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Research Article

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Bacterial Nano-polymer Production to Produce Edible Coating and Films

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ABSTRACT

This study was performed to evaluate the separation of cellular biopolymer production for batch fermentation of bacterial Acetobacter spp and Leuconostoc spp to produce edible film-forming suspension solution at different treatments B20%, C40%, D60% and E20%, F40% and G60% treatments loaded on CMC and control A0%. preparation of nano-polymer production to produce an edible film with a higher potential to carry active ingredients such as silver $AgNo_3$, which was added with different concentrations of 0.25, 0.50, and 0.75% wt to the edible film. A study estimation of rheological properties of treatments and suspended solutions to determine the best ones for use in edible films and their application in food science were studied. The permeability, particle size, zeta potential, mechanical properties, and color were evaluated in addition to electron microscopy of the tested films. It was found that the best measurements were the film-forming nan emulsions solutions are decreased B20% 111.6 nm, E20% 262.0 nm then followed treatment C40% 571.0 nm and F40% 928.9 nm as compared to in edible films nano-polymer were A0% 1417nm. This bacterial biopolymer product produced in the production of nanoparticles is capable of forming films and coatings with valid fence properties versus the transport of gases such as color, oxygen, CO_2, and water vapor. The elongation, tensile strength, solubility, and thickness are remarkable mechanical properties as tensile strength and elongation increase, % solubility with treatment B20%, C40% and D60% than in treatment E20%, F40%, and G60.

Key words: *Biopolymer product bacterial, Nanomaterials, Edible coating, Film*

INTRODUCTION

A group of bacteria Acetobacter can oxidize sugars, sugar alcohols, and ethanol, producing acetic acid as the main end product. They generally contain bacterial exopolysaccharides. Acetobacter species are capable of synthesizing cellulose, having many uses in some fermented food products. This strain Gluconacetobacter xylinus appeared under the number I-2281 [1]. It was isolated from vinegar fermentation. It produces (GAX) soluble polystyrene and contains rhamnose, glucose, mannose, and glucuronic acid, its acetane-related structure is produced by some strains of Acetobacter xylinus Abdelhamid *et al*., (2020) [2]. It generally contains the bacterial Leuconostoc spp. in both glucosyltransferases (GTFs) and fructosyltransferases (FTFs) primarily produced by Leuconostoc species. Plant or animal origin, including polysaccharides and proteins, have been studied. Moreover lipids, their derivatives, and their compounds [3] and [4] because of their abundance, regenerative susceptibility, environmental friendliness, and good film properties [5]. CMC polysaccharides

derived from water-soluble cellulose, Youssef *et al*. (2019) are the most common polysaccharides plentiful and economical to manufacture biodegradable packaging films [1, 6, 7]. The solubility of CMC in water indicates that it contains CMC particles. Various additives such as nanoparticles, plant extracts, essential oils, etc. have been used to impart functionality of packaging materials to increase shelf life by ensuring the safety and preservation of packaged foods [8]. The work aims to produce a bacterial nanopolymer to produce edible membranes. Study of rheological, mechanical, permeability, color, and scanning electron microscopy properties.

MATERIALS AND METHODS

Materials

Lavender oil (Lavendula - Officinalis) was obtained from Ginapharm, Germany in precision cultivation in the central laboratory of the Department of Food and Packaging Engineering. Chemicals such as ethyl alcohol with a concentration of 95% and CMC products for a British chemical company were used in these experiments. Glycerol and sodium hydroxide were purchased from Al-Jumhuriya Chemical Company. MRS was acquired by Acmatic Chemicals and Laboratories. Silver Ag No3 was obtained from New Jersey USA, Na2HPO4 from Win Lab, (UK). Glucose, Citric Acid Elephant Bio-Engineering Co., LTD, China.

Producers strain by using bath type batch fermentation of bacterial Acetobacter spp and Leuconostoc spp and then two treatments they were divided into two groups as follows:

The first bacterial Acetobacter spp: Collecting batch fermentation kinetics of exogenous sugars (EPS) and Gluconacetobacter xylinus (GAX) from Acetobacter spp using low-cost substrates. The name "(EPS) and (GAX)" was the first to describe the formation of extracellular polysaccharides by Acetobacter spp. Taken from the Microbiological Resource Center (Cario MIRCEN) for culturing bacteria and obtaining “EPS and GAX”, the liquid base was used for standardizing the culture medium containing the following composition (g/l): it contained the following components: 20 g/glucose, 5 g/l: L yeast extract, 5 g/L peptone, 2.7 g/L Na2HPO4, 1.15 g/L citric acid, then incubated at 30°C for 4 days, then kept at 4°C after that [9]. They generally contain Acetobacter spp genera in both (EPS) and (GAX).

