported by Hagenimana et al. (2007) . The results show that the forming solution exhibits trend of non-Newtonian pseudoplastic behavior at different treatment A0, B20%, C40% and D60% and fits the power low equation $\tau = k\gamma^n$ → (1) Where : τ: shear stress, Pa γ: shear rate 1/sec, k: consistency index, n: flow behavior index. The results observed that apparent viscosity decreased as shear rates increased at different temperatures A0, B20%, C40% and D60% and figure (1,2). The results observed that sample A0 exhibited non-Newtonian pseudoplastic behavior as previously discussed by (Suisui et al., 2016) who found that the apparent viscosity increased with increasing concentrations of pullulan natural film. The dispersibility of the pullulan natural film in aqueous solutionis reflected by the rheological properties,which are important for their application both when they are used alone and when they are part of a mixture. The viscosity values pullulan natural film suspensions containing different concentrations the aqueous suspensions of pullulan natural film exhibited shear-thinning behavior, indicating that they were non-Newtonian fluids. All samples A0, B20%, C40% and D60% exhibited the same trend, as previously discussed by Wittmar et al (2012) who stated that was strongly affected by ionic liquids. While, Shows a representative flow curve shear stress increased with increasing shear rate at different temperatures 25 and 70°C, and the extend of downward bending curvature decreased as the concentration of blend suspentions was decreased. Apparent viscosity decreased with increase of k at each level of concentration (Hagenimana et al .2007), (Ding et al. 2005).

Treat-ments	Viscosity			Shear Stress		
	k	N	R2	k	N	R2
A0%	0.1958	0.637	0.928	0.1953	0.637	0.9609
B20%	0.01937	0.473	0.885	0.01937	0.473	0.924
C40%	0.0289	0.637	0.900	0.0289	0.637	0.9747
D60%	0.343	0.698	0.911	0.0343	0.698	0.8594

Table 2: *Relation between consistency index (k) and flow behavior index(n) at different treatment's A0, B20%, C40% and D60% properties pullulan edible natural film and coatings:*

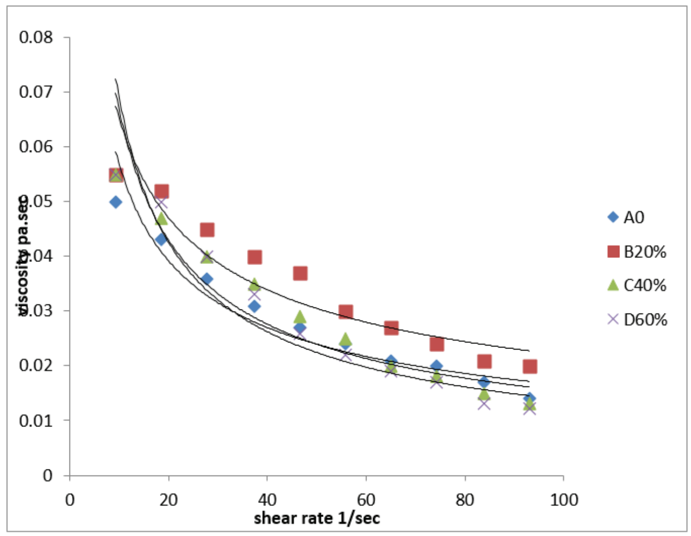

Figure 1: *Shear rate and Shear Stress of pullulan edible natural film and coatings.*

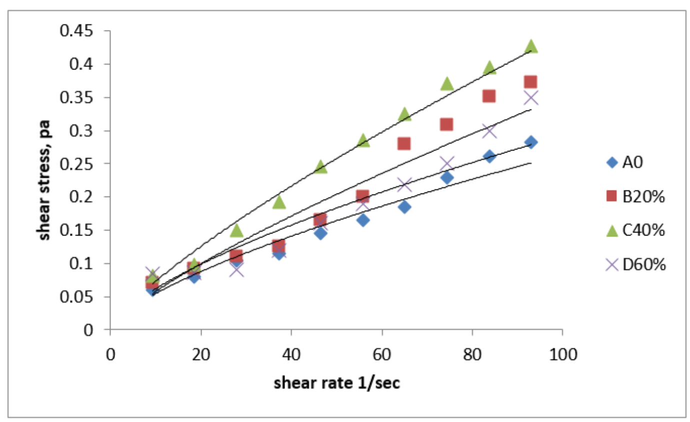

Figure 2: *Shear rate and Shear Stress of pullulan edible natural film and coatings.*

