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Nano-Encapsulation of Bioactive Oils through Emulsion Polymerization as Antimicrobials and its adhesion to packaging films

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ABSTRACT

Bioactive essential oil components, aldehydes, terpenes and phenols can be encapsulated into nanosized emulsified droplets. Nano-encapsulated bioactive oils emulsions were prepared by high amplitude ultrasonic homogenization with food grade ingredients. The effect of delivery system on the antimicrobial activity of different oils components, e.g., carvacrol, lemon grass and cinnamaldehyde, was investigated by determining the antimicrobial activity of three different groups of microorganisms (Lactobacillus delbrueckii, Saccharomyces cerevisiae and Escherichia coli). The incorporation of bioactive oils into nano-encapsulation emulsion resulted in a significant increase in the antimicrobial activity, which significantly depended on physicochemical properties of the delivery systems, such as emulsifier composition and the mean droplet size. A simulation for food ingredients was performed using bioactive oils in presence of different emulsifiers was performed to examine their adhesion to different hydrophobic packaging films (polypropylene, metallized polypropylene and low density polyethylene). The effect of surface hydrophobicity on the adhesion to packaging films was studied. The increased hydrophobicity increases the adhesion to packaging films.

Keywords: Packaging, Antimicrobial activity, Nano-Encapsulation, Bioactive Oils, Emulsion and Adhesion

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INTRODUCTION

The use of environmental controls to inhibit pathogens or saprophytes is an important approach to preserving food which can compete with aseptic treatment and packaging, removal of microorganisms mechanically by washing or filtration and annihilation of microorganisms by chemical or physical sanitization. Further, this is a contribution that benefited most from the modern developments of nanotechnology [1].

Moreover, there is a great demand for antimicrobial products in food packaging, e.g., the recent studies have revealed that the presence of campylobacter on 34% of packaging and its survival in many gas mixtures that commonly used in gases packaging of food [2]. Therefore, as a result of the increased resistance of bacteria against antibiotics, there is a great interest for preparation of antimicrobial products and find acceptable explanation to the mechanism of bacterial adhesion to the different substrates.

Specifically, the addition of natural compounds inhibits microbial growth such as essential oils, a significant inhibitory or fungicide/bactericidal activity [3].

Unfortunately, the addition of chemically reactive species to complex food systems, such as antimicrobial agents, may cause negative effects on the integrity or physical stability of the food chemistry, in addition to the degradation of the biological activity of bioactive compounds [4]. Therefore, the amount of antimicrobial compounds needed to hinder microbial growth within the imposed limits by food regulations exceeds the amounts that ensure a minimal alteration of the product's qualitative properties.

Currently, nano-encapsulation of the essential oils reflects an efficient approach to the increased physical stability of the active substances; protects them from the interactions with food ingredients and increases their bioactivity. This may due to the subcellular size which induces passive mechanisms of absorption through the cell membrane. Additionally, the nano-encapsulation can increase the concentration of bioactive compounds in food where microorganisms are preferably located, e.g. liquid-solid interfaces or water-rich phases [3]

Furthermore, it was reported that many oils of antimicrobial effect can be considered as valuable alternative that can be used to oppose foodborne pathogens, meanwhile they may reflect excellent preservative effects [5]. These oils require the use of high concentrations to achieve an equivalent antimicrobial efficiency in food products.

Many encapsulation systems of different formulations, size distributions and effects were examined for the delivery of different essential oils in foods. E.g., the use of a micrometric emulsion was examined to reduce the volatility of the used antimicrobial agent, hence protecting active compounds against environmental factors [6].

The improvement of the antimicrobial activity of the encapsulated essential oils into liposomal delivery systems is considered a proof of these concepts [7-8]. Moreover, the encapsulated eugenol and carvacrol into nanometric surfactant micelles also enhances the antimicrobial activity [9].

Adhesion of emulsions and oils on different packaging surfaces like glasses and stainless steel have been studied [10]. Moreover, the adhesion of food materials to industrial equipment and packaging surfaces reviewed [11]. In addition to, there are many studies have been performed to this topic and also were applied to precise food products.

This work focuses on the formulation and fabrication of nanoemulsion-based delivery systems for the encapsulation of selected bioactive essential oils. Some bioactive essential oil components were tested and compared to a mixture of terpenes extracted from Melaleucaalternifolia. Also, it aims to improve the antimicrobial activity and to measure these antimicrobial activities inside packaging materials and measure the adhesion power of the nano-emulsion formulations of these bioactive essential oils to different hydrophobic packaging films.