The second Leuconostoc spp: the group was started with the other by using bath type batch fermentation bacterial Leuconostoc spp genera produce ether (GTFs) and (FTFs). It was taken from the Microbiological Resources center (Cario MIRCEN) to cultivate the bacterial and get " GTFs and FTFs ", the liquid base of the standard culture medium containing the following composition (g/L) was used: MRS, it was incubated at 30 °C for 4 days and preserved at 4 °C thereafter [10]. They generally contain bacterial Leuconostoc spp genera produced in both (GTFs) and (FTFs). Then sterilized for 15 minutes at 121°C cooled and then adjusted to pH 6.8 the filled culture was transferred to a 250ml beaker containing a 50ml culture, then the beaker was incubated at 30°C for 48 hours in an incubation rotary shaker (200 cycles per minute) (5% volume/volume) of these cultures were used for inoculation and production for all fermentation studies. 1 ml of cultures were then transferred to a fresh medium and cultured under the same conditions described above for 96 h [11]. The biopolymer product bacteria (11,000 rpm, 20 min at 4 °C) was centrifuged to separate the cellular biopolymer product bacteria from the supernatant liquid. “EPS and GAX” or “GTFs and FTFs” were precipitated from the supernatant using 96% ethanol and centrifuged at 11,000 rpm for 20 minutes [12].

Methods

Preparation and characterization of silver nanoparticles

Preparation of Silver nanoparticles by extracting 1mL/L of lavender oil, adding to prepared 45 mL (0.002 M AgNO3), and placing in 100 mL beakers at room temperature in dark glass for some period after one hour the formation of silver particles according to [13] and Abdel Khafar (2020) [14].

Materials used for edible coatings and films were divided into seven groups, A0= Edible films glycerol B20% =Edible films+20% (Nano biopolymer product acetobacter sp) C40%=Edible films+40% (Nano biopolymer product acetobacter sp) D60%=Edible films (Nano biopolymer product acetobacter sp) E20%=Edible films+20% (Nano biopolymer product Leuconostoc sp) F40%=Edible films+40% (Nano-biopolymer product Leuconostoc sp) G60%=Edible films+(Nano biopolymer product Leuconostoc sp). The composition of the solution mixture was modified to the film-forming solution described above by adding 1 ml/L of lavender oil. Then, it was added to the solution and left in the mixture for 30 minutes. Simultaneously, the required quantity

of action silver Ag No3 in a ratio of 0.25, 0.50, and 0.75%. Synthetic nanoparticles loaded on (CMC) films and about 100 ml of deionized distilled water were added using an ultrasonic bath for 30 min. To completely dissolve the CMC, add a suspension of Ag No3 to it and mix in a homogenizer of unification. The film's seven biopolymer product bacterial edible coating and films were prepared from CMC by suspension solution consisting of 4% w/v in 10mL alcohol 95% plus 100 mL of deionized distilled water with the incorporation to contain 1.2% v/v glycerol and Solution adjusted pH to 7 with mixtures that were manually homogenized, Then the solution was heated in a magnetic stirrer at 75 ± 2 °C for 30 min and then filter the edible film solution and pour it on Teflon plates, then separate dried films from the plates according to Priyadarshi *et al*. (2021) [15].

Physical and mechanical and rheological properties of prepared separate cellular biopolymer product bacterial Acetobacter spp and Leuconostoc spp nano-polymer production to produce edible coating and film solution.

1. *Rheological measurements:* The parameters (shear rate and shear stress) were measured using a Brookfield Engineering lab. The spindle sc4-18
2. *Determination of nanoparticles Zeta size:* Device Type Malvern Made in UK and Model: Zeta sizer nano series (Nano ZS). Size range (nm): 0.6: 6000 nm Size and zeta range (mV): (-200: 200mV).
3. Microscopy electron scanning: Measurement of edible film nano biopolymer product bacterial using microscopy electron scanning electron using a High Precision Scanner Electron Microscope INSPECT S 150A SPUTTER COATER SEM Schematic Overview – Quanta FEG250 with field emission gun, FEl company Netherlands Machine type inspect S.
4. *Film thickness:* Measured with a digital Micrometer (Mitutoyo type Digital Indicators The company's models: pk-1012 E, Japan) Aboul anean (2021) [16].
5. *Color:* Inner color measurements L and edible biopolymer membrane values were measured with a Colorimeter Minolta chromameter Cyril number CR-200 Swarup Roy and Jong-Whan Rhim (2021) and color measurement using a scale Portable colors based on the L*a*b*color scheme [17]. The color difference can be calculated as follows:

$$\Delta E^* = \sqrt{(L^* - L^*_s)^2 + (a^* - a^*_s)^2 + (b^* - b^*_s)^2} \quad (1)$$

Where: L*, a*, and b* represent the values of the film samples, L*s, a*s, and b*s for the standard whiteboard L*s = 54.3, a*s = -0.5 and b*s = 44.2 values represent as background and take three readings for each film by Maria-Ioana Socaciu *et al*. (2020) and $\Delta E = \sqrt{(L)^2 + (a)^2 + (b)^2}$ where ΔL, a and b show the difference between each color value of the film sample [18]. According to Munir *et al*. (2019) [19].

