Using effect of nano emulsion based on plant extracts as edible coating on prolonging the shelf life of fresh zucchini

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ABSTRACT

Nanoemulsion-based edible coatings technology is considered a valuable alternative to improve fresh fruits and vegetables quality. Therefore, the aim of this research is to study the preparation and characterization of Nano edible coating which contains sodium alginate with Nano laurel or lemongrass extract as the antimicrobial agents and antioxidants, and their effects of these Nano emulsions compared with the emulsions from the same materials on the quality attribute of fresh zucchini. The results showed that the Nano-emulsion of extracts had more effect than emulsions of the same extracts on microbial growth and anti-oxidation activity. The effects on quality of zucchini were studied during the storage at ambient temperature (25±2°C) for 6 days and cold storage (5±1°C) for 18 days. The Nano edible coating exhibited a beneficial effect on the quality during different storage periods, retarding moisture loss, reducing hardening and microbial growth. After 3 days of storage at ambient temperature, uncoated sample showed greatest loss (P≤0.05) in weight (25.66%) compared with the coated samples, especially with Nano laurel extract (5.18 %). Also, at 12th days of cold storage, the highest loss was recorded in uncoated sample (19.15%), while the lowest weight loss was observed in sample with Nano laurel extract (2.50%). In general, the samples coated with sodium alginate with Nano emulsion laurel extract had the best treatments in all quality characteristics and extended the shelf life of fresh zucchini from 6 to 18 days compared to uncoated sample (control) at the ambient or cold storage temperatures.

Keywords: Edible coatings; Nanoemulsion; Emulsion; Sodium alginate; Lemon grass extract; Laurel extract, Zucchini.

INTRODUCTION

To reduce the rate of desiccation, gas exchange and related oxidation reactions, many food products can be protected with edible coating, an environmentally friendly method (Nawab *et al*., 2017). According to Ramos *et al*. (2013), edible films can supplement or work in concert with other elements to enhance food quality overall, prevent food from external microbiological contamination, lengthen food shelf life and possibly increase packing material effectiveness. According to reports, the using of edible coatings for fruits and vegetables improved colour and flavour retention during storage, increased product shelf life, slowed moisture content, firmness loss and delayed products ageing. (Rojas-Graü *et al*., 2009; Dhall, 2013; Ciolacu *et al*., 2014).

A polysaccharide extracted from marine brown algae called sodium alginate is frequently used as a thickening ingredient in the food industry. It also possesses film-forming capabilities. (Krochta *et al*., 1994).

Several active substances may also be added to polymer matrix from edible coatings in order to improve quality or safety. (Acevedo-Fani *et al*., 2017). Edible coatings improved with Nano emulsion may help to increase the shelf life of horticulture produce by reducing moisture migration, gaseous exchange and oxidative reactions, as well as controlling pathogenic growth and physiological diseases. (SalviaTrujillo *et al*., 2015; Sessa Ferrari *et al*., 2015).

The characteristics of EO laden coating solutions are improved using Nano emulsion-based colloidal systems. Oil-in-water Nano emulsions are produced by dispersing lipid Nano droplets with a diameter of 10 to 100 nm in an aqueous solution. (McClements, 2011). The advantages of Nano emulsions include enhanced stability, enhanced physicochemical properties, masking the taste or odor of the core material to have less of an impact on the organoleptic properties of food and enhanced biological activity of EO by increasing the surface area to allow using lower doses of EO. (McClements and Rao, 2011). Recently, Studies on the application of EO-loaded Nano emulsions in active edible coatings to prolong the shelf life of green beans have been published (Donsì *et al*., 2015; Severino *et al*.,

2015, 2014), fresh-cut Fuji apples (SalviaTrujillo *et al*., 2015), fish fillets (Wu *et al*., 2016).

In this regard, Nano emulsions made from polysaccharides like alginate and plant extracts as antimicrobial agents may be employed for the development of edible coating, which may be regarded as a new generation of edible packaging.

The technology employed from the farm to commercialization affects the vegetable quality. The lowering of vegetable metabolic activity is the primary goal of the post-harvest processes of packaging and cold storage, which subsequently extends the post-harvest life of these items. Postharvest and distribution losses account for more than 40% of losses from fruits and vegetables. Even though the point in the value chain where they happen differs, these losses are the same in both developing and developed nations. Losses happen in retail sales and consumption in industrialised nations, whereas postharvest and processing losses happen in developing nations. (Gustavsson *et al*., 2011). Additionally, the losses increase with the length of the time between harvest and consumption (Kader, 2008). The lack of protection during shipping increases mechanical damage, which may be prevented if packed. Infections brought on by bumps and wounds can increase losses, especially if they occur during prolonged storage at high temperatures. (Antonia Mirian *et al*., 2020).

Zucchini (*Cucurbita pepo L.*) It is distinguished by a rapid rate of respiration, a non-climacteric respiratory patterns, and perishability. Because they are harvested at a young stage of development with a thin cuticle that is easily damaged during harvest and subsequent handling, zucchini fruits are particularly prone to water loss. Cuts, punctures, abrasions, and other skin-damaging injuries are major issues that can result in irregular cellular growth and microbial decomposition.

The objective of this study was to produce edible coating with a high potential to carry active plant extracts nanoparticles loaded on sodium alginate emulsion and study characterization of Nano emulsion which included particle size and zeta potential, as well as to study the effect of Nano coating on the following quality attributes: pH, acidity, TSS (%), weight loss and microbial growth during the storage periods to select the best edible coating for improving storability fresh zucchini under different storage conditions.

MATERIALS AND METHODS

Materials:

Plants:

Fresh Zucchini (*Cucurbita pepo L.*) "*Kavili F1*" were obtained from (Obour market, Egypt). Zucchini were harvested on April, 2021 at the same ripening stage. Fruits were immediately transported to the food technology laboratory of the Food Science and Technology Department at Al-Azhar University. The fresh Zucchini used in this investigation were in maturity stage, and they were free from any mechanical damage or pests.

Fresh herb of laurel (*Laurus nobilis*) and lemongrass (*Cymbopogon citratus*) were collected at July 2020 from Farm in El-Kanater El-Khairia, Qalyubia, Egypt. Samples from each plant were then shade dried and subjected to extraction.

Chemicals:

Food-grade Sodium alginate which was used to form the edible coatings, it was obtained from Morgan Company for Chemicals, Cairo, Egypt. Calcium chloride was used for initiating crosslinking reaction with sodium alginate to form the continuous edible coating on the fruit surface, it was obtained from El Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. Glycerin and sodium hypochlorite were obtained from El-Gomhouria chemical company, Cairo, Egypt. Tween 80 (Non-ionic surfactant used as an emulsifier). It was purchased from El Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Microorganism strains used in this study:

The following food-borne pathogens bacterial strains *Staphylococcus aureus, Bacillus cereus* (gram positive) and *Escherichia coli, Salmonella typhimurium* (gram negative), also, the fungal strains *Aspergillus flavus* and *Aspergillus niger* were obtained from the Microbiological Resources Center, (Cairo MIRCEN) Egypt.

Methods:

Extraction of bioactive compounds from plant Leaves:

The air-dried ground (80 mesh) of plant leaves (20g for each sample), was added to 200 ml of a mixture of ethanol (CH_3OH) and water (H_2O) at ratio 80:20 v/v for 6 hours at ambient temperature (25°C±2) in an orbital shaker in a water bath in separate experiments. The extracts were separated from the residues by

filtering through Whatman No.1 filter paper. The residues were extracted twice with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed from mixture solvents under pressure at 45°C, using a rotary evaporator. The dried concentrated extracts were weighed to calculate the yield, and then stored in a refrigerator at 4 ±1°C according to (Bushra et al., 2009).