MATERIALS AND METHODS

2.1. Emulsion preparation and characterization

The tested antimicrobial compounds are Lemon grass, Carvacrol and Cinnamaldehyde (Sigma-Aldrich, Germany) and a mixture of terpenes that was received as a gift from the oils and fat department in National Research Center, Egypt. In emulsion fabrication, sunflower oil was added to the essential oils. Soy lecithin Solec IP, Tween 40, glycerol oleate (Sigma-Aldrich, Germany), CLEARGUM® CO 01, pea proteins F88M and sucrose esters P-1770 (generous gifts from Genetech, Egypt) were used as emulsifying agents.

All oils (sunflower oil or essential oil) in water nano-emulsions were prepared using an homogenization technique. High Shear Homogenization was used to prepare primary emulsions using an Ultra Turrax T25 (IKA Labortechnik, Germany) at 24000 rpm for 5 min.

Droplet size distribution was determined by DLS at 25°C (Malvern Instruments, UK). From the DLS data, the average droplet diameter (z-average) was determined. The samples were diluted with Millipore water to a suitable concentration prior to any measurements being taken. Each measurement was replicated twice, and the mean and the standard deviation were calculated.

2.2. Microbial inactivation tests

All experiments were performed on three different microbial strains, including Saccharomyces cerevisiae (ATCC 16664), Escherichia coli (ATCC 26) and Lactobacillus delbrueckii (ATCC 4797), were grown to a stationary phase

in an aerated incubator (Haeraeus Instruments) S. cerevisiae yeast was grown in MRS broth at 30°C for 2 days, E. coli in Tryptone Soya broth at 30°C for 16-24 h and L. delbrueckii was grown in in MRS broth at 32°C for 2 days.

Inactivation tests of the chosen three microorganisms in the presence of encapsulated antimicrobial agents were performed at 0.1% antimicrobial concentration, which was previously shown to be as a sufficient concentration to elucidate a bacteriostatic or a bactericidal action when compared to a control where sunflower oil was chosen instead of antimicrobial agents [12].

The microorganisms were centrifuged at 6500 rpm for 5 min at 4°C and re-suspended in sterile distilled water to a final concentration of 104 cfu/ml in test tubes, where the nano-emulsions were added to the desired final antimicrobial concentrations.

After that, the test tubes were incubated at 32°C for S. cerevisiae and L. delbrueckii and at 30°C for E. coli. After 2 h and 24 h, the surviving cells were simply evaluated by the standard plate count method. In brief, 1 ml of each sample was used to prepare decimal dilutions, that were plated in duplication with Plate Count agar for E. coli and MRS agar for S. cerevisiae and L. delbrueckii. The plates were then incubated at 30°C for 24 h for E. coli and at 32°C for 48 h for S. cerevisiae and L. delbrueckii.

2.4. Contact angle of packaging films:

Contact angles of non-metallized films before and after pre-treatment were measured by the sessile drop technique using a DSA 10 goniometer (Kruss GmbH, Germany). MilliQ-H2O was used as test liquid with a drop volume of 10 µl. All values are the average of five measurements per sample.

2.4. Nano-emulsion adhesion to packaging film:

The amount of adhered nano-emulsion to the hydrophobic packaging films (Wad) was measured by a simple designed device according to (Omar, Saber, 2014). The prepared nano-emulsion was simply held back at the middle to of a 80o tilted surface of packaging films (polypropylene, metallized polypropylene and low density polyethylene). Consequently, it was allowed to flow down and the weight remaining on the film surface after flow had stopped was finally measured using digital precision balance, then the adsorbed weight was calculated according to equation 1.

W_{ad}=([(solid weight before flow – solid weight after flow)])/((surface area)) $g.m^{-2}$ [1]

RESULTS AND DISCUSSION

Stable nano-emulsions encapsulating the antimicrobial compounds were produced by different formulations and fabrication methods. Generally, the nano-emulsions contained 3% w/w of the active compounds, when essential oil were used, that were mixed in the oil phase (sunflower oil), or 3% w/w active compounds, when the mixture of terpenes was used (table1).

TABLE-1
FORMULATION AND DROPLET DIAMETER OF THE NANO-ENCAPSULATED EMULSION WITHDIFFERENT EMULSIFIER.