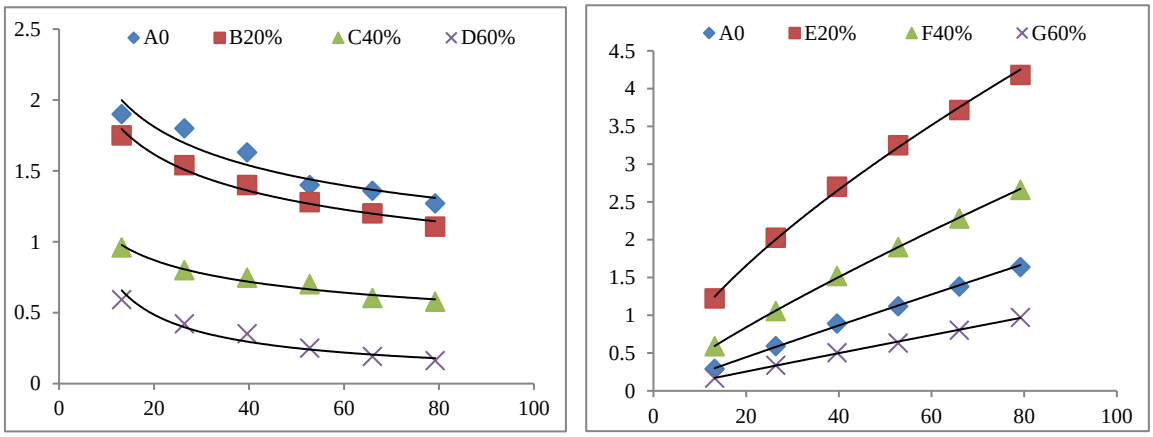
6. *Light transmittance:* Measurement of the barrier and transparency of the films by measuring the light transmittance ratio at the wavelength (T280) and 660 nm, respectively Priyadarshi *et al*., (2021) [15]. Then the value of the absorbance is converted to the transmittance ratio. Maria-Ioana Socaciu *et al.* (2020) [18].
7. *% Solubility in water:* The edible film samples were first dried in a desiccator containing calcium chloride and then the dry sample of 500 mg nano-polymer film was immersed in beakers containing 50 mL of distilled water at room temperature over 24 h with gentle spin rocker incubation. % weight loss = initial dry weight-final dry weight x100/initial dry weight according to [20].
8. *Measuring mechanical properties of biopolymer product edible films.*
   Measurement of tensile properties (tensile strength, elongation) using a type CT3 texture analyzer. The nano edible film at different treatments. It was cut into 2 x 6 cm strips.
9. *Measuring water vapor permeability (WVP).* The water vapor transfer rate (WVTR) and water vapor permeability were determined using the following: $WVPR = \frac{\Delta m}{\Delta t A} WVP = WVPR. L/\Delta RH$ Where, Δm/t is the moisture gain weight each time (g/s), A is the film surface area m2, L is the film thickness (mm) and ΔRH is the difference in relative humidity. *(ASTM E96–95).*
10. *Gas permeability measurement:* The gas permeability (O_2 and CO_2) was measured at 30°C in a designed stainless cell using the Witt Oxybaby Headspace Gas Analyzer (O_2/CO_2) Gas Tester Model, following the method described by [16]. The gas permeability P was calculated according to the following equation: P = QX/AtΔp where P is the gas permeability (m^3/m. day. mmHg) Q is the amount of diffused gas m^3 X is the film thickness and the film area m^2 t is the time, day and p is the pressure difference across the nanoparticles at treatments.

RESULTS AND DISCUSSION

Rheological properties of prepared separate cellular biopolymer product bacterial Acetobacter spp and Leuconostoc spp nano-polymer production to produce edible coating and film solution.

The prepared study of rheological properties such as shear rate, shear stress, and viscosity of the samples was measured by preparing a nano-cellular biopolymer product separated from the bacteria to produce an edible film with different treatments A0% (B20%, C40%, and D60%) and (E20%), F40% and G60%) and different shear rates (13.2, 26.4, 39.6, 52.8, 66.00, 79.2 1/sec). **Figure 1** Explains the relationship between shear rate, apparent viscosity, and shear stress for different samples. The results indicated that as the shear rate increased, the apparent viscosity decreased so that all samples behave in a pseudoplastic behavior. On the other hand, K decreased with increasing concentration of biopolymer production for batch fermentation of acetobacter sp (B20%, C40%, D60%). In the same manner (E20%, F40%, and G60%) biopolymer production for quantitative fermentation of Leuconostoc sp bacteria produces an edible membrane-forming suspension solution, and higher than the control sample A (0%) which has a consistency index (3.676). Flow behavior index (n) increased with increasing concentration of biopolymer production for batch fermentation of Acetobacter sp (B20%, C40%, D60%) to produce edible membrane-forming suspension solution and did not give a trend for batch fermentation of Leuconostoc sp bacteria and higher than control with Samples A (0%)

that has a blow behavior (0.847). according to Abu Al-Anyan (2021) [16]. The reaction stops because the particles tend to vibrate at lower and higher temperatures and also break down larger molecules into smaller ones. The graphical representation showed that the solution exhibited typical non-Newtonian properties of the behavior of the pseudoplastic fluid. Also, the viscosity of each solution showed a high value when the shear rate decreases linearly with increasing shear stress.



a) b)

Figure 1. Shear rate and viscosity of nano biopolymer production to produce edible film-forming suspension solution of different treatments.