Preparation of Nano emulsion:

Nano emulsion extract was formulated according to the method of (Ghosh *et al*., 2013) with some modifications. Non-ionic surfactant Tween80 and distilled water were used for preparation of Nano emulsion. Concentration of extract (5% v/v) and Tween80 (1.5% v/v) were fixed for both of emulsion and Nano emulsion formulations. Coarse emulsion was prepared by gradually and continuous adding of EO and surfactant to water with shaking at 3000 rpm. After completion of the addition of the aqueous phase to the oil phase, the resulting dispersion was continued to be stirred for 30 min, homogenized by homogenizer (Model: SHM2 / EURO, Made in USA, v230,50-6- Hz, w700) at 6000 rpm for 10 min. Then, the coarse emulsion was subjected to ultrasonic emulsification using a 20 kHz Sonicator (ULTRASONIC CLEANER, capacity 5L, v220, Italy). The operation power was adjusted to 200 W and a sonotrode containing a piezoelectric crystal with a probe diameter of 15 mm was applied. Sonicator probe was symmetrically dipped into coarse emulsion in depth of 25 mm, and the sonication process was carried out for 15 min. The temperature difference between initial coarse emulsions to final Nano emulsion was less than 10 C. Then, the coarse emulsion and formulated Nano emulsion were characterized in ambient temperature (25±2°C).

Characterization of Nano emulsion

Visual inspection:

The formation of opalescent different color suspension in the reaction mixture was used as a visual indicator to confirm the synthesis of Nano emulsion according to the method of Morris *et al*. (2011).

Measurement of Particle size and z-potential:

The droplet size of the developed Nano emulsion solutions were determined by using dynamic light scattering (DLS) with a zetasizer laser diffractometer (Model: SZ-100 Nano particle analyzer) at 633 nm at ambient temperature (25±2°C) (Acevedo-Fani *et al*., 2017). Nano particle were measured on the following nano- devices Malvern Zetasizer nano series (Nano ZS), UK, size range (nm):0.6 – 6000 nm and zeta range (mv): (-200 - +200). Nano particle analyzer (SZ-100) is based on Dynamic Light Scattering (DLS), which determines the scattering intensity which naturally fluctuates due to the brownian motion of macromolecules or particles in suspension. The system had dual solid state laser diodes at 633 nm (near-infrared) wave length as optical light sources with refractive index of 1.487. To avoid multiple scattering effects, samples were diluted with Milli Q water (1:10) prior to analysis.

Antimicrobial activity of Nano emulsion against microorganisms:

Antibacterial activity test:

The antibacterial activity of Nano emulsion was determined by the agar well diffusion method against the previously mentioned gram-negative and gram-positive strains according to the method of Mokhena and Luyt (2017). In this method, sterile nutrient agar medium was prepared. Bacterial strains used in the present experiment were spread over the agar plate. The plates were allowed to dry. Under aseptic conditions, two holes with 5mm diameter were made in each plate using a sterilized tip, subsequently, a 100µl (100, 300 and 500µg/mL) of the nanoparticle suspension was introduced into holes. The plates were allowed to stand for 1h or more for diffusion to take place and incubated at 37 ºC for 48 hrs. and then examined for evidence of zones of inhibition, which appear as a clear area around the holes. The diameter of such zones of inhibition was measured for each organism was recorded and expressed in millimeters**.**

Antifungal activity test:

Antifungal activity of Nano emulsion suspensions was evaluated against fungi strains (*A. flavus* and *A. Niger*) by using agar well diffusion method as described by Balouiri *et al.* (2016). The test was carried out by inoculating a Potato Dextrose Agar (PDA) medium with each strain individually. Then 15-20 ml of the inoculated medium were poured into Petri dishes and settled down on ambient temperature (25±2°C) till solidification. Under aseptic conditions, two holes with 5 mm diameter were made in each plate using a sterilized tip, and a volume 100µl (100, 300 and 500 µg/mL) of the nanoparticle suspension were added to each hole. The plates were incubated aerobically at 25ºC for 3-5 days. The zone of inhibition was calculated

by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

Total phenolic, total flavonoid contents and antioxidant activity of Nano emulsion:

Total Phenolic content:

The total phenolic content was determined as the method described by Singleton *et al*. (1999). Lemongrass leaves extract as much as 0.5 mL was mixed with 2.5 mL reagent Follin Ciocalteu 10% (which had been dissolved in distilled water) and 2.5 mL of NaHCO3 7.5%. The mixture was further incubated for 45 minutes at a temperature of 45 °C. Absorbance was measured at a wavelength of 765 nm. A standard calibration curve used gallic acid (0, 15, 20, 25, 30 and 35 mg/L). Furthermore, each standard gave the same treatment with the extracts of leaves samples. The phenol content is expressed in mg gallic acid/g extract.

Total flavonoid content:

The total flavonoid content was determined using the Dowd method (Meda, A. *et al*., 2005). 2mL of 2 % aluminium trichloride (AlCl3) in methanol was mixed with the same volume of the extract solution (0.1 mg/mL). Absorption readings at 415 nm using spectrophotometer were taken after 10 minutes against a blank sample consisting of a 2 ml extract solution with 2 mL methanol without AlCl3.The total flavonoid content was determined using a standard curve with rutin (25 - 200 µg/2ml methanol) as the standard. Total flavonoid content is expressed as mg of rutin equivalents (QE)/ g of extract.

Antioxidant activity by DPPH radical scavenging activity.

The (DPPH)1,1-diphenyl-2 picryl- hydrazyl is a stable free radical and it is widely used to assess the radical scavenging activity of antioxidant component. The reduction of DPPH radical was made, following the methodology reported by Sundararajan *et al.* (2016). Briefly, DPPH solution was prepared in methanol (Exactly 0.00394 g DPPH was weighed and diluted with 100 mL of 95 % methanol to obtain 0.1 mmol/100 mL solution) and 1 ml of this solution was added to 3.0 ml of Nano emulsion solution of 25, 50, 75 and 100 µg ml^{-1}). The solution was incubated for 30 min. at dark conditions at ambient temperature (25±2°C) and absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Model: CT-2200-S/N: RE1310004- Germany). The control solution was prepared by mixing ethanol and DPPH radical solution. The reduction of the DPPH radical was calculated as a percentage of inhibition by equation:

DPPH inhibition (%) = Ac – As/ Ac x100

Where: Ac is the control absorbance, and as is the absorbance of the sample.

Preparing of coating solution that contains Nano emulsion.

Sodium alginate (SA) 2% (w/v) solution was prepared according to Poverenov *et al*. (2014) with slight modifications. The SA powder was suspended in milli-Q water and kept on hot plate magnetically stirred at 70 °C for 2 h to get completely uniform solution without any lumps. Glycerol (1.5%) was added as plasticizer. Then, extract emulsion and Nano emulsion were added with constant stirring to reach final concentration of extract to 0.5% wt. After 20 min stirring at 1100 rpm, the solutions were characterized and applied for coating of the tested samples.

Application of prepared coatings solutions in food system.

Vegetables was selected based on fruit length, firmness, and freedom from mechanical damage. Prior to coating, the fruits were washed, rinsed, and surface dried by being kept under fan at ambient temperature (25±2°C). These vegetables were then divided into 6 groups: one group was only dipped in distilled water (C), the second group was coated with sodium alginate (SA), the third group was coated with sodium alginate incorporated with lemongrass extract (SA+LGE), the forth group was coated with sodium alginate incorporated with laurel extract (SA+LUE), the fifth group was coated with sodium alginate incorporated with Nano emulsion lemongrass extract (SA+ NANO LGE), and the sixth group was coated with sodium alginate incorporated with Nano emulsion laurel extract (SA+ NANO LUE). The Prepared vegetables were treated dipping in CaCl2 (2%) solution added with glycerol (1.5%) to form the cross linking reaction with Sodium alginate to form coating film over the Zucchini vegetables, then, dipping in one liter of respective coating solution for 3 min. The coated zucchini vegetables were then dried for 20 min, and packaged in foam trays (125 x 80 x 40 mm), then wrapped with polypropylene stretch film with venting holes 20 µm of thickness, (Creative Forming, Inc., Ripon, Wis., U.S.A.). Finally, the samples were stored at ambient temperature (25 ± 2 °C) and in cold storage (5 ± 1 °C) in triplicates. The control

(untreated) and treated samples were periodically analyzed every 3 days during storage at ambient and cold temperature until the samples are destroyed. The preparation method was used as the methods described by Salvia-Trujillo *et al*., 2015; Robledo *et al*., 2018.

