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Preparation of Antibacterial Food Active Package Nano-Biocomposite Edible Film Containing Pectin and Cinnamon Essential Oil Nano-Emulsion.

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ABSTRACT

Edible films based on active ingredients can be used to protect food products. Plant essential oil (EO) reduces the risk of pathogen growth but their low water solubility limits the application in foods. Nano-emulsion can be improving water dispersion, are much more stable and improved delivery of active ingredients than regular emulsions. Nano-composites are being extremely investigated to add interesting characteristics in food stuffs packages. This study aimed to produce biodegradable nano-biocomposites from cinnamon and pectin nano-emulsion for application as active packaging to improve food quality and safety. The nano-composites were analyzed through: Mechanical properties, water vapor permeability (WVP) and bactericidal activities. For mechanical and water vapor permeability analyzes an interesting improve in the film properties were noticed that: In the antibacterial activity test the zone of inhibition with disk diffusion against two strains namely Gram-positive bacteria *Staphylococcus aureus, Listeria monocytogens,* and two strains Gram-negative bacteria *Salmonella typhimurium, Escherichia coli* was analyzed. The test revealed better results for smaller nano-particles.
Keywords: Nano-composite; Cinnamon essential oil; Active Packaging; Pectin; Nano-emulsion; antimicrobial properties.

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INTRODUCTION

Nano-emulsions are met stable submicron oil-in-water dispersions. Nano-emulsions play as delivery factors for lipophilic bioactive compounds such as flavors and antimicrobial agents in the food industry, for drug in the pharmaceutical production, for pesticides in agrochemical industry and as vehicle for skincare [1]. Nano-emulsions are a suitable way to delivery bioactive compounds in food products because of compatibility with ingredients and interesting microorganism inhibition [2].

The application of edible coatings can protect food products from external environment enhance the quality of food, resulting in shelf-life extension and in protecting consumers from foodborne outbreaks [3]. In spite of, active packaging changes conditions to expand shelf-life duration or to ameliorate some properties of food products [3], so that it gives functional properties, innovative alternative for food processing and productions.

Active packaging announces an innovative and feasible alternative for food processing and distribution, which is by gradually liberation antimicrobials and consumed with the food.
A rout in which active packaging interacts with food is by gradually liberation antimicrobials agents from packaging material instead of adding them into food matrix [4-5-6].

For example, pectin is a heterogeneous grouping of acidic structural poly saccharides, it may do serve as a material in matrix for production of friendly environmental and biodegradable edible film and to contribution exploit fruit processing wastes. Now, it increasing trend in using underutilized food processing byproducts and wastes for edible film production [7].

Pectin is a polysaccharide present in the cell wall of all higher plants, widely used as an ingredient in food products: jams, jellies, confectionery products, bakery fillings, as a stabilizer in milk products or even as a thickener improver for texture in sauces and other products [8]. By the absorption of water is possible to form films with pectin [9]. As a polysaccharide without danger for ingestion there are uses of this film in edible packages [10-11-12].

Antioxidants, antimicrobials, nutraceutical compound [13], and essential oils (EOs) from most of spices (garlic, lemongrass, cinnamon, clove bud, and oregano), can be combined with edible films to modify the functionality of films against food pathogens [14-15-16-17-18].

The application of apple-based edible films can reduce growth of pathogenic bacteria (e.g., *Escherichia coli*) when combined with lemongrass, oregano, or cinnamon EO, even though their major constituents (citral, carvacrol, and cinnamaldehyde, respectively) and minimized growth of *Listeria monocytogenes*, and *E. coli*, *Salmonella enteric* [19], delivered a better antimicrobial activity [20-21]. Also, edible films containing EO or certain oil compound (OC) may have improved barrier properties [22] as an antioxidative barrier [23-24] and mechanical properties [15].