Physical and mechanical properties of prepared separate cellular nano biopolymer production for batch fermentation of bacterial to produce edible film

It can be seen that the treatment in the prepared membranes is separate from the bacterial nano-cellular biopolymer to produce an edible film from this **Table 1** where the results indicated that the thickness values at different treatments (B20%, C40%, and D60%) and (E20%, F40%, G60%) gradually increases in thickness with increasing concentration. It was also observed that the concentration of separate cellular nano-biopolymer samples from acetobacter sp. B20% 62 um, C40% 65 um and D60% 67 um was less thick than the concentration of samples E20% 64 um, F40% 67 um and G60% 72 um. A nanopolymer produced from bacteria (Leuconostoc sp) in comparison with the A0% thickness value of 78 um of control where it was higher than the coefficients. It was found that it decreases with increasing concentration in both tensile strength and elongation in all different parameters. This result is a reaction with composite films or gelatin to build a strong biopolymer film compared to sago starch films and its solubility in water where it was found that the melting rate was 17.40-23.12% while the water vapor permeability was 0.47-1.37 g. / m 2. 24 h film thickness and CMC-based mechanical film preparation. The thickness of the elegant CMC membrane was 79.4 µm, which was slightly increased by the addition of ZnONPs. The TS value of film-added grape seed extract (GSE) was much higher

than that of ZnONPs, and when GSE was added to ZnONP, the tensile strength periodically increased [15]. The high film elongation has long been a desirable feature of film for use in food applications [14]. An illustration of similar findings by [18]. All major factors greatly influenced the mechanics of the film as the chitosan film below has an 18% higher elongation than the chitosan film, as well as the incorporation of thymol, which reduced the layer of chitosan, [17].

Table 1. Mechanical properties, permeability, and thickness of edible film

Treatments	Thickness Um	Tensile strength N/M^2	Elongation %	$M^3.M/M^2\times10^{-7}$day.mmHg O_2	$M^3.M/M^2\times10^{-8}$day.mmHg Co_2	Water vapors $g/m^2$24hr	solubility in water %
A0%	78	134.43	50.45	45	68	39	50
Biopolymer production from bacterial (acetobacter sp) to produce edible film-forming							
B20%	62	156.33	76.89	35.24	36.65	14.22	35.67
C40%	65	142.43	65.67	37.34	41.56	22.38	37.66
D60%	67	138.14	60.67	40.45	47.12	27.48	40.56
Biopolymer production from bacterial (Leuconostoc sp) to produce edible film-forming							
E20%	64	148.46	72.43	39.43	47.11	16.54	39.18
F40%	67	139.45	68.15	44.14	56.27	27.83	40.75
G60%	72	135.32	53.78	48.32	63.14	36.54	47.79

Determination of particle size distribution and Zeta potential produced edible film of prepared separate cellular nano biopolymer production for batch fermentation of bacterial on edible films the solution formed:

Particle size

The results obtained are shown in **Table 2** and **Figure 2**, when treatments (E20%, F40%, G60%) were higher than the concentration of treatments (B20%, C40%, D60%) in both urinary contrast index (PdI) and the hydrodynamic diameter of the particle size (nm), it was found that the peaks were (0.474, 0929, and 0.495) and (111.6, 571.0 and 1070) for (B20%, C40%, and D60%), respectively. While it was found that the peak was (20%, F40%, G60%) in the peak (0.329, 0.887 and 0.664) and (262.0, 928.9 and 1332) respectively, compared with the primary control samples A0% working volume. The distribution (nm) in both the polydispersity index (PdI) 0.202 and the hydrodynamic diameter of the partial size (nm) 1419. A decrease of about 200 nm in the particle size around the charges was also observed in the aqueous solution from the treatment, where the nanosize appears around 450 nm, which, as the particle size decreases, the zeta potential value decreases. Suspended particles with zeta potentials above +30 or less than -30 mV repel each other because they are considered stable but if the zeta potentials are between +30 to -30 mV they tend to attract each other [21].

Zeta potential

The results in **Table 2** and **Figure 3** showed that the zeta potential distribution and zeta deviation (mV) of the treatment were recorded at the peak (17.0, -38.5, 10.4) and (3.32, 4.04, and 3.70). (B20%, C40%, D60%) respectively, while it was found that the recorded number recorded the concentration coefficients of zeta voltage distribution and zeta deviation (mV), (E20%, F40%, G60%). The treatment was at peak (49.6, 50.5, and 50.1) and (4.48, 3.95, and 4.19), respectively, compared to the A0% primary control samples in both the 21.6 zeta voltage distribution and the 3.83 (mV) Zeta Deviation (mV) (mV). It is usually obtained by measurement and conversion from electrical kinetics of particles, tests, and materials. Zeta potentials with an absolute value greater than 30 mV indicate the "medium to good" stability of colloidal systems. The higher the zeta potential, the better the scattering stability [20].

Table 2. Measured particle size and zeta potential of nanotechnology prepared separate cellular nano biopolymer production for batch fermentation of bacterial on edible films solution formed from it

Treatments	particle size distribution(nm)		Zeta potential(mv)	
	Poly Dispersity index Pdi	Hydrodynamic Diameter nm	Z-Potential	Z- Deviation
A0%	0.202	1419	21.6	3.83
Biopolymer production from bacterial (acetobacter sp) to produce edible film-forming suspension solution				
B20%	0.474	111.6	17.0	3.32

C40%	0.929	571.0	-38.5	4.04
D60%	0.495	1070	10.4	3.70
Biopolymer production from bacterial (Leuconostoc sp) to produce edible film-forming suspension solution				
E20%	0.329	262.0	49.6	4.48
F40%	0.887	928.9	-50.5	3.95
G60%	0.664	1332	50.1	4.19