Physiochemical analyses:

Physiochemical parameters:

Weight loss (%):

The weight of the coated and uncoated Zucchini vegetable during storage periods was measured by monitoring the weight changes. The weight loss was calculated as the percentage loss of initial weight as reported by (Qin *et al.*, 2015) by the following equation:

Fruit weight loss (%) = [(initial weight - weight at sampling date/ initial weight)] x 100.

Total soluble solids:

Total soluble solids (TSS) were determined by the refractometric method at ambient temperature (25±2°C) using refractometer (Model: MA871, Romania). The results were expressed as % TSS according to AOAC (2016).

Total titratable acidity:

Total titratable acidity was measured for fresh samples as mentioned in the official method of the AOAC (2005). It was expressed as citric acid using sodium hydroxide N/10 and phynol phythaline as indictor. Total titratable acidity is expressed as g per 100g,

pH value:

The pH value was determined by pH meter. For the measurement of pH value, 5.0 g of the different treatment samples were separately homogenized with 45 mL distilled water and pH value was measured by a digital pH-meter equipped with gold glass electrode (Model-3505.UK). The pH-meter was calibrated by standard solution of pH 4 and 7 before use. The pH value was determined according to AOAC (2016)

Microbiological Aspects:

Total plate count:

Total colony count of bacteria was estimated using plate count agar medium according to the procedures, Described (FAO/WHO, 1995) Inoculation and pour plating; 1ml of each dilution was pipetted into each of appropriately marked duplicate Petri dishes. 15-20 ml of plat count agar medium was poured into each Petri dish, cooled to 45ºC mixed thoroughly and allowed to solidify. The plates were incubated at 37ºC for 24-48 hours. After incubation, dishes showed convenient numbers of colonies that were counted.

Psychrophilic bacterial count:

Psychrophilic bacterial count was estimated as described in typical procedure of the total bacterial count method, except incubation was carried out at 7ºC for 5 days in refrigerator according to American public Health Association (AP.H.A., 1976).

Yeast and mold counts:

The yeasts and molds were determined using the methods for the microbial examination of foods as described by American Public Health association (A.P.H.A., 1992) using potato dextrose agar medium, incubation at 20-25 ºC for 3-5 days. If excessive growth develops, count colonies first after 3 days and then again after 5 days, and reported as mold and yeast count per/g.

Statistical analysis:

The data were statistically analyzed by using the Statistical Package for Social Science (SPSS) computer program software; (version 20.0 produced by IBM Software, Inc. Chicago, USA) of completely randomized design as described by Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Characterization of Nano emulsion:

Visual inspection of Nano emulsion:

By visual examination of Nano emulsions, the results were observed where they appeared almost transparent after being homogenized and sonication whereas the coarse Macro emulsion was opaque.

Particle size and zeta potential of Nano emulsion:

The average of droplet size and distribution of the primary and Nano emulsions were evaluated in the current investigation. Depending on the kind of EO, variations in primary and Nano emulsion droplet sizes were seen. Several techniques, including as solvent displacement, membrane emulsification, phase inversion, high pressure homogenization, microfluidization, high-speed mechanical and ultrasonication, can be used to create Nano emulsions, which have droplet sizes between 20 and 1000 nm. (Huang *et al*., 2010; Walker *et al*., 2015; Jin *et al*., 2016).

Particle size of Nano emulsion from laurel oil:

The results Shows in Fig. (1), the average droplet size for Nano emulsions and the dispersion of their droplet sizes. The Nano emulsions Laurel extract had an average droplet size of 50.89 nm. There were noticeable variations in emulsion droplet size and size distribution depending on the essential oil used in the creation of Nano emulsions. However, a prior work (Ozogul *et al*., 2017) discovered that the laurel EO Nano emulsion had droplet sizes in the 66.02 nm range. Another study showed that depending on the effect of the oil content and composition, the hydrodynamic size of the essential oil of *Laurus nobilis* emulsion droplets ranged between 239.5 and 357.0 nm. (Reis *et al*., 2019).

Particle size of Nano emulsion from lemon grass extract:

The results Shows in Fig. (2), the average droplet size for Nano emulsion and the dispersion of their droplet sizes. The Nano emulsions containing lemon grass extract had an average droplet size of 584.8 nm. There were noticeable variations in emulsion droplet size and size distribution depending on the essential oil used in the creation of Nano emulsions. (Nagah *et al*., 2020) found that the droplet size distribution for the ultrasonically generated Nano emulsion of lemongrass oil resulted in the development of nano emulsions with a small droplet size of about 275 nm.

Zeta potential of Nano emulsion from laurel oil:

As reported by Behl *et al*. (2011); Honary and Zahir (2013), zeta potential (ZP) of nanoparticles can be used to understand and foretell particle interactions. The zeta potential is the nanoparticle surface charge value that affects particle stability or speeds up particle flocculation, two key characteristics of adsorbents. The mean zeta potential of the Nano emulsion from laurel extract was showing good stability with – 48.6 mV as shown in Fig. (3). It indicates that the Particle size for Nano emulsion could be expected to be stable for longer time.

Zeta potential of Nano emulsion from lemon grass extract:

As reported by Behl *et al*. (2011); Honary and Zahir (2013), zeta potential (ZP) of nanoparticles is an effective instrument for comprehending and foretelling particle interactions. The zeta potential is the measure of a nanoparticle's surface charge, which affects particle stability or speeds up flocculation of particles, two key characteristics of adsorbents. The mean zeta potential of the Nano emulsion from lemon grass extract was showing good stability with + 23.5 mV as shown in Fig. (4).It indicates that the Nano Particle size could be expected to be stable for longer time.

Antibacterial activity of emulsion and Nano emulsion:

Laurel extract.

The effect of Nano emulsion from laurel extract on growth of tested bacteria and their comparison with effect of emulsion laurel extract is showed in Tables (1, 2). Nano emulsion from laurel essential oil demonstrated varying degrees of inhibition against the growth of the microorganisms under investigation. The inhibition zones of Nano emulsion from laurel extract were ranged from 14 to 23 mm., while the inhibition zones for emulsion laurel extract were ranged from 13 to 18 mm at 500 µg/mL concentrate. Merghni *et al*. (2016), studied the effectiveness of EO as an antibacterial agent against *Staphylococcus aureus* strains, achieving inhibition zones (halos) ranging from 6.75 to 16.5 mm, a similar pattern to that reported in the current study for the emulsion.

Lemon grass extract:

The results indicate, in Tables (3, 4), the impact of the Nano emulsion from lemon grass extract on the growth of the tested bacteria and their comparison to the impact of the emulsion from lemon grass extract. The growth of the examined microorganisms was inhibited to varying degrees by Nano emulsion from Lemon grass extract. The inhibition zones of Nano emulsion from Lemon grass oil were ranged from 13 to 19 mm. ,while the inhibition zones for Lemon grass extract emulsion were ranged from 11 to 15 mm at 500 µg/mL concentrate. Both *Staphylococcus aureus* and *Bacillus cereus* had the highest microbial sensitivity and this inhibitory effect increased with increasing Nano emulsion concentration. The lemongrass and extract are effective against a wide variety of disease-causing microbes (Avoseh *et al*., 2015).