EOs are liquids from plants material with oily and aromatic characteristics, which have interesting antibacterial activities against food pathogens and food spoiling microorganisms. Being a natural product makes this product attractive for use as a food preservative in the food industry, as consumers have become weary of synthetic additives recently [25-26]. Essential oils also have interesting characteristics in film formation [27]. Among the properties of essential oils are antifungal, antiviral and antibacterial activities [28-29-30-31-32]. A way to use nano-emulsions of essential oils is using ton film in contact with the desirable protected product obtaining inhibition of microorganism growth [33].

Several studies demonstrated that plant essential oils (EOs) including cinnamon (*Cinnamomum zeylanicum*) EO inhibit or delay the growth of pathogenic microbes [34-35-36-37-38-39-21].

Many food formulations included Cinnamon EO, thus it fits desirable sensory characteristics [40]. This EO is illustrated as generally recognized as safe (GRAS) for human consumption [41]. Antimicrobial activity effect of EOs contain certain oil compounds (OCs) in higher ratio, which are themselves known to exhibit pronounced antimicrobial properties, such as cinnamaldehyde in cinnamon EO [42].

The objective of the present study is to test mechanical properties, water vaporperme ability, thermal and antibacterial analysis of a pectin film with dispersed cinnamaldehyde nano-emulsion. The focus on this study is to produce and characterizes an edible pectin film with dispersed cinnamon nano-emulsion in order to use it as package material.

MATERIALS AND METHODS

Materials

The emulsifying agent, Polyoxyethylene (20) sorbitanmonooleate, commercially known as Tween80, cinnamaldehyde (>93%). Low methyl ester pectins (LMP) were kindly provided by CP Kelco (Atlanta, Ga., U.S.A.).

Methods

Emulsion preparation

Direct oil-in-water (O/W) emulsions were prepared by adding 2% (w/v) of cinnamaldehyde and 1.5 % (w/v) of Tween 80 to ultrapure water, followed by mixing in a T25 Ultra-Turrax® (IKA®Werke GmbH and Co, Staufen, Germany) at 12,000, or 16,000 rpm for 4 min to obtain different droplet sizes.

Particle size and short-term stability

The average particle size and size distributions of the emulsion droplets were determined by dynamic light scattering in a Zeta sizer Nano Series twice: 1 and 72 h after its preparation.

Film preparation

Solutions of either LMP 2.3% (w/v) were prepared by dissolving powdered pectin in ultrapure water and mixing until complete solubilization, the solutions were also incorporated with previously formed emulsions, producing by 12000 and 16000 rpm. The compositions of the film-forming solutions are presented in Table 1. In this technique, a portion of the film suspension is poured onto acrylic plates, and then, dried in a room temperature. Dried films were cut, peeled from the casting surface, and stored folded in aluminum foil within air-tight sealed plastic bags. Four films were produced for each treatment.

Mechanical properties

Film thickness was measured with a Mitutoyo digital micrometer (Mitutoyo Corp., Kanogawa, Japan) to the nearest 0.01 mm, at five random positions. The mechanical attributes tensile strength, elastic modulus, and elongation at break were measured using an EMIC Universal Testing Machine according to standard method of American Society for Testing and Materials [43]. The films were equilibrated at 50 ± 2% relative humidity (RH) for 48 h before testing. A 0.1 kN load cell was used and the cross-head speed was set at 10 $mm.min^{-1}$. At least 8 replicates were performed for each sample.

Water vapor permeability

Water vapor permeability (WVP) was determined by modification of the ASTM [43], a gravimetric method to determine the relative humidity (RH) at the film underside, according to McHugh [44]. Samples without defects were shaped into circles and mounted in poly (methyl methacrylate) flat-bottom cups with 50.8 mm-dia openings, which were filled with 6 ml, deionized water to create a high percentage RH environment inside the cups. Four screws symmetrically located around the cup circumference were used to seal the cup base to a ring, and silicon sealant was used to prevent water vapor loss through gaps. The cups were held in temperature-controlled cabinets at 25 ± 1ºC containing fans to ensure a uniform 0% RH maintained by anhydrous calcium sulphate. Eight weights were periodically taken for each test cell after steady state was reached. WVP of each film was calculated according to McHugh [44] and the final value, in $g\ mm\ K^{-1}\ Pa^{-1}\ h^{-1}\ m^{-2}$, was given as the mean and standard deviation of 5 replications.