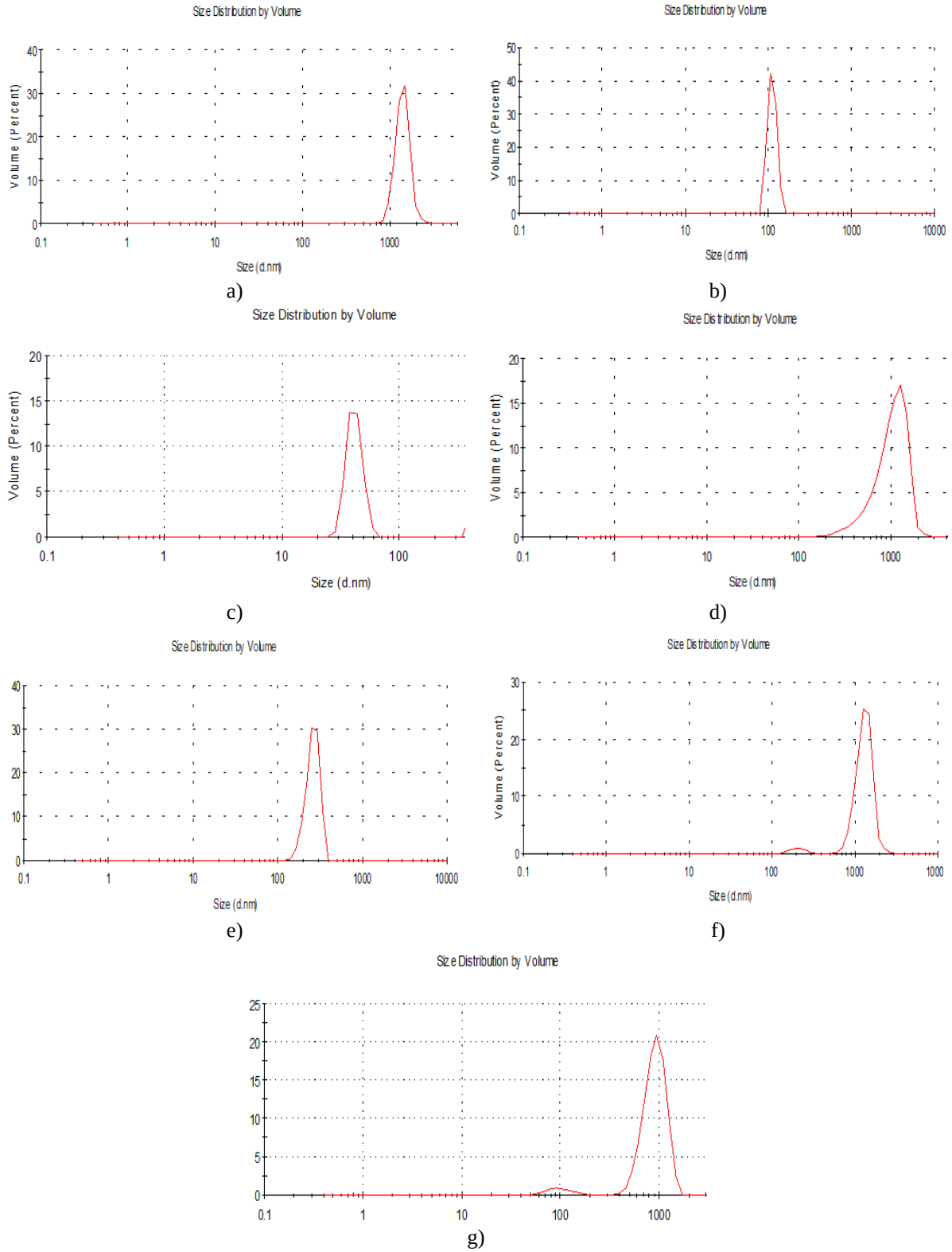


Figure 2. The particle size of biopolymer to produce edible film-forming suspension solution for different treatments.