Physical and mechanical properties of prepared pullulan to produce edible natural films

The obtained results are recorded in table (3), it can be noticed that the A0% treatment, had the highest thickness value, 109 um in films prepared pullulan to produce edible natural films. The thickness values of edible natural film B20% 55.52 um treatment was lower than thickness values C40% 60.25 and D60% 68.32 um. From the results; it can be observed that the lowest value of tensile strength (26.22 and 35.26 N.M.M^2), elongation (23.21 and 25.14 %), Oxygen (25.42 and 30.44 M^3.M/M2 X$^{10-7}$), CO2 (35.80 and 41.52 M^3.M/M^2 X$^{10-8}$), water vapors permeability 6.29 and 7.42 [g/m2.24hr] and solubility (39.10 and 40.18%) was recorded for the treatment B20% and C40%. Also, A0 and D60% treatment showed higher tensile strength (48.45 and 45.20 N.M.M^2), elongation (35.21 and 42.65 %), Oxygen (45.23 and 66.25 M^3.M/M2 X$^{10-7}$), CO2 (56.25 and 79.35 M^3.M/M^2 X$^{10-8}$), Water vapors permeability [8.78 and 9.77 [g/m2.24hr] and solubility (45.20 and 49.45 %). This finding attributed to the interact as strongly with composite films or gelatin in the biopolymer film structure compared with water solubility of sago starch films , It was found that the solubility rate was of 17.40 23.12% while, Water vapor permeability 0.47- 1.37 g/m^2. 24hr According to Alebooyeh et al., (2012). Patricia et al., (2010) High film elongation is always a desirable characteristic if the film is to be used for food applications Chen, (1995). Similar results were reported by Franciele et al., (2013). All main factors significantly affected the mechanical of the film as follows: a) the chitosan film had an 18% higher elongation than the chitosan film) and In addition to incorporating the with the thymol, this reduced the chitosan film, Nelson et al. (2016). (ASTM). (2003).

Treatments	Thickness Um	Tensile strength (N/M^2)	Elongation (%)	Oxygen (O2) Permeability M^3.M/M^2×10^{-7} day.mmHg	CO2 Permeability M^3.M/M^2 ×10^{-8} day.mmHg	Water vapors Permeability [g/m^2.24hr]	"% Solubility" loss in weight after dipping in water and drying
A0%	109	48.45	35.21	45.23	56.25	8.78	45.20
B20%	55.62	26.22	23.21	25.42	35.80	6.29	39.10
C40%	60.25	35.26	25.14	30.44	41.52	7.42	40.18
D60%	68.32	45.20	42.65	66.25	79.35	9.77	49.45

A0 = Edible films + glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan
C40% = Edible films + 40% pullulan D60% = Edible films + 60 pullulan

Table 3: *The thickness, mechanical properties and permeability of prepared pullulan edible natural films.*

Microstructure of the produced pullulan edible natural films using scanning electron microscopy (SEM) technique

The microscopic images of four edible natural films from pullulan are presented in figure (A0, B20%, C40% and D60%). The edible natural films give films characterised with smooth surface and rought bottom averge size from 10 to 40 um polygonal crystals spherical morphology. Also contains films has homogeneous structure with some micro granules embedded in a continuous matrix. However, the addition of pullulan edible natural films produced films the characterised with smooth surface figure (3). Also, addition of antioxidant gave the thickness of produced films increase the degree of creamy color. The best pullulan edible natural films treatment image characterization was B average droplet size range (10um) polygonal crystals morphology followed by C average droplet size range (20 um) spherical followed by D average droplet size range (40um) ellipsoidal polygonal and spherical morphology. as compared to A treatment hexagonal. Generally, the scanning microscopy study may be useful for recognizing the microstructure and morphology of the produced films which can be help in choosing the proper film formuls for coating and packaging. Purposes .Also, the color appearance of produced films may be of important because it could affect consumer acceptant of coated items. Nelson caro et al. (2016).