Antifungal activity of emulsion and Nano emulsion:

From the results of Tables (1, 2), Nano emulsion from laurel extract revealed different levels of growth inhibition for *A. flavus* and *A. niger.* The inhibitory zones brought on by the laurel extract -derived Nano emulsion was 17 and 19 mm for *A. niger* and *A. flavus,*

respectively, against 14 and 15 mm for inhibition zones of laurel oil emulsion. Naik *et al*. (2010) stated that gram positive organisms were discovered to be more sensitive to lemon grass extract than gram negative species.

Also, from the results of Tables (3, 4), Nano emulsion from Lemon grass extract revealed different levels of growth inhibition for A. flavus and A. niger. The Nano emulsion from lemon grass extract produced inhibition zones that were 15 and 16 mm for type *A. flavus* and *A. niger* respectively, against 13 and 14 mm for inhibition zones of emulsion from Lemon grass oil. The *Laurus nobilis* EO demonstrated modest antifungal activity in the current study, which supported earlier studies on the EO's antifungal potential, such as those by Guynot. *et al*. (2005), who also discovered that *Laurus nobilis* EO had antifungal properties against the development of *Aspergillus sp*. However, Tajkarimi *et al*. (2010), showed lesser antibacterial effects.

Total phenolic, Total flavonoids content and Antioxidant activity of tested emulsion and Nano emulsion:

Laurel oil:

From Table 5, it is noticed that the phenolic content in Nano emulsion from laurel extract was found to be as 99 mg/g, and in the emulsion from laurel extract was found 81 mg/g, these results are significant and indicate the richness of the Nano emulsion from laurel essential oil with phenolic compounds.

From Table 5, it is noticed that the content of flavonoids in Nano emulsion from laurel extract was 63 mg/g, and was higher which had higher than that obtained by laurel extract emulsion which recorded 48 mg/g.

Nano emulsion from laurel extract exhibited a significant scavenging activity versus the emulsion made from laurel extract (P 0.05), as in (Table 5). According to the DPPH scavenging, Nano-emulsion made from laurel extract had higher antioxidant activity than emulsion made from laurel extract. From Table 5, it is noticed that the radical scavenging activity was 90% for Nano emulsion from laurel extract, where it recorded 79% for laurel oil emulsion. This is in line with the findings of Elmastas *et al*. (2006), who studied the antioxidant properties of ethanolic extracts from laurel leaves (at 60 g/L-1), finding that 92% of the radical DPPH was inhibited.

Lemon grass oil:

From Table 6, it is noticed that the phenolic content in Nano emulsion from Lemon grass extract had higher (72 mg/g) than that obtained by Lemon grass extract emulsion (49 mg/g).

From Table 6, it is noticed that the flavonoids content in Nano emulsion from Lemon grass extract had higher (34 mg/g) than that obtained by Lemon grass extract emulsion (27 mg/g).

The Nano emulsion from Lemon grass extract demonstrated a substantial scavenging activity (P 0.05), when compared to an emulsion made from Lemon grass extract as shown in Table 6., where the DPPH scavenging indicated that Nano emulsion from Lemon grass essential extract had superior antioxidant activity than emulsion from Lemon grass extract. From Table 6, it is noticed that the radical scavenging activity was 84% for Nano emulsion from Lemon grass extract, while it was 70% for emulsion from Lemon grass extract. Sah *et al*. (2012), reported that the total phenolic compounds in ethanol extract 40% of lemongrass leaves was 67.28 mg GAE/g.

Effect of treatments on physicochemical quality criteria for zucchini during storage periods:

Weight loss (%):

The results are shown in Table (7), and it is clear from this table that the weight loss increased with increasing the storage period at ambient and cold temperatures for all samples. while during storage periods at ambient temperature (25±2°C), in both treated and untreated samples there was an upward trend in weight reduction. The control sample (without coating) displayed the largest weight losses (P 0.05) after 3 days of storage it was 25.66% with compared to the coated samples with SA, SA+ LGE, SA+ NANO (LGE), SA+LUE and SA+ NANO (LUE) which reached to 7.25, 6.78, 5.69, 6.61 and 5.18 %; respectively.

After six days of the storage periods at ambient temperature, the samples that received treated with SA experienced the greatest weight decrease (P 0.05) (20.12%). The samples that were treated with SA+ NANO (LUE) experienced the least weight loss (17.36%). When samples kept at cool temperature of (5±1°C), the data revealed a similar pattern of weight loss starting on the third day as indicated in Table (7). At the third

day of storage, the weight loss for control sample reached 3.78%. while the samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE) reached 1.18, 0.87, 0.58, 0.82 and 0.34%, respectively. Moreover at the 12th day of cold storage, the largest losses (P 0.05) were recorded in uncoated sample (19.15%). while the samples treated by SA+ NANO (LUE) and SA+ NANO (LGE) showed the least weight loss (P 0.05), 2.50 and 2.83%; respectively as portrayed in Table (7).

After 18 days of cold storage periods, certainly the coating significantly decrease (P≤0.05) weight loss in all samples, in treated samples, the average of weight loss ranged from 4.86 to 7.13%. The results indicate that the lowest losses (P≤0.05) were noted in samples coated by SA+ NANO (LUE) (4.86 %).

Finally, it can be concluded that the weight losses continuously increased (P≤0.05) in each of the tested samples during various storage periods. Application of coatings showed that an important effect on weight loss (%). Also, Nano-coating leaded to a significantly higher decrease in weight losses compared with control and other samples coating. It turns out that, SA+ NANO (LUE) had a best effect on delaying weight loss for fresh zucchini as shown in Tables (7).

The results generally supported earlier research that claimed coatings' capacity to act as semipermeable barriers to oxygen, carbon dioxide and moisture decreased weight loss by reducing respiration, water loss, and oxidation processes (Gol *et al*., 2013).

Total soluble solids (TSS):

TSS content, a crucial quality metric, represents the solute concentration inside cells. An increase in TSS content is advantageous for preserving the equilibrium of permeation between the protoplasm and the environment and for improving the tolerability of vegetables and fruits to chilling. (Agopian *et al*., 2011).

From the data in Table (8), the initial values showed Brix degrees (5.11 to 5.14%) within the range above mentioned.

TSS content in coated zucchini samples did not vary much while being stored at the ambient temperature, however untreated samples underwent significant variations in TSS concentration where the control sample's TSS content increased from 5.14 to 6.50% after 3 days from storage, compared to coated samples which reached to 5.53, 5.49, 5.35, 5.44 and 5.29 % in the samples treated by SA, SA+ LUE, SA+ Nano (LUE), AS+ LGE and SA+ Nano (LGE); respectively.

After 6 days of storage at ambient temperature, the observed increases in TSS for samples treated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE, and SA+ NANO (LUE) were 6.12, 6.01, 5.76, 5.88, and 5.63%, respectively.

Results in Table (8) for TSS% to samples stored at 5±1°C, indicated a similar pattern of increased TSS starting on the third day. Where the TSS concentration of all samples gradually increased (P 0.05) as storage times were prolonging.

After 12 days from storage, the TSS concentration of the control sample rose from 5.14 to 6.80% and the samples spoiled after 13 days, while TSS concentration for coating samples ranged from 5.49 to 5.90% on the same day. Also, after 18 days from cold storage periods, the increment rates in TSS % were less in treated samples with Nano emulsion, where it was 5.97 and 6.00 for sample treated with SA+ NANO (LUE) and SA+ NANO (LGE) respectively whereas it was 6.05, 6.12 and 6.52 of the other tested samples.

The TSS values show a tendency to high with maturity because of mass loss, which aggregates and concentrates the solids, as well as because of biosynthesis, polysaccharide degradation or even by the significant water loss by the plants, which leads to their accumulation (Oshiro *et al*., 2012).

PH value*:*

Table (9) shows that there were no appreciable pH variations between control samples and the treated samples at the beginning of storage or throughout various storage times. During storage at the ambient temperature (25±2°C), pH values of uncoated and coatings samples ranged from 6.11 to 6.13 at initial storage.