Bacterial cultures

The tested microorganisms were provided from the culture collections of the Microbiological Dept. National Research Center (NRC) Dokki, Giza, Egypt. These include two strains Gram-positive bacteria *Staphylococcus aureus* (ATCC 43300), *Listeria monocytogens* (ATCC 35152), and two strains Gram-negative bacteria *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 27325).

Microbiological analysis

To investigate the antibacterial activity of films, 1 cm diameter disks were cut from different composite bioactive films and placed on inoculated tryptone soy agar (TSA) medium. 0.1 ml of diluted inoculum (10^7 CFU/ml) of test organism was spread on tryptone soy agar (TSA) plates. Dishes were incubated at 37ºC for 24 hours. Data was expressed as growth inhibitory zone diameter (mm) for three replicates [45].

Statistical analyses

Data were analyzed using Minitab 14.12.0 (State College, PA) by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests at 95% confidence level.

RESULTS AND DISCUSSION

The visual properties of Nano-emulsions (NEs) had analogous to other published studies by Rao and Mcclements, [46]; Ghosh [1]. For instance nano-particle size was estimated by DLS measurements, and the results are shown in Table (1) focus that the function of stirring speed effect on the nano-emulsion sizes may be explained based on the fact that an increase in the stirring speeds, and consequently in the agitation of the system, also due to the formation of smaller nano-particles. High-forces inputs create deforming that overcome pressure and brake droplets into smaller ones [47].

Nano-emulsion size remained unchanged in range 72 h after emulsification (Table 1), illustrating that the stability will not be lost through droplet adhesion. In nano-emulsions, the sedimentation is less likely to occur, compared to conventional emulsions. This fact is favorable to work with nano-emulsion of essential oils. The films formed with pectin and cinnamon nano-emulsion showed good handling, homogeneity and continuity properties [48]. Table (2) shows the strain values for the different studied films. It is observed that the films with the highest elongation are the films with lesser nano-emulsion included. With the addition of cinnamon nano-emulsion in pectin films increases the elongation value. The elongation increased in the film is an important factor because it is directly related to the increased adhesion strength and tenacity of the films. Tenacity measures the amount of energy a material can absorb before fracturing. More tenacious films exhibit a greater deformity capacity before rupture [5].

Table 1: Particle size of cinnamon aqueous emulsions submitted to different homogenization speeds.

Rotation speed (rpm)	1 h		72 h	
	D^a (nm)	PdI^b	D^a (nm)	PdI^b
12000	122.85 ± 8.47^d	0.216 ± 0.023^g	119.50 ± 3.07^d	0.205 ± 0.020^g
16000	42.35 ± 2.05^e	0.227 ± 0.014^g	46.98 ± 0.25^e	0.220 ± 0.019^g

D^a (nm): cumulant mean (z-average) particle size, reported as mean value ± standard deviation.
PdI^b, polydispersity index, reported as mean value ± standard deviation.
$D^{c\text{-}e}$ (nm) values followed by different letters are significantly different (p<0.05).
$PdI^{f\text{-}g}$ values followed by different letters are significantly different (p<0.05).

Table 2: The strain values for the different films studied.

Film	Elongation (%)
LP	18.13 ± 2.72
LP12000	43.00 ± 2.95
LP16000	58.05 ± 3.02

The interaction of nano-emulsions and polymeric matrix can influence the mechanical properties of the film [49]. The tensile strength (TS) of films containing different nano-emulsions is shown in Figure (1). The results showed that incorporation of nano-emulsion into film caused a significant increase of TS compared to that of control (without nano-structure).

This unimportant fortified attitude because carvacrol had already been exhibited yet [17] and cinnamon EO in pectin/apple mash films [20] and garlic EO in alginate edible films [50].