	TP, %	CIN, %	LG, %	CA, %	Sun Flower, %	Emulsifiers, %	Droplet size, nm
SA0	3	---	---	---	---	3, soy lecithin	79
SA1	---	3	---	---	7	3, soy lecithin	193
SA2	---	3	---	---	7	3, Pea protein	224
SA3	---	3	---	---	7	1, glycerol +1, Tween 40	241
SA4	---	---	3	---	7	3, soy lecithin	249
SA5	---	---	3	---	7	3, Pea protein	184
SA6	---	---	3	---	7	1, glycerol +1, Tween 40	236
SA7	---	---	---	3	7	3, soy lecithin	219
SA8	---	---	---	3	7	3, Pea protein	227
SA9	---	---	---	3	7	1, glycerol +1, Tween 40	236

Table 1 reports all prepared nano-emulsions that physically was stable over 4 weeks with no visible creaming or any significant variation in mean droplet diameter.

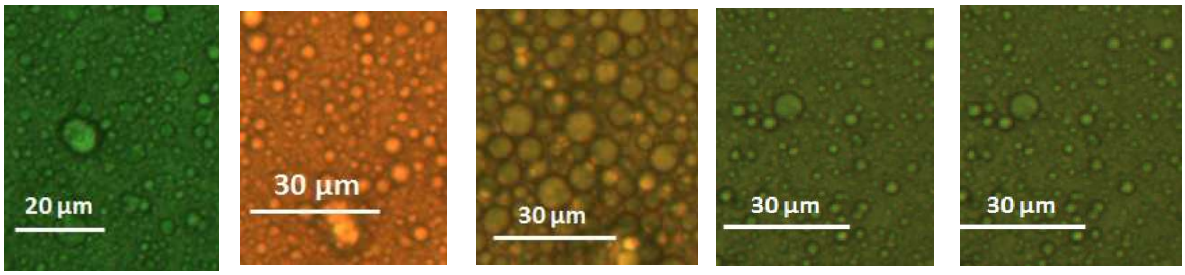

Fig. 1.Morphology nano-encapsulated emulsion of bioactive oils stabilized by soy lecithin

The microscopic pictures of the resultant nano-encapsulated emulsion with only soy lecithin as emulsifier (as the lowest droplet size were obtained) are shown in Fig. 1, for different bioactive oils. It is observed that particles are homogeneous with a spherical form.

The antimicrobial activity of incorporated essential oils in nano-emulsion-based delivery systems was evaluated on three different microorganism species in comparison with control nano-emulsions, where the essential oil was replaced by sunflower oil. Specifically, the results were reported as incremental inactivation that caused by the encapsulated formulation and were calculated as the ratio between the survival after 2 h of microorganism exposure to the Nano-encapsulated antimicrobial agents (0.1% w/w concentration of the active compound) and the survival of microorganisms exposed to the control nano-emulsions for the same time. The reported incremental inactivation was limited to 100 to enhance the readability of the graph.

The increase of antimicrobial activity significantly depends not only on the antimicrobial itself, but also on the Nano-emulsion formulation. Mostly, limonene-based formulation was not significantly active against E. coli, with the exception of SA5 and SA6 Nano-emulsion formulations. On the other hand, carvacrol oil shows an incredible bactericidal effect for all formulations, followed by cinnamaldehyde and finally by the terpenes mixture.

The antimicrobial activity of the nano-encapsulated agents against L. delbrueckii, showed that carvacrol was very active (especially SA8 and SA9 formulations, with an incremental inactivation >100), while other delivery systems exhibited a mild but positive antimicrobial activity.

In this study, all of the tested nano-emulsion based delivery systems reflected a significant and high antimicrobial activity, except the terpenes mixture that exhibited a mild antimicrobial activity. Moreover, the observed inactivation data refers to a very short exposure, during which some of the chosen essential oil expressed a bacteriostatic instead of bactericidal activity.

It is clear that the antimicrobial activity of cyclic hydrocarbons is restricted by their solubility, and only those molecules that are dissolved in the aqueous phase can be available for interaction with cells [13]. Consequently, the essential oil components, e.g., carvacrol must be dissolved in concentrations approaching or exceeding their maximum solubility to demonstrate bactericidal activity [14]. In contrast, limonene is characterized by a lower solubility than carvacrol and only exhibits bacteriostatic activity only if its concentration in the aqueous phase is increased, e.g., by favorable partitioning between a selected lipid phase and the aqueous, or by solubilization in appropriate surfactant micelles.

The TP formulations added to orange juice was inoculated with L. delbrueckii at two different concentrations (0.1% and 0.5% w/w) and was followed by evolution of the microbial population over 16 days. The results of the accelerated shelf life illustrates that after 2 days, the total inactivation of the initial microbial load of 103 CFU/ml in the chosen orange juice was already reached for the terpenes at concentrations of 0.5% w/w. When terpenes reached a concentration of 0.1% w/w, microorganism growth was delayed by 5 days in comparison to the control.