Also, Table (9) showed that pH values significantly rose (P 0.05) for all samples throughout the duration of storage. In comparison to treated samples, the control sample had the highest pH values at the end of storage.

In this instance, the pH value of the control sample rose from 6.12 to 6.53 after 3 days, whereas coated samples rose at slower rates than uncoated samples at the same earlier storage times without significant differences. After 6 days of storage periods, the pH values

were 6.28 and 6.31 for sample treated by SA+ NANO (LUE) and SA+ NANO (LGE); respectively, while it reached (6.61, 6.53 and 6.50) for samples treated by SA, SA+ LGE and SA+ LUE; respectively according to Table (9).

Table (9) shows that for all treatments the changes in pH values at cooling temperature were less than those changes at ambient temperature. The pH value of control sample increased from 6.12 to 6.68 after 12 days from storage and spoiled after 13 days, whereas the pH value for coated samples ranged from 6.26 to 6.38 at the12th days. Additionally, compared to uncoated samples, the pace at which pH values climbed was lower in coated samples. Also, after 18 days of cold storage periods, the increment rates in the pH value were less in treated samples with Nano emulsion, where it was 6.44 and 6.49 for sample treated with SA+ NANO (LUE) and SA+ NANO (LGE) respectively, whereas it was recorded 6.54, 6.57 and 6.60 for the samples treated with SA+ LUE, SA+ LGE and SA; respectively.

In a related study, Dhital *et al*. (2017) found that pH increased during storage, which may be related to the effect of greater O2 levels on fruit respiration rates.

Titratable acidity:

Data are shown in Table (10) They show the effects of treatments on the titratable acidity content of zucchini. The titratable acidity contents were 0.16 to 0.18g malic acid /100g for zucchini samples at the beginning of storage. TA contents are significantly different in samples treated during storage at ambient temperature (25±2°C). After 6 days of storage, the lowest decrease in TA was noted in samples treated with SA+ NANO (LUE) and SA+ NANO (LGE) 0.14% and 0.13%, respectively.

In relation to sample storage at a cold temperature, the results showed a similar trend in TA reduction beginning on the third day, as shown in Table (10). The TA values for control samples reached 0.09 g/100g after being stored for 12 days,while, the coated samples with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE) reached 0.13, 0.14, 0.15 0.14 and 0.15 %, respectively at the same day.

After 18 days of storage, the lowest decrease in TA was noted in samples treated with SA+ NANO (LUE) (0.13%). Acid organics concentrations throughout storage tended to decline, and postharvest alterations depend on the acid, tissue, handling, and storage. (Kays, 1991).

Microbiological aspects:

Total bacterial count:

From results of Table (11), it could be noticed that total bacterial count (TBC) of all samples were influenced by coatings treatments during periods of storage at the cooling temperature (5±1°C)) or ambient temperature (25±2°C). Table showed that TBC of zucchini samples coated were lower than uncoated sample during storage periods. TBC of uncoated and coated samples were ranged from 2.00 and 2.13 log CFU/g, at initial storage.

During storage at the ambient temperature, all studied samples' TBC rose linearly as storage times increased; control samples, in particular, grew more quickly than the other coated samples. Additionally, during the storage periods, all coating treatments reduced the growth of TBC. The initial TBC values for control samples increased to 5.98 log CFU/g after three days of storage at ambient temperature, compared to coated samples with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE), which were 3.74, 3.57, 3.40, 3.52 and 3.37 log CFU/g, respectively.

The control sample spoiled on the sixth day of storage, while the TBC values for all the samples that were looked at ranged from 4.71 to 5.95 log CFU/g. Table (11), which dealt with the storage of samples at a cold temperature, showed that starting on the third storage day, the TBC grew more quickly in uncoated samples than in coated samples. On the third day, the initial values of TBC for control samples increased to 3.46 log CFU/g compared to samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE), which were 3.02, 2.79, 2.66, 2.73 and 2.58 log CFU/g; respectively. Also, during storage up to 12 days, uncoated sample reached to (6.00 log CFU/g) and spoiled after this day, while all the tested coated samples did not exceed the permissible limit, the values of TBC ranged from 4.45 to 4.80 log CFU/g at the same day.

After 18 day of cold storage, TBC counts for samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE were 6.00, 5.84, 5.06 and 5.59; respectively higher than the samples coated with SA+ NANO (LUE) which recorded 4.92 log CFU/g.

Finally, these results found that the samples coated with SA, SA+ LGE and SA+ LUE lower than uncoated and higher than Nano coating

SA+ NANO (LGE) and SA+ NANO (LUE) in TBC during the storage. This might be as a result of the Nano coating's ability to effectively stop microbial growth. It is significant to note that for both cultivars, the total mesophilic and psychrophilic cell load was consistently below the threshold value established by the French Regulation. ($5x10^7$ UFC/g) (Corbo *et al*., 2004).

***Psychrophilic bacteria count**:*

It was observed that the changes in Psychrophilic Bacteria Count (PSC) during storage at cold temperatures ((51°C) followed a pattern that was identical to the changes in Total Bacterial Content (TBC) of all samples. All of tested samples' PSC progressively increased with during storage, particularly control samples were increased faster than the other coated samples as shown in Table (12). Furthermore, during the storage durations, all coating treatments slowed the growth of PSC.

The initial PSC values for uncoated samples increased to 2.65 log CFU/g on the third day of storage, compared to the samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE), which were 2.42, 2.38, 2.20, 2.32 and 2.14 log CFU/g; respectively.

Also, while in storage up to 12 days uncoated sample reached to 5.08 log CFU/g and spoiled after 13 days,while all tested coated samples did not exceed the permissible limit, the values of PSC ranged from 3.95 to 4.15 log CFU/g on the same day. After 18 days of cold storage, the values of PSC for samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE were (4.90, 4.77, 4.55 and 4.63 respectively) higher than the samples coated with SA+ NANO (LUE) which recorded 4.48 log CFU/g. It is significant to note that for both cultivars, the total mesophilic and psychrophilic cell load was consistently below the threshold value established by the French Regulation. ($5x10^7$ UFC/g) (Corbo *et al*., 2004.

***Mold and yeast counts**:*

The yeast and mold counts are a crucial factor in determining the quality of fresh vegetables and fruits. Table (13) shows the variation in yeast and mold counts for coated and untreated samples throughout storage. It was clear from Table that the coating treatments used during storage conditions had an impact on the mold and yeast counts.

Mold and yeast count increased linearly over the course of storage at ambient temperature for all studied samples; control samples in particular increased more quickly than coated samples. Additionally, during the storage periods, all coating treatments slowed the growth of yeasts and molds. Also, results showed that mold and yeast count of zucchini samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE) were lower than uncoated samples at the 3rd days of storage. Mold and yeast count for uncoated samples were 2.96 log CFU/g., while coated samples ranged from 1.20 to 1.69 log CFU/g under the same conditions as shown in the Table.

Uncoated sample spoiled on the sixth day of storage,while the yeast and mold counts for coated samples with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE) were 3.75, 3.49, 3.35, 3.46 and 3.30 log CFU/g, respectively.

Table (13) showed that starting on the sixth storage day, the mold and yeast counts rose more quickly in uncoated samples than in coated samples when samples were stored at a cold temperature. The initial mold and yeast counts increased to 2.73 log CFU/g, after 6 days of storage for uncoated samples compared to the coated samples with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE), which were 1.59, 1.45, 1.36, 1.42 and 1.30 log CFU/g; respectively.

Uncoated samples reached 3.93 log CFU/g on the 12th day of storage, then spoiled after 13 days, but compared to the uncoated samples, all covered samples had reduced levels of mold and yeast. As indicated in Table (13), the mold and yeast count of coated samples ranged from 2.01 to 2.30 log CFU/g. After 18 days of storage periods, the yeast and mold counts for samples coated with SA, SA+ LGE, SA+ NANO (LGE), SA+ LUE and SA+ NANO (LUE), were 3.81, 3.68, 3.38, 3.55 and 3.22 log CFU/g; respectively.