Applications of choosen proper nanomaterials of edible films to guava fruit.

Physico-chemical and microbiological of coated guava fruit during storage period

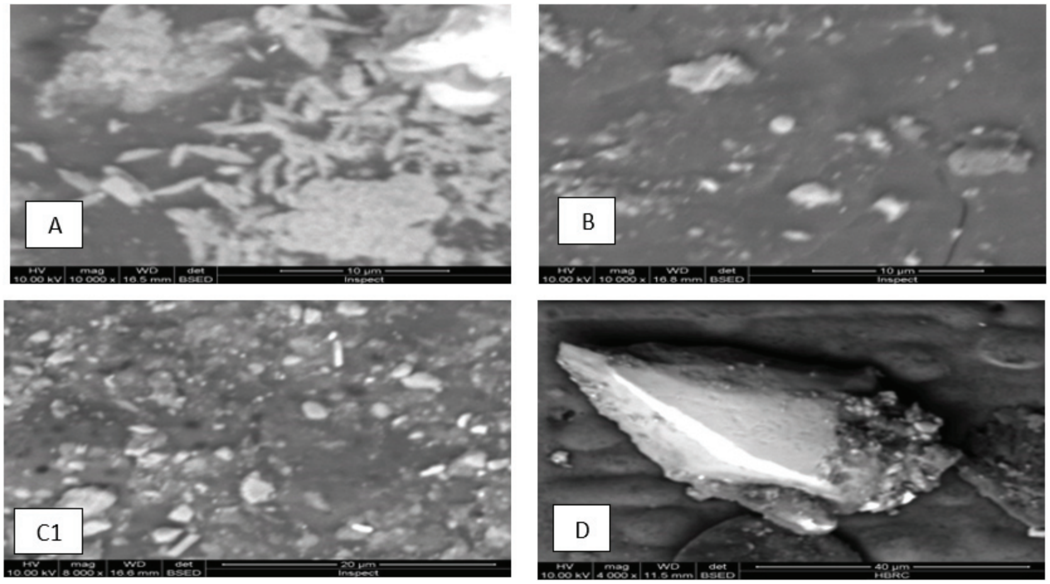

Figure 3: *A0% = Edible films+glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan C40% = Edible films + 40% pullulan D60% = Edible films + 60 pullulan*

Weight loss percentage

The results obtained are presented in table (4), from this table; it could be observed that the weight loss was increased with increasing the storage period at cooled temperature for all samples (A0%, B20%, C40% and D60%) and also found that the increase in the percentage of loss was in the A0%. It was also found that the treatments (B20%, C40% and D60%) were low in the percentage of weight loss compared to other treatments A0. As noted that the values of the losses are increasing gradually treatments (B, C, and D) respectively. The lowest of them was the reduction in weight loss treated B20%. Loss of weight in fresh fruit and vegetable is mainly due to the loss of water caused by transpiration and respiration processes. Most likely edible coating and film caused reduction of moisture condensation on the fruit surface (Zhu et al., 2008). Normally, the weight loss occurs during the fruit storage due to its respiratory process, the transference of humidity, and some processes of oxidation (Ayranci and Tunc, 2003).

Total soluble solids (TSS)

The results obtained with guava coated with coating materials used are presented in table (5), from this table; it could be observed that the gradual increase in total soluble solids (TSS) during the complete storage period at cooled temperatures. The TSS was reduction in control as compared to other treatments. As noted the values of the increasing gradually treatments (A0%, B20%, C40% and D60%) respectively. The results of natural metabolic processes occurred in fruits during storage as a results of moisture losses from the fruit and/or hydrolysis of starch to from (ripening) which lead 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 fruit continued to loss their resistance to puncture, TSS continued increase Saleh et al., (2005), the total soluble solids significantly ($p<0.05$) increased with storage time in all treatment with the exception of fruit covered with the bilayer film, which had no significant change with time. The foam tray wrapped with PVC film showed a small increase in total soluble solids (Wand et al, 2006) 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 stored moisture of the stored products decreases during the storage period and consequently soluble solid amount of the stored product increases Selcuk Arin and Serap Akdemir., (2004).