The addition of the nano-emulsion slightly altered Bx and pH. It was observed during the storage period, unless significant microbial growth appeared (data not reported). Furthermore, the impact of the addition of the terpenes mixture on fruit juice color can be considered acceptable at both tested concentrations, with only slight color

deviations being induced. The stable color over the storage time for all the systems indicates that there is no significant microbial growth occurring.

Furthermore, all formulations (as shown in table 1) consist of sunflower oil that added to the essential oils and the same amount of emulsifiers (soy lecithin, Tween 40, glycerol oleate, pea protein). Our previous studies showed that the adhesion to packaging films was affected by the philicity of the examined films [15]. In this study, the packaging films (LDPE, PP and MPP) are hydrophobic and were chosen as they are the most used packaging films in the market.

TABLE-2
CONTACT ANGLE MEASUREMENTS OFPACKAGING FILMS (LDPE, PP AND MPP)

Packaging film	Contact angle
LDPE	97.6
PP	103.4
MPP	96.2

The results of contact angle measurements (table 2) of the chosen packaging films (LDPE, PP and MPP) reflects that lowest hydrophobic film is MPP, while PP is the highest hydrophobic film and LDPE is of moderate hydrophobicity if it is compared to other films. Moreover, from fig. 2, 3 and 4 it is clear that the adhesion to packaging films was affected by hydrophobicity of the films. Where, the highest adhesion to packaging film was for PP (highest hydrophobic film) and MPP is of the lowest adhesion.

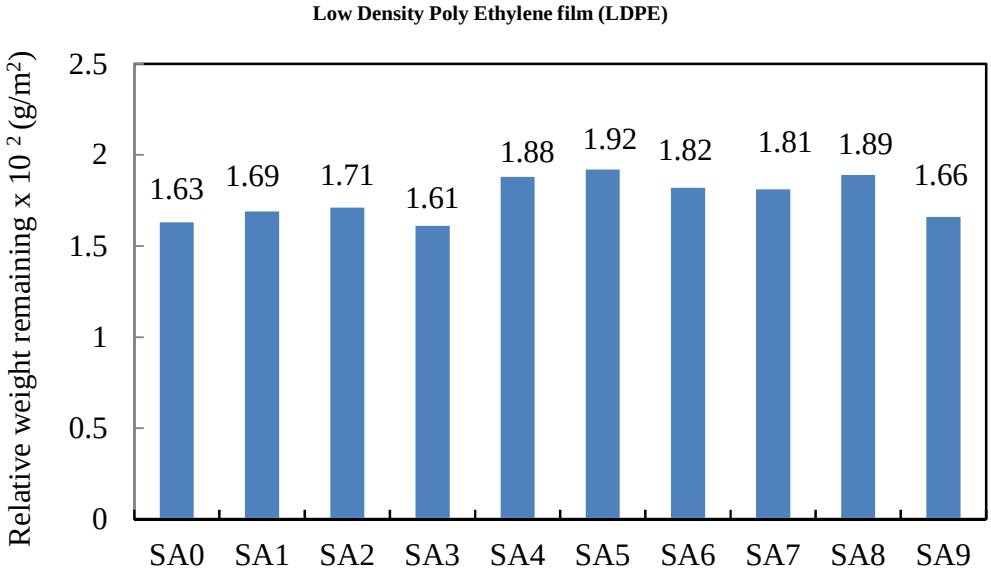

Fig. 2. Effect of low density polyethylene (LDPE) film on the adhesion (g/m^2)

As it is shown in fig. 2, the lowest adhesion to packaging film (low density poly ethylene film, LDPE) was SA3, while the highest adhesion to LDPE film was SA5. The results in fig. 3 reflects that the adhesion to polypropylene film (PP) was higher than LDPE and has the same trend, where SA3 is of the lowest adhesion to PP film and SA5 is of the highest adhesion to PP film.