Generally, these results showed that, the samples coated with emulsion were lower in microbial load than uncoated samples and higher than the samples coated with Nano emulsion during the storage periods. This might be as a result of the Nano coating's ability to effectively stop microbial growth.

According to reports, the edible coatings based on emulsion containing orange peel essential oil which act as antibacterial compounds are leaded to reduce microbial populations (Settanni *et al*., 2012; Randazzo *et al*., 2016).

CONCLUSION:

Finally, these results indicate that the SA+ NANO (LGE) and SA+ NANO (LUE) can be utilised effectively as edible covering to prolong shelf-life of zucchini while maintaining its physicochemical and microbiological properties during storage.

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Table 1: Effect of Laurel extract emulsion on growth of tested microorganisms.

Concentration (µg/mL)	Diameter of inhibition zone (mm)					
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Salmonella typhimurium*	*Aspergillus flavus*	*Aspergillus niger*
100	8	7	5	6	7	5
300	10	11	7	9	8	7
500	18	17	13	14	15	14

Table 2: Effect of Laurel extract Nano emulsion on growth of tested microorganisms.

Concentration (µg/mL)	Diameter of inhibition zone (mm)					
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Salmonella typhimurium*	*Aspergillus flavus*	*Aspergillus niger*
100	9	8	6	7	8	6
300	11	12	8	9	10	9
500	23	18	14	15	19	17

Table 3: Effect of Lemon grass extract emulsion on growth of tested microorganisms.

Concentration (µg/mL)	Diameter of inhibition zone (mm)					
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Salmonella typhimurium*	*Aspergillus flavus*	*Aspergillus niger*
100	8	7	5	6	5	5
300	9	10	6	8	7	6
500	15	12	11	12	14	13

Table 4: Effect of Lemon grass extract Nano emulsion on growth of tested microorganisms.

Concentration (µg/mL)	Diameter of inhibition zone (mm)					
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Salmonella typhimurium*	*Aspergillus flavus*	*Aspergillus niger*
100	8	7	5	6	7	6
300	10	11	7	9	9	7
500	17	19	13	14	16	15

Table 5: Total phenols, Total flavonoids and Antioxidant activity of emulsion and Nano emulsion of laurel extract.

Treatment	Total phenols(mg/g)	Total flavonoids(mg/g)	Antioxidant activity (%)
Laurel extract	81	48	79
Nano Laurel extract	99	63	90

Table 6: Total phenols, Total flavonoids and Antioxidant activity of emulsion and Nano emulsion of Lemon grass extract.

Treatment	Total phenols(mg/g)	Total flavonoids(mg/g)	Antioxidant activity (%)
Lemon grass extract	49	27	70
Nano Lemon grass extract	72	34	84

Table 7: Effect of storage periods at ambient and cold storage temperature on weight loss (%) of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Ambient temperature (25±2°C)					
	Control	SA	SA+LGE	SA+ Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	0	0	0	0	0	0
3	25.66^{aA}±0.57	7.25^{bB}±0.43	6.78^{bB}±0.28	5.69^{cdB}±0.11	6.61^{bcB}±0.17	5.18^{dB}±0.05
6	ND	20.12^{aA}±0.57	19.28^{abA}±0.57	18.67^{bA}±0.11	19.05^{abA}±0.23	17.36^{cA}±0.11
Cold storage temperature (5±1°C)						
0	0	0	0	0	0	0
3	3.78^{aD}±0.34	1.18^{bF}±0.23	0.87^{bcF}±0.11	0.58^{bcF}±0.05	0.82^{bcF}±0.11	0.34^{cF}±0.05
6	5.73^{aC}±0.28	1.98^{bE}±0.23	1.63^{bE}±0.17	0.98^{cE}±0.05	1.56^{bE}±0.11	0.61^{cE}±0.05
9	9.90^{aB}±0.23	2.95^{bD}±0.17	2.40^{cD}±0.11	1.93^{deD}±0.04	2.34^{cdD}±0.11	1.60^{eD}±0.02
12	19.15^{aA}±0.11	4.10^{bC}±0.05	3.40^{cC}±0.11	2.83^{dC}±0.04	3.28^{cC}±0.05	2.50^{eC}±0.05
15	ND	5.45^{aB}±0.05	4.62^{bB}±0.11	3.87^{cB}±0.04	4.48^{bB}±0.11	3.52^{dB}±0.11
18	ND	7.13^{aA}±0.05	6.14^{bA}±0.08	5.17^{dA}±0.04	5.94^{cA}±0.03	4.86^{eA}±0.08

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected. Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$).

Table 8: Effect of storage periods at ambient and cold storage temperature on total soluble solids (TSS %) of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Ambient temperature (25±2°C)					
	Control	SA	SA+LGE	SA+ Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	5.14^{aB}±0.03	5.11^{aC}±0.02	5.13^{aC}±0.02	5.12^{aC}±0.01	5.12^{aC}±0.01	5.11^{aC}±0.005
3	6.50^{aA}±0.05	5.53^{bB}±0.04	5.49^{bB}±0.03	5.35^{cdB}±0.02	5.44^{bcB}±0.03	5.29^{dB}±0.01
6	ND	6.12^{aA}±0.04	6.01^{bA}±0.03	5.76^{dA}±0.01	5.88^{cA}±0.02	5.63^{eA}±0.01
Cold storage temperature (5±1°C)						
0	5.14^{aE}±0.03	5.11^{aG}±0.03	5.13^{aG}±0.01	5.12^{aG}±0.01	5.12^{aG}±0.01	5.11^{aG}±0.005
3	5.59^{aD}±0.02	5.25^{bF}±0.02	5.21^{bcF}±0.02	5.17^{cF}±0.01	5.18^{cF}±0.01	5.15^{cF}±0.01
6	5.87^{aC}±0.01	5.43^{bE}±0.01	5.36^{cE}±0.02	5.30^{dE}±0.01	5.32^{cdE}±0.01	5.27^{dE}±0.01
9	6.26^{aB}±0.02	5.68^{bD}±0.01	5.52^{cD}±0.01	5.47^{deD}±0.01	5.49^{cdD}±0.005	5.43^{eD}±0.01
12	6.80^{aA}±0.02	5.90^{bC}±0.02	5.69^{cC}±0.01	5.62^{dC}±0.01	5.67^{cdC}±0.01	5.49^{eC}±0.005
15	ND	6.22^{aB}±0.01	5.86^{bB}±0.02	5.80^{cB}±0.01	5.83^{bcFB}±0.01	5.74^{dB}±0.005
18	ND	6.52^{aA}±0.01	6.12^{bA}±0.01	6.00^{cdA}±0.01	6.05^{cA}±0.02	5.97^{dA}±0.01

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected. Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$).