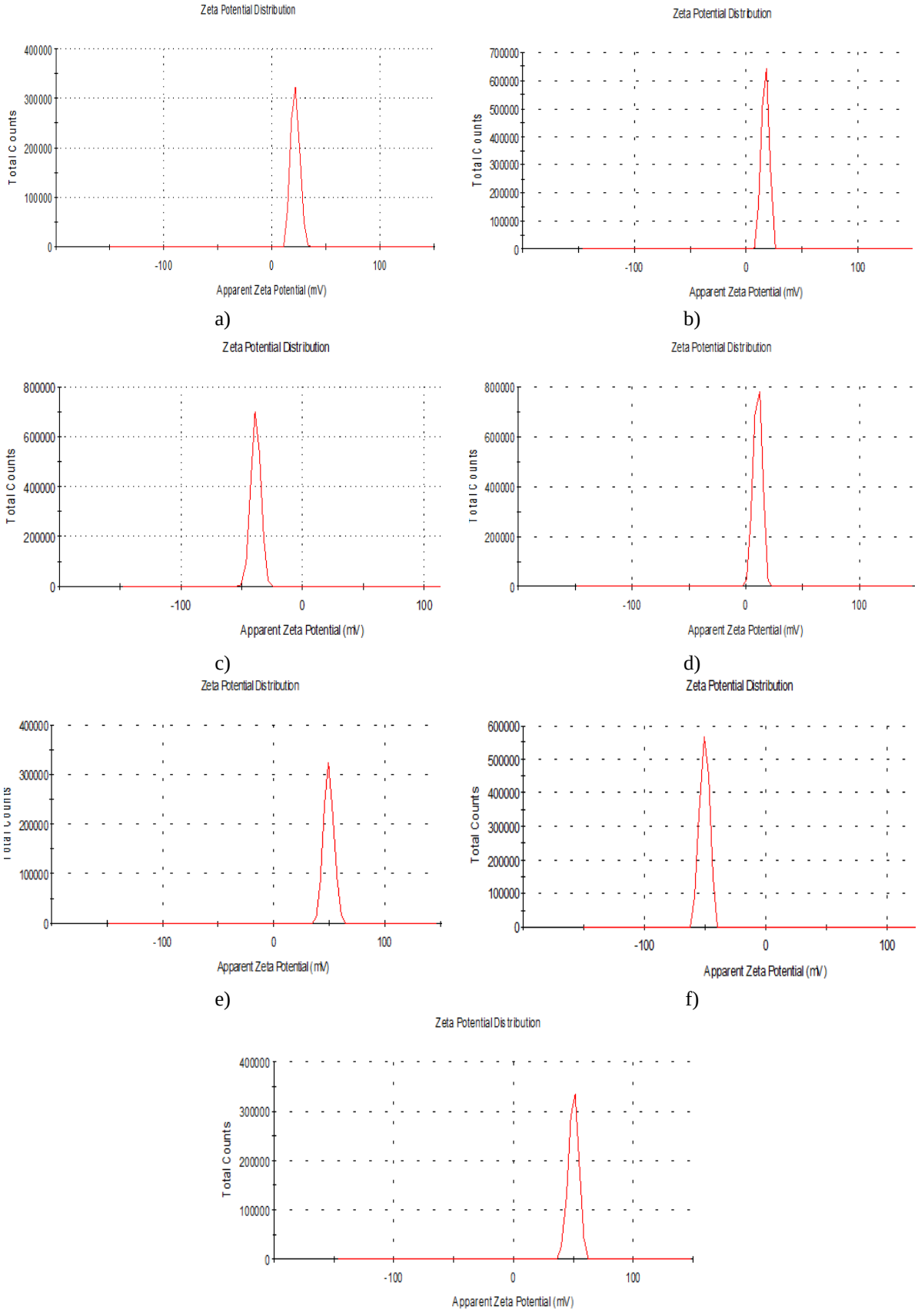
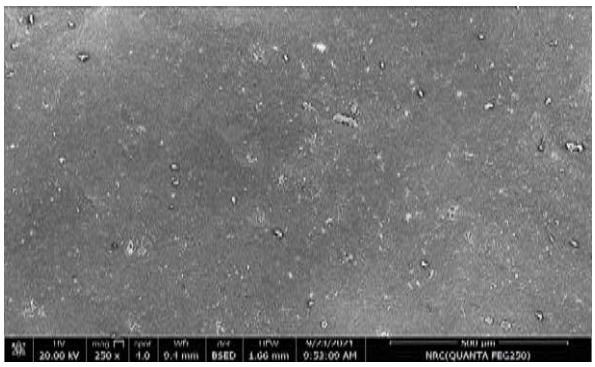


Figure 3. The Zeta potential of biopolymer to produce edible film-forming suspension solution for different treatments.

Microstructure of prepared nano biopolymer production for batch fermentation of bacterial to produce an edible film using scanning electron microscopy (SEM) technology:

Explain these micrographs of seven edible films of nanoscale biopolymer, and after taking the cross-section, the surface morphology was examined using SEM images **(Figure 4)**, and the surface roughness was estimated using the biopolymer. SEM images showed that the edible films were intact and smooth without any noticeable fragmentation on the surface which was found to rise with increasing concentration. It was also observed that the treatments had the highest value of the micrographs recorded for the E20%, F40%, and G60% treatment, 30, 100, and 200 µm respectively, compared to the treatment (B20%, C40%, and D60%), 20, 50 and 200 µm on the Consecutive and control A0% 500 µM without addition. The best edible biopolymer film contains a homogeneous solution with some embedded fine grains and intact smooth crystal morphology in a continuous matrix. However, adding bacteria to produce such a natural nano biopolymer produces membranes. The smooth surface and some coarse ridge bed images have a B band (20 µm) average droplet size followed by C biopolymer films. A transparent, polygonal, spherical elliptical appearance has an average droplet size (50 µm), and the D-treatment is homogeneous with average bubble droplets. Scale size (200 µm) compared to the average roughness of the droplet band (500 µm). Our results agree with these obtained by [15, 22].



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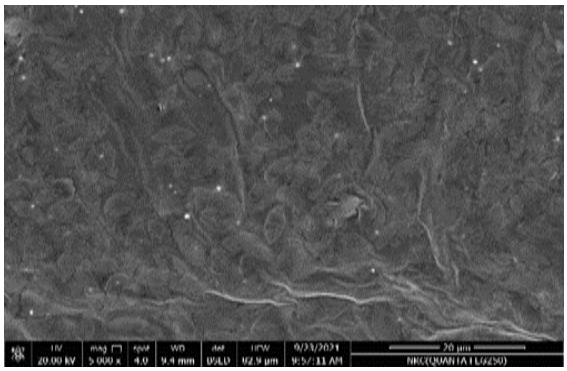