Chemical similarity makes particle-matrix with strong interaction, and when sprinkled, the nano-particles size act as setting centers, reducing the chain mobility and enhance an improvement in tensile strength.

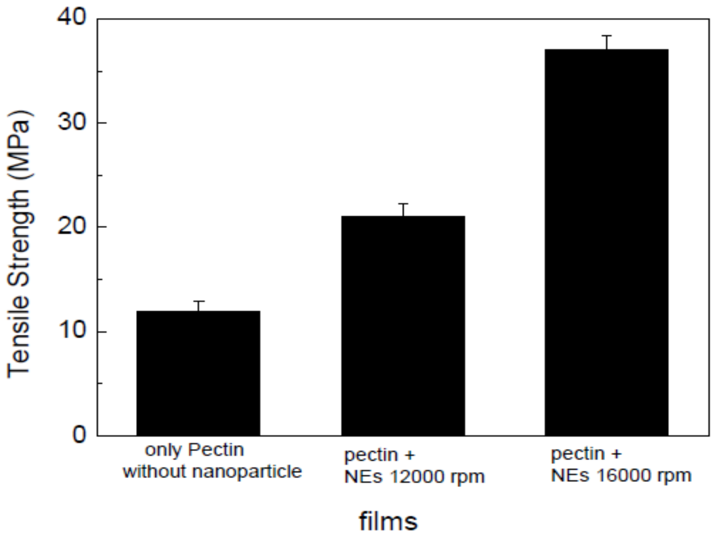

Figure 1: Tensile property of pectin films with nano-emulsion.

Nano-emulsion with increased sizes has decrease in the resistance. On the other hand, when the sizes of these nano-emulsions were high, adding them to film may induce the development of a heterogeneous structure with the presence of discontinuous areas, producing low tensile strength which can be demonstrated by the greater particle size structure and smaller TS. In the study Li *et al.*, [51], the authors suggested that an increase in the aggregation of the molecules (a more compact molecular structure) increases the tensile strength. Such an argument also applies here, where the smaller nano-particles disperse more easily in the matrix, facilitating interactions with the matrix as a whole and favoring compaction, thereby increasing the tensile strength values.

In most cases, the function of a pack in the industry reduces the interaction between the environment and food. Also, slowing loss of the water increase its food shelf life. The estimation of WVP already provides additional information that is useful when identifying potential applications for these films. Thus, WVP should be as low as possible.

Table (3) presents the WVP of all samples in this study. The WVP of films incorporated with different nano-emulsions had significantly difference compared to control ($p > 0.05$). The relative humidity at the film underside was not significantly different (70.9 ± 1.0% RH) for the three different films as follow:

Table 3: The effect of presence nano-emulsions on WVP and RH % at film.

Film	WVP ($g\ mm\ K^{-1}Pa^{-1}\ h^{-1}\ m^{-2}$)	RH at film underside (%)
LP	3.14 ± 0.02	72.2 ± 0.2
LP12000	1.50 ± 0.11	71.1 ± 1.0
LP16000	1.20 ± 0.10	70.9 ± 1.0

Nano-particles in the films reduce the WVP of the pectin films. The decrease on the nano-emulsion size causes a decrease in the values of WVP. WVP value of pectin film without nano-particle is about 3.14 ± 0.02 g mm K^{-1} Pa^{-1} h^{-1} m^{-2}. Incorporation of nano-emulsion decreased the WVP of pectin films to a considerable extent. The structure of films including nano-emulsion was more hydrophobic and compact influencing a reducing of water permeability of pectin films.

Essential oil's chemical structure plays a significant act in the barrier properties of edible films. Hydrophobicity can be explained variation observed by addition of different plant EOs. Nano-emulsion with lower size particle present a greater reduces in values of WVP. This fact is due to the presence of more hydrophobic structures in the matrix, dispersed in more homogenous way [52].