Table 9: Effect of storage periods at ambient and cold storage temperature on the pH values of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Ambient temperature (25±2°C)					
	Control	SA	SA +LGE	SA +Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	6.12^{aB}±0.06	6.12^{aC}±0.05	6.11^{aC}±0.05	6.12^{aB}±0.03	6.13^{aC}±0.04	6.11^{aB}±0.02
3	6.53^{aA}±0.07	6.36^{abB}±0.06	6.33^{bB}±0.05	6.21^{bAB}±0.04	6.29^{bB}±0.05	6.19^{bAB}±0.03
6	ND	6.61^{aA}±0.05	6.53^{abA}±0.04	6.31^{cA}±0.02	6.50^{bA}±0.02	6.28^{cA}±0.01
Cold storage temperature (5±1°C)						
0	6.12^{aD}±0.06	6.12^{aE}±0.06	6.13^{aD}±0.05	6.12^{aE}±0.04	6.11^{aE}±0.05	6.11^{aE}±0.04
3	6.28^{aC}±0.05	6.17^{abDE}±0.04	6.15^{abD}±0.04	6.13^{bDE}±0.02	6.15^{abDE}±0.03	6.12^{bDE}±0.02
6	6.38^{aBC}±0.05	6.22^{bDE}±0.04	6.20^{bD}±0.04	6.17^{bDE}±0.02	6.19^{bDE}±0.03	6.14^{bDE}±0.02
9	6.49^{aB}±0.05	6.29^{bCD}±0.05	6.26^{bCD}±0.03	6.23^{bCD}±0.01	6.25^{bCD}±0.02	6.19^{bCD}±0.01
12	6.68^{aA}±0.04	6.38^{bBC}±0.04	6.35^{bcBC}±0.02	6.29^{bcBC}±0.02	6.31^{bcC}±0.02	6.26^{cBC}±0.01
15	ND	6.48^{aAB}±0.04	6.46^{aAB}±0.03	6.38^{abB}±0.04	6.43^{abB}±0.01	6.33^{cB}±0.01
18	ND	6.60^{aA}±0.02	6.57^{aA}±0.04	6.49^{abA}±0.02	6.54^{aA}±0.02	6.44^{bA}±0.02

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected. Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$).

Table 10: Effect of storage periods at ambient and cold storage temperature on titratable acidity content of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Ambient temperature (25±2°C)					
	Control	SA	SA +LGE	SA +Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	0.18^{aA}±0.02	0.18^{aA}±0.01	0.17^{aA}±0.01	0.17^{aA}±0.01	0.17^{aA}±0.01	0.16^{aA}±0.005
3	0.10^{bB}±0.01	0.14^{abAB}±0.01	0.15^{aA}±0.01	0.16^{aAB}±0.01	0.15^{aAB}±0.01	0.16^{aA}±0.01
6	ND	0.10^{bB}±0.01	0.12^{abA}±0.01	0.13^{abB}±0.005	0.12^{abB}±0.01	0.14^{aA}±0.005
Cold storage temperature (5±1°C)						
0	0.18^{aA}±0.02	0.18^{aA}±0.01	0.17^{aA}±0.01	0.18^{aA}±0.01	0.18^{aA}±0.01	0.17^{aA}±0.01
3	0.15^{aAB}±0.01	0.17^{aA}±0.01	0.17^{aA}±0.01	0.17^{aAB}±0.005	0.17^{aAB}±0.01	0.17^{aA}±0.005
6	0.13^{bBC}±0.01	0.16^{abAB}±0.01	0.16^{abAB}±0.01	0.16^{abAB}±0.005	0.17^{aAB}±0.005	0.17^{aA}±0.01
9	0.12^{aBC}±0.01	0.15^{aABC}±0.01	0.15^{aABC}±0.01	0.16^{aAB}±0.005	0.16^{aABC}±0.01	0.16^{aAB}±0.01
12	0.09^{bC}±0.01	0.13^{aABC}±0.01	0.14^{aABC}±0.01	0.15^{aBC}±0.01	0.14^{aBCD}±0.01	0.15^{aAB}±0.005
15	ND	0.11^{aBC}±0.01	0.12^{aBC}±0.01	0.13^{aCD}±0.005	0.13^{aCD}±0.01	0.14^{aAB}±0.005
18	ND	0.10^{bC}±0.01	0.11^{abC}±0.01	0.12^{abD}±0.005	0.11^{abD}±0.005	0.13^{aB}±0.01

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected. Means values in the same row (as a capital letter) or column (as a small letter) with different letters are significantly different ($p \leq 0.05$).

Table 11: Effect of storage periods at ambient and cold storage temperature on total bacterial count (log CFU/g) of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Ambient temperature (25±2°C)					
	Control	SA	SA +LGE	SA +Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	2.13	2.08	2.00	2.00	2.01	2.00
3	5.98	3.74	3.57	3.40	3.52	3.37
6	ND	5.95	5.18	4.83	5.06	4.71
Cold storage temperature (5±1°C)						
0	2.07	2.04	2.01	2.01	2.01	2.00
3	3.46	3.02	2.79	2.66	2.73	2.58
6	4.64	3.50	3.38	3.19	3.35	3.07
9	5.81	3.97	3.62	3.48	3.55	3.39
12	6.00	4.80	4.75	4.60	4.73	4.45
15	ND	5.85	5.19	4.93	5.06	4.68
18	ND	6.00	5.84	5.06	5.59	4.92

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected.

Table 12: Effect of storage period at cold storage temperature on psychrophilic bacteria count (log CFU/g) of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Cold storage temperature (5±1°C)					
	Control	SA	SA +LGE	SA +Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	2.04	2.03	2.01	2.00	2.01	2.00
3	2.65	2.42	2.38	2.20	2.32	2.14
6	3.51	3.19	3.06	2.94	3.00	2.82
9	4.47	3.84	3.71	3.59	3.67	3.41
12	5.08	4.15	4.10	4.03	4.06	3.95
15	ND	4.56	4.50	4.32	4.38	4.25
18	ND	4.90	4.77	4.55	4.63	4.48

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected.

Table 13: Effect of storage periods at ambient and cold storage temperature on mold and yeast count (log CFU/g) of coated fresh Zucchini.

Storage period (days)	Storage treatments					
	Ambient temperature (25±2°C)					
	Control	SA	SA +LGE	SA +Nano (LGE)	SA+ LUE	SA+ Nano (LUE)
0	0	0	0	0	0	0
3	2.96	1.69	1.50	1.26	1.38	1.20
6	ND	3.75	3.49	3.35	3.46	3.30
	Cold storage temperature (5±1°C)					
0	0	0	0	0	0	0
3	1.22	1.07	0	0	0	0
6	2.73	1.59	1.45	1.36	1.42	1.30
9	3.18	1.95	1.81	1.70	1.78	1.67
12	3.93	2.30	2.18	2.07	2.12	2.01
15	ND	2.85	2.66	2.56	2.60	2.47
18	ND	3.81	3.68	3.38	3.55	3.22

SA: sodium alginate, SA + LGE: sodium alginate incorporated with lemon grass extract, SA+ LUE: sodium alginate incorporated with laurel extract, SA + Nano (LGE): sodium alginate incorporated with Nano lemon grass extract, SA+ Nano (LUE): sodium alginate incorporated with Nano laurel extract, ND: No detected.

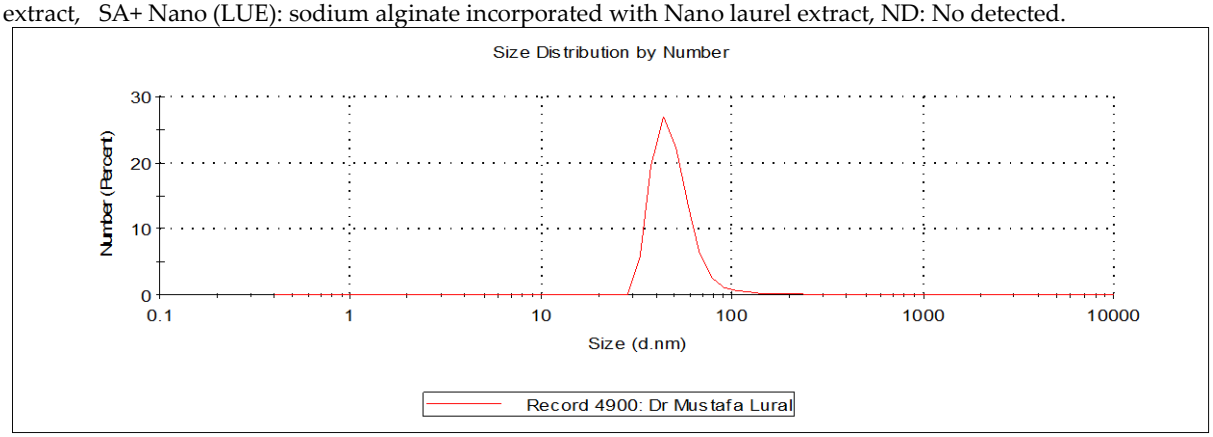

Figure 1: Particle size of Nano emulsion from laurel extract.