Total acidity

The changes in total acidity of guava were determined during storage period at cooled temperatures. The obtained results are recorded in table (6). The results indicated that the total acidity gradually decreased with increasing of storage period at cooled temperature. The decrease of acidity during storage demonstrated fruit senescence. The same authers outlined that coatings may be slowed the changes in titratable acidity and effectively delaying fruit senescence. This was probably because the semi-permeablity 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. (Djedidi et al, 2003) found that the reduce of organic acid concentration with decreasing acidity during period of storage demonstrated fruit ripening and senescence. This may be caused by the respiration activites in tomatoes. (Artes et al, 2000) found that the pH value of peppers increased with increasing of storage period to 12 week at 5ºC. While, the titratable acidity tend to decrease during storage in all treatment. As it is known, when fruits stored moisture of the stored products decreases during the storage period and consequently total acidity amount of the stored product increases Selcuk Arin and Serap Akdemir., (2004).

Treatments	A0%	B20%	C40%	D60%
Zero time	0	0	0	0
5	1.46	1.12	1.25	1.39
10	2.65	1.56	2.10	2.50
15	4.28	2.20	2.25	3.44
20	5.62	3.73	4.23	4.53
25	-	4.83	5.50	5.65

A0% = Edible films + glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan C40% = Edible films + 40% pullulan D60% = Edible films + 60 pullulan (-) a spoiled reject samples

Table 4: *Effect pullulan edible natural film and coatings on weight loss (%) of guava fruit during storage.*

Treatments	A0%	B20%	C40%	D60%
Zero time	8.50	8.3	8.2	8.0
5	8.70	8.90	8.65	8.21
10	9.45	9.85	8.26	8.10
15	10.28	10.50	9.85	9.60
20	10.45	11.95	10.66	10.45
25	-	12.10	10.95	10.68

A0 % = Edible films + glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan C40% = Edible films + 40% pullulan D60% = Edible films + 60 pullulan (-) a spoiled reject samples

Table 5: *Effect of pullulan edible natural film and coatings on total soluble solids (TSS) of guava fruit during storage.*

Treatments	A0%	B20%	C40%	D60%
Zero time	0.48	0.48	0.48	0.48
5	0.46	0.45	0.44	0.43
10	0.43	0.42	0.41	0.40
15	0.39	0.38	0.36	0.35
20	0.32	0.31	0.30	0.29
25	-	0.29	0.26	0.25

A0 % = Edible films + glycerin + Tripolyphosphate (TPP) B20% =Edible films + 20% pullulan C40% = Edible films + 40%pullulan D60% = Edible films+60pullulan (-) a spoiled reject samples

Table 6: *Effect of pullulan edible natural film and coatings on total acidity (%) of guava fruit during storage.*

Color changes

The color changes was measured recording lightness (L* value), chroma (intensity of color) and hue angle (hº). Lightness of the guava was affected by storage time (table, 7) the results indicated that the guava gradually decreased with increasing of storage period at cooled temperatures .The lightness (L*) gradually decreased during storage in both coated A0% and treatments (B20%, C40% and D60%) coated guava. The highest decrease in lightness was observed in A0% coated guava.Changes in the hue-angle (ho) value of coated fruit with storage time were slight and only became significant at the end of the storage period .Chroma was reduced by around 30% for control and 10% for coated fruit . Changes in the hue-angle (ho) value of coated fruit with storage time were slight and only became significant at the end of the storage period .Chroma was reduced by around 30% for control and 10% for coated fruit. It has been observed that uncoated fruits are significantly darker than coated fruit throughout the storage period. The chitosan concentration of the coating solution gave rise to significant differences in fruit colour by the end of the storage period, surface colour of fruits (Rayees Ahmad Shiekh et al., 2012). The packaging and storage was good enough to product the grapes and the color changing of the bunches were acceptable Selcuk Arin and Serap Akdemir., (2004). These results were agreements with those obtained by Colla et al (2006), they found that guava fruits treated with edible coating delayed fruits senescence in which the external and internal color was lighter than that of uncoated fruits. Thus, the senescence delay, evidenced by the decrease in color changes, demonstrates the effectiveness of this coating.