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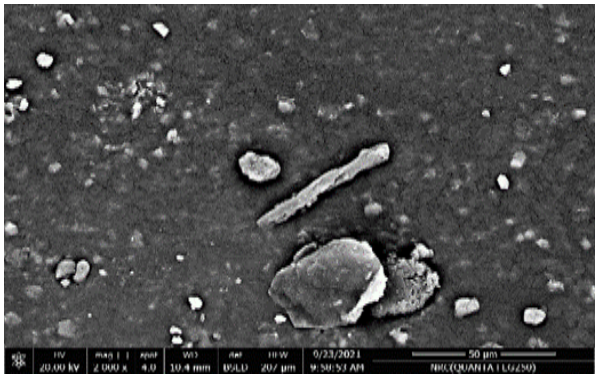

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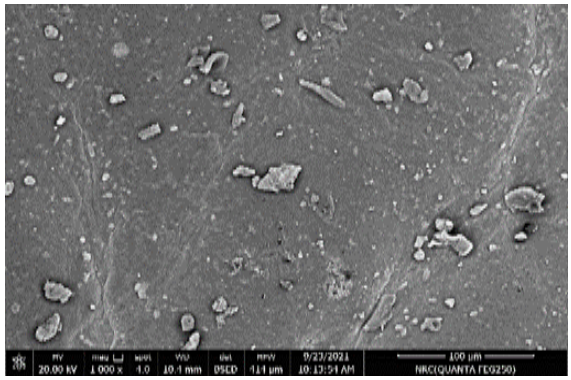

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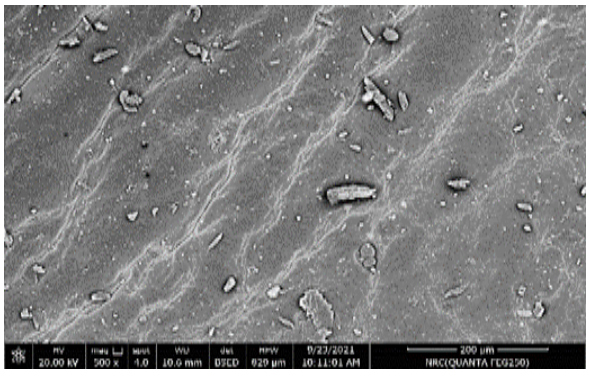

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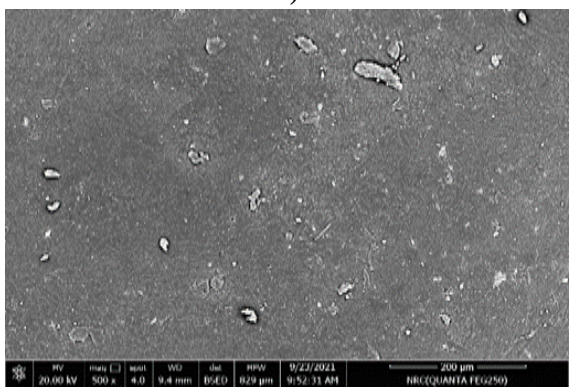

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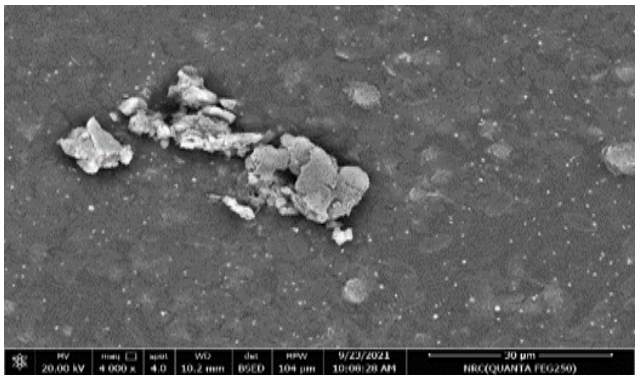
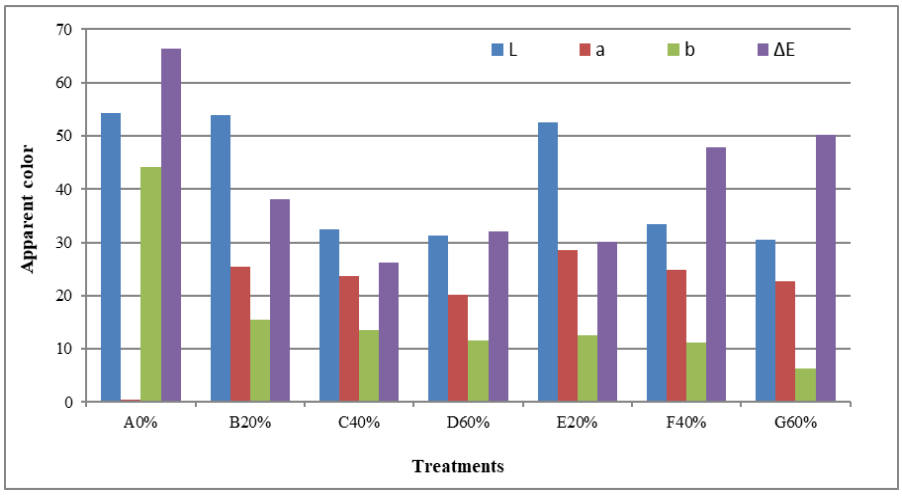


g)

Figure 4. Microstructure of prepared l to produce an edible film using (SEM) at different treatments

Determination of color apparent & transmittance light of the edible films prepared separate cellular nano biopolymer production for batch fermentation of bacterial on edible films.