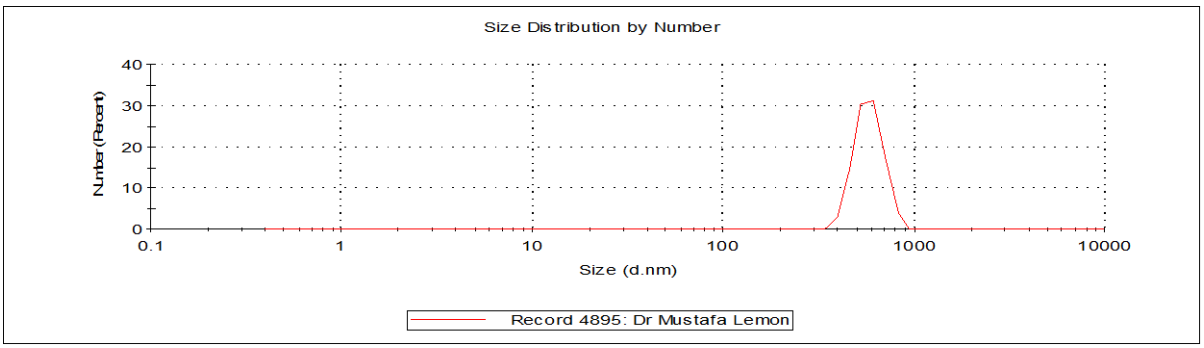

Figure 2: Particle size of Nano emulsion from lemon grass extract.

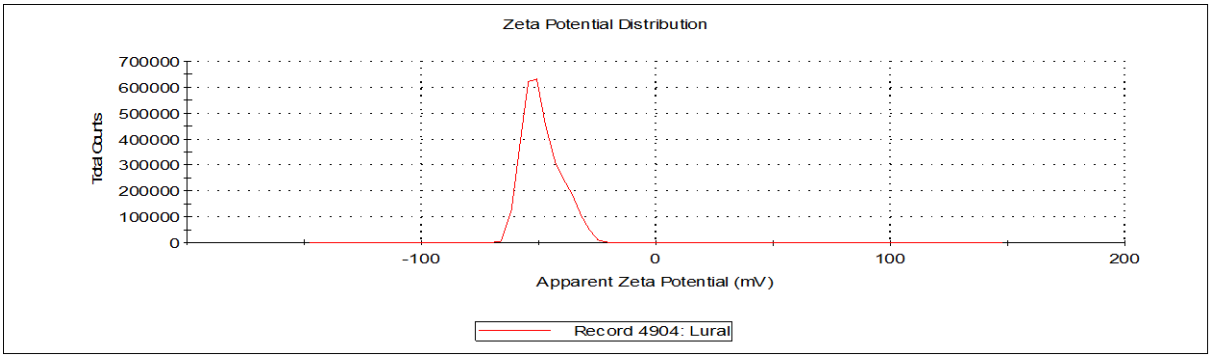

Figure 3: Zeta potential analysis of Nano emulsion from laurel extract.

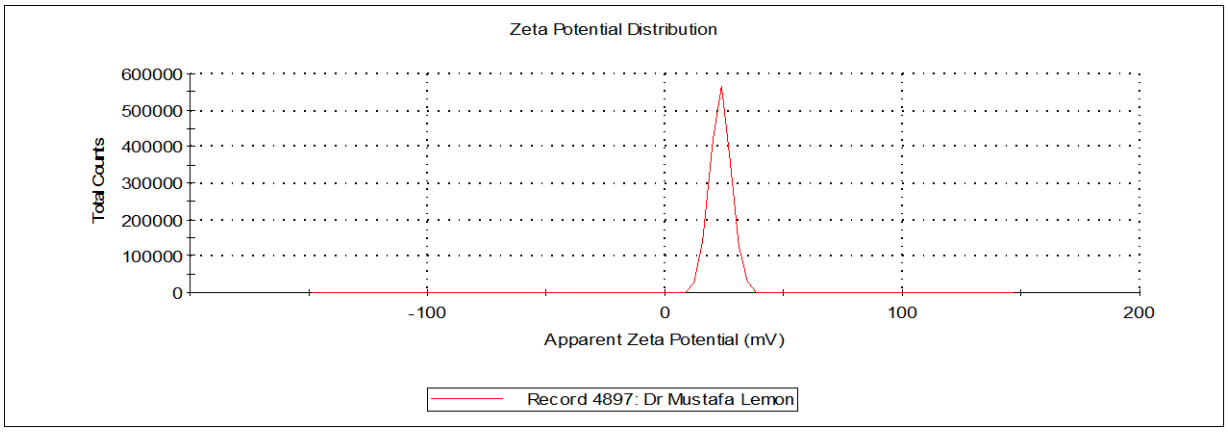

Figure 4: Zeta potential analysis of Nano emulsion from lemon grass extract.

تأثير استخدام مستحلب النانو القائم على المستخلصات النباتية كغلاف صالح للأكل على إطالة العمر الافتراضي للكوسة الطازجة

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الملخص العربي

تعتبر تقنية الأغلفة القابلة للأكل القائمة على مستحلب النانو بديلاً لتحسين جودة الفواكه والخضروات الطازجة. لذا كان الهدف من البحث هو دراسة تأثيرالمستحلب المكون من كل من مستخلص الغارأو مستخلص عشب الليمون مع الجينات الصوديوم فى صورته العادية وكذلك المستحلب فى الصورة النانونية لهذه المستخلصات مع الجينات الصوديوم كمواد مضادة للنمو الميكروبى وكذلك كمواد مضادة للاكسدة, ثم دراسة تأثير استخدام الأغلفة القابلة للأكل من تلك المستحلبات (الصورة العادية مقارنة بالصورة النانونية لتلك المستحبات) على صفات الجودة وإطالة فترة الصلاحية للكوسة الطازجة أثناء تخزينها على درجة الحرارة المحيطة (25±2°م) وكذلك بالتبريد (5±1°م). وقد أظهرت النتائج أن مستحلب النانو من المستخلصات كان أكثر تأثير من المستحلب العادى من نفس المستخلصات على النشاط الميكروبى والتضاد للأكسدة, كما دلت النتائج أن الأغلفة القابلة للأكل كان لها تأثير كبير على جودة الكوسة الطازجة خلال فترات التخزين المختلفة فقد أدت لتأخير فقدان الرطوبة وتقليل التصلب وخفض نمو الأحياء الدقيقة, فقد ظهر أعلى النسب (25.66٪) فى فقدان الوزن بعد 3 أيام من التخزين على درجة حرارة المحيطة (25 ± 2 درجة مئوية) في العينة بدون أغلفة مقارنة بالعينات المغلفة بألجينات الصوديوم مع مستحلب النانوى من مستخلص الغار (5.18٪). أما بعد 12 يوم من التخزين بالتبريد فقد سجل أعلى نسبة فقد للعينة الكنترول (19.15٪) بينما وصلت نسبة الفقد إلى 2.50٪ بالنسبة للعينات المعاملة بألجينات الصوديوم مع المستحلب النانوى لمستخلص الغار. كما ظهر أن العينات المعاملة بألجينات الصوديوم مع المستحلب النانوى لمسخلص الغار أفضل المعاملات فى كل صفات الجودة وإطالة فترة الصلاحية لثمار الكوسة الطازجة من 6 إلى 18 يومًا مقارنة بالعينة الكنترول على درجة حرارة الغرفة أوعلى درجة حرارة التبريد.

الكلمات الاسترشادية: الطلاءات الصالحة للأكل، مستحلب النانو، مستحلب، ألجينات الصوديوم، مستخلص عشب الليمون، مستخلص الغار، الكوسة.