Treatments	A0%	B20%	C40%	D60%
Zero time	20.46	20.60	20.41	20.35
5	20.50	21.58	20.18	20.10
10	18.11	19.55	18.20	17.18
15	17.25	18.88	17.52	16.12
20	15.25	17.20	16.25	15.76
25	-	16.86	15.16	15.50

A0 %= Edible films + glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan C40% = Edible films + 40% pullulan D60% = Edible films+60pullulan (-) a spoiled reject samples

Table 7: *Effect of pullulan edible natural film and coatings on color [hue angle (h)] of guava fruit during storage.*

Treatments	A0%	B20%	C40%	D60%
Zero time	51.20	51.65	51.45	51.35
5	46.35	48.85	47.20	46.81
10	39.55	42.14	41.86	40.20
15	32.80	34.10	33.25	32.24
20	25.17	26.85	24.26	23.18
25	-	18.40	16.20	14.55

A0 % = Edible films + glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan C40% = Edible films + 40% pullulan D60% = Edible films + 60 pullulan (-) a spoiled reject samples

Table 8: *Effect of pullulan edible natural film and coatings on Firmness (N) of guava fruit during storage.*

Firmness

The obtained results are recorded in table (8). Show the effect of using pullulan edible natural film and coatings on firmness of guava during refrigerated storage. The results in table (8) indicate that the guava fruit decreased with increased period of storage days for both treated and control. At the end of storage, control fruit clearly showed the lowest firmness. Guava treated with CaCl2 carried by soy protein or gluten film significantly delayed the loss of fruit firmness compared to control. The favorable effect of CaCl2 treatment in reduction of firmness loss of guava during storage may be due to the stabilization of membrane systems and formation of Ca-pectats, which increase the rigidity of the middle lamella and cell wall to increase resistance for polygalacturonase activity Poovaiah., (1986). It could be responsible for delaying ripening which resulted in the reduction of firmness loss during storage.(Patricia et al., 2005) found that increasing respiration activates increased water loss and most likely decreased potential texture depression. Changes in the hue-angle (ho) value of coated fruit with storage time were slight and only became significant at the end of the storage period .Chroma was reduced by around 30% for control and 10% for coated fruit. It has been observed that uncoated fruits are significantly darker than coated fruit throughout the storage period. The chitosan concentration of the coating solution gave rise to significant differences in fruit colour by the end of the storage period, surface colour of fruits (Rayees Ahmad Shiekh et al., 2012). The packaging and storage was good enough to product the grapes and the color changing of the bunches were acceptable Selcuk Arin and Serap Akdemir., (2004).

Total count

The use of pullulan edible natural film and coatings on which have antimicrobial activity extend the shelf-life of guava. The results are shown in table (9) shows the changes in total bacterial counts of guava pullulan edible natural film and coatings during storage periods. The data indicates that total counts gradually increased with increasing the cold storage period of guava at different treatments (A , B , C and D) of pullulan edible natural film and coatings . The results in table (9) showed that the microbial load increases with the increase of storage in all storage treatments and the increase in the Ao% higher than other treatments. Storage lasted only 20 days compared to treatments 25 days of storage. It was also found that the treatments (B, C and D %) are lower in microbial load than treatments Ao%. The counts reached to 6. 35– 7.10 × 10^1 CFU/g after 25 days of storage for in both coated and uncoated samples, as compared with the initial counts 1.80 × 10^1 CFU/g .Chitosan edible coatings extend the shelf life of the fruits and vegetables by minimizing the rate of respiration and reducing the water loss. Chitosan coating offers a defensive barrier against bacterial contamination and loss of moisture from the surface of food products, thus extending their shelf life. With limited increase in the concentration of chitosan coating, the beneficial effect of chitosan on postharvest life and quality of the food is enhanced. (Rayees Ahmad Shiekh et al., 2012). The addition of nanoparticales material as antimicrobial agent to coating emulsion improved the fruit microbial quality and decreased microbial counts (Lee, et al 2004) found that the coating treatment of fruit and vegetable allowed a limited gases exchange and respiration, moreover, prevent the occurrence of fermentation process and minimized the microbial count. Also, they added that and respiration, moreover, prevent the occurrence of fermentation process and minimized the microbial count. Also, they added that high microbial counts, appeared after three weeks in control samples stored at room temperature.