The results obtained are presented in **Table 3** and **Figures 5a and 5b**. From the curves illustrated in the figures, it is clear that the apparent color increased with increasing concentration at different treatment's in both (B20%, C40%, and D60%) and (E20%, F40%, and G60%) of prepared separate cellular nano biopolymer production for batch fermentation of bacterial to produce an edible film. It was also noted that the treatments were higher value of apparent color was recorded for the treatment, B20%, (53.8, 25.4, 15.4 and 38.07), C40%, (32.4, 23.6, 13.6 and 26.18), D60%, (31.2, 20.2, 11.5 and 32.05) for L, a, b and ΔE respectively, while that the treatment, E20%, (52.6, 28.5, 12.5 and 30.15), F40%, (33.4, 24.8, 11.2 and 47.91), G60%, (30.5, 22.6, 6.4 and 50.28) for L, a, b and ΔE respectively as compared A0% for L, a , b and ΔE was (54.3, -0.5, 44.2 and 9.95). On the other hand, the barrier and transparency of the films were evaluated by measuring the percent light transmittance at (T280) and 660 nm respectively. It was found that the treatments decrease with increasing concentration at different treatment's in both (B20%, C40% and D60%) and (E20%, F40% and G60%) compared to the control sample, and the results were as follows B20%, (17.9 and 67.2), C40%, (16.6 and 60.4), D60%, (9.4 and 44.6) for (T280) and 660 nm respectively, while that the treatment, E20%, (17.6 and 66.4), F40% (16.2 and 58.3), G60%, (9.1 and 85.7) for (T280) and 660 nm respectively as compared A0% for T280 and 660 nm was 18.4 and 9.95. Interestingly, the addition of nanomaterial did not significantly reduce the transparency of the film (by less than 10 %) but significantly reduced the UV light transmittance depending on the concentration of nanomaterial. When 2 wt% of nanomaterial was added, the T280 of the film was reduced by more than 50 %. These results indicate the addition of nanomaterial significantly increased the UV barrier property without sacrificing the transparency of the film. The UV blocking property of pectin/agar films containing nanomaterial is mainly attributed to the UV light absorption function of nanomaterial respectively, according to Swarup Roy and Jong-Whan Rhim (2021), Maria-Ioana Socaciu *et al*. (2020) and Munir *et al*. (2019) [17-19].



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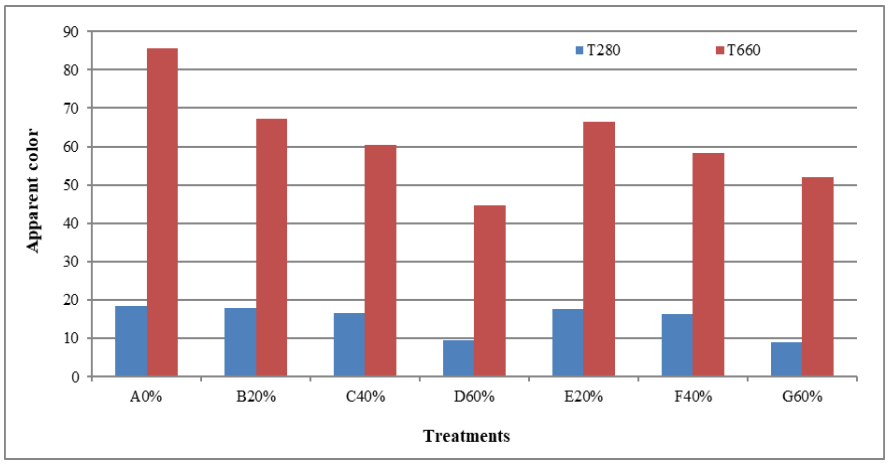

b)

Figure 5. a) Measurement of apparent color (Minolta Chroma) of the edible films. b) Measurement of apparent color (Light transmittance) of the edible films.

CONCLUSION

It can be concluded that the best treatment of samples was edible membranes (B20% and E20%) followed by (C40% and F40%) compared with the control group A0% without addition was the lowest. Other physical and chemical properties were studied for example rheological properties and partial size distribution, effective zeta, color, and scanning electron microscopy films. Effect of adding nanomaterials to edible membranes to extend product shelf life, reduce risks of pathogen growth and improve food quality. Production of a bacterial biopolymer from Acetobacter spp by batch fermentation to obtain exogenous polysaccharides and Gluconacetobacter xylinus. The Leuconostoc spp of Leuconostoc spp bacterial genera for batch fermentation produces ether Glucosyltransferases and Fructosyltransferases using low-cost nanopolymer production substrates to produce edible coatings and films. Polysaccharides are extracted from polysaccharides to preserve the film from oxidation and microbial contamination, maintain the stability of the film and extend the shelf life of food, as this substance prevents microbial growth and oxidation and improves the quality of the film with acceptable organoleptic properties Suitable properties of the barrier (carbon dioxide, oxygen, and water) Microbiological Biochemical The chemical-physical stability Public health safety Effective carrier of antioxidants, flavor, color, food additives or antimicrobial, could indicate that these results may be useful for application in the food industry with simple production technology and low cost.

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