EOs can be classified the microbiological activity in three grade of inhibition: weak activity (zone of inhibition ≤ 12mm), moderate activity (12mm ≤ zone of inhibition ≤ 20mm) and strong activity (zone of inhibition ≥ 20mm) [53-54]. The results of microbiological activity obtained in Table (4) have been done by disc diffusion method for Gram-positive and Gram-negative bacteria. In the results it is possible to see that the particle size of the aqueous emulsion interferes in the microbiological activity, obtaining better results with smaller sizes of aqueous emulsion, it's submitted at 16000 rpm homogenization speed.

Table 4: Inhibition zone of nano-composites active films against G^+ and G^- bacteria.

Bacterial strains	LP 12000	LP 16000
Staphylococcus aureus (ATCC 43300) (G^+)	26±2	35±3
Listeria monocytogens (ATCC 35152) (G^+)	28±4	38±2
Escherichia coli (ATCC 27325) (G^-)	18±1	22±2
Salmonella typhimurium (ATCC 13311)(G^-)	20±2	23±3

Results are expressed as mean ± standard deviation

The highest inhibition halos were noticed for the gram positive (G^+) bacteria. The distribution Nano-particles can be better dispersed into the Nano-compounds surface favoring their interactions with the culture [5-55].

Among the tested bacteria, this is illustrated in Table (4), which presents that G^+ bacteria (*L. monocytogenes* and *S. aureus*) were more greater inhibition zones than G-ones (*S. typhimurium* and *E. coli*) due to the active compound of cinnamaldehyde that migrate from films and penetrate microbial cells.

G^- Bacteria have an outer lipophobic membrane comprising lipopolysaccharide molecules that role as block to hydrophobic compounds (e.g., cinnamaldehyde). But in G^+ bacteria such membrane is absent allowing that cinnamaldehyde to infiltrate bacteria cell faster and led to presenting the active compound with highly inhibition effect.

This minimize, though does not block, the penetration of lipophilic compounds [56-57]. Such membrane is absent in G^+ bacteria, allowing cinnamaldehyde to infiltrate bacteria cell faster and hence presenting the active compound with highly inhibition effect. Comparable notice were demonstrated by Fisher and Phillips [56], Ojagh *et al.*, [58], and Peng and Li [59].

Smaller droplets improved the antimicrobial activities which attributed to a better ability of the active compound of cinnamaldehyde. This is supported by Huang *et al.*, [60], as a characteristic of nano-emulsions over native emulsions the facilitated penetrated of phytochemicals through cell wall; give rise to increase bioavailability by elevated phytochemical compounds in plasma.

Antimicrobial activity mentioned in previous studies on essential oils influence in culture media showed some inhibitory effect on certain pathogen bacteria [61-62]. Antibacterial activity of rosemary essential oil contra some food-borne pathogens in liquid media was reported by Seydim and Sarikus, [62] and Pintore *et al.*, [63].

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CONCLUSION

The nano-composites film has many characteristics as good tensile strength and barrier properties against pathogens were successfully obtained and used in foodstuff production package. Presence of cinnamon essential oil increased the tensile strength of the film from 12 ± 0.9 MPa to 37 ± 1.4 MPa. The decrease of WVP observed in the nano-composite system means an interesting improvement for product quality and shelf stability. The antibacterial activity provided by cinnamaldehyde against food pathogens was uncommonly improved by droplet size reduction due to increased surface area. Depending on the results presented here, we can emphasize the significance of having higher bacterial inhibition with the same amount of active compound in an effort to match consumers' demands for food products with lower preservative contents but still safe to consume. We also demonstrated a bactericidal potential with some bacteria for the nano-composite films, in which the nano-particle size interferes in the zone of inhibition. The latter indicates that the Pectin/cinnamon nano-composite can be used in food packaging as antibacterial agent in internal coating. The essential oils nano-emulsion perhaps recommended for manufacturing of packaging materials based preservatives for longest time of shelf life of food items during post-harvest processing also stimulates the interest in future work in the treatment of burns and cuts in the medical field regarding antimicrobial activity.

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