Treatments	A0%	B20%	C40%	D60%
Zero time	1.80	1.65	1.74	1.78
5	2.65	2.40	2.55	2.60
10	3.86	2.95	3.12	3.40
15	5.60	4.26	4.95	5.10
20	6.98	5.25	5.80	6.55
25	-	6.35	6.95	7.10

A0 % = Edible films + glycerin + Tripolyphosphate (TPP) B20% = Edible films + 20% pullulan C40% = Edible films + 40% pullulan D60% = Edible films + 60 pullulan (-) a spoiled reject samples

Table 9: *Effect of pullulan edible natural film and coatings on total count of guava fruit during storage.*

Moulds and yeast

The changes in moulds and yeast counts of fresh guava were determined during cold storage. The obtained results are shown in table (10), The results indicated that the moulds and yeast counts gradually increased with increasing the storage period at cold temperature in both samples packaged in pullulan edible natural film and coatings. The samples Ao% indicates higher mould and yeast counts than coated ones. The moulds and yeast counts reached to 2.35–2.66 × 10^1 CFU/g after 25 days of storage period in samples, as compared with the initial counts 0.0 × 10^1 CFU/g. This is may be due to the increasing of RH in refrigerating champer and suitability of the refrigerator temperature for yeast growth according to (Koide and Shi, 2006). This is may be due to improvement of postharvest life, in combination with temperature and relative humiditly management. In addition, bio-films and coatings, by themselves are acting as carriers of foods additives (i.e.: antioxidants, antimicrobials), and have been particularly considered in food preservation due to their ability to extend the shelf life (Sallam, 2007).

Conclusion

This investigation was carried out as trial to use pullulan in the preparation of pullulan natural edible film and coatings for guava during storage at 8 ± 1°C for 25 days .The prepared coating was evaluated through determination some physical ,chemical, microbial properties. Also, the stored coated guava were evaluated. The results indicated that a film with concentration of antimicrobials action of extract Luria leaves / acetic acid and (TPP) to pullulan natural edible film have the potential to provide a safe edible films decreased microbial growth and consequently prolonged the shelf life of guava fruit, As well as improved the physiochemical changes properties of the guava fruit. The substances used in this experiment were, film with glycerin A(0%), film with pullulan B(20%) , film with pullulan C (40%) and film with pullulan D (60%) on quality attributes and prolong shelf- life of guava fruit up to 25 days of storage. Production of pullulan natural edible film and coatings from polysaccharides extracted from polysaccharides in order to preserve the film from oxidation and microbial contamination maintain the stability of the film and extend the shelf life of guava, as this substance inhibits microbial growth and oxidation and improves the quality of the film acceptable sensory characteristics appropriate barrier properties (CO2, O2 and water)Microbial, biochemical and physicochemical stability Public health safety Effective carrier for antioxidant, flavor, color, nutritional or anti-microbial additives it could be indicate that these findings may be of application benefit in the field of food industry Simple technology for production and Low cost.

Treatments	A0%	B20%	C40%	D60%
Zero time	0	0	0	0
5	0.50	0.25	0.37	0.43
10	1.58	1.20	1.42	1.49
15	1.77	1.60	1.65	1.70
20	2.30	1.95	2.10	2.30
25	-	2.35	2.46	2.66

A0 % = Edible films + glycerin + Tripolyphosphate (TPP) B20% =Edible films + 20% pullulan C40% = Edible films + 40%pullulan D60% = Edible films + 60pullulan (-) a spoiled reject samples

Table 10: *Effect of pullulan edible natural film and coatings on moulds and yeast of guava fruit during storage.*

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