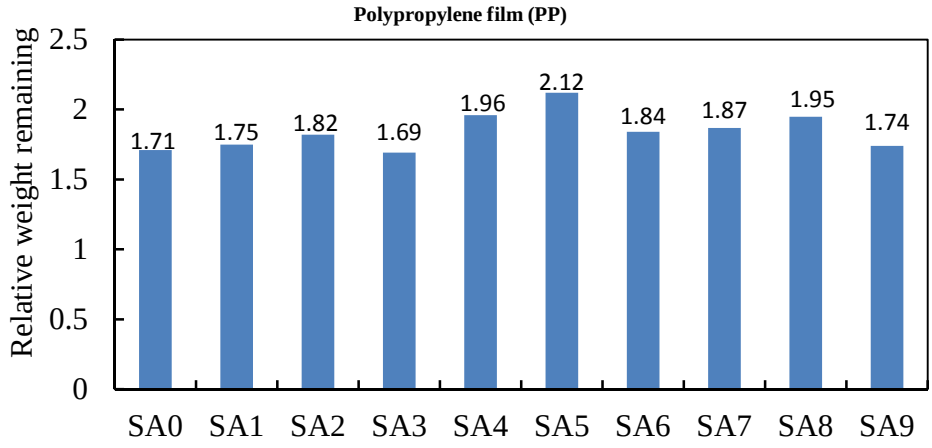

Fig. 3. Effect of non-polar (polypropylene) film on the adhesion (g/m^2)

In addition, as shown in fig. 4, the adhesion to metallized polypropylene (MPP) was the lower than that of LDPE and PP. Besides, the lowest adhesion to MPP film was SA3 and the highest adhesion was SA5.

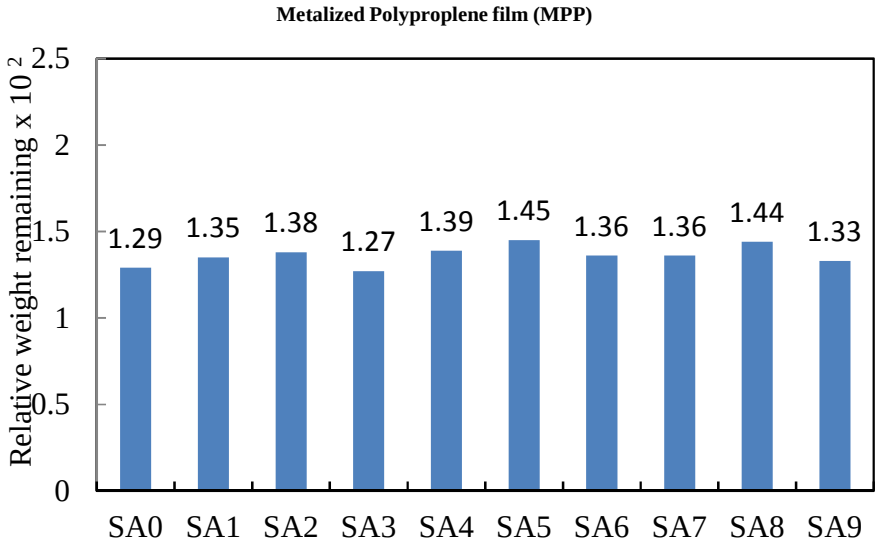

Fig. 4. Effect of metalized polypropylene film on the adhesion (g/m^2)

Generally, the lowest adhesion to packaging films was SA3 for all films, while it was the highest using SA5 for all films. Moreover, the lowest adhesion was observed by using MPP films, while the highest was detected for PP films. Additionally, as it shown if fig 2, 3 and 4 the highest adhesion was for (SA4, SA5 and SA6) formulations, while the lowest was for (SA1, SA2and SA3) formulations for all films (LDPE, PP and MPP). This means that the needed desorption energy is expected to be greater for (SA4, SA5 and SA6) formulations than (SA1, SA2 and SA3). Additionally, the greatest desorption energy was for PP films, while the lowest was for MPP films.

CONCLUSION

Encapsulation of some essential oils to nano-emulsion was investigated as a route to improve the quality and safety of foods through addition of natural preservatives. The resulting antimicrobial activity significantly depended on the essential oil components and on the nano-emulsion formulation. Carvacrol oil was the most active essential oil components, while the addition of Tween 40/Glycerol monooleate or sucrose esters as emulsifiers were the greatest

advantageous in nano-emulsion formulations. The mixture of nano-encapsulated terpenes was tested in orange juice. The addition of low concentrations of the nano-encapsulated terpenes delayed microbial growth (0.1% w/w) or totally inactivated the microorganisms (1 % w/w) while only slightly varying the organoleptic properties of the fruit juices. Metallized polypropylene film was considered to be of the lowest recycling cost due to its lowest adhesion power; hence it is easier to be cleaned than other films (PP and LDPE). This means that it requires the lowest desorption energy, while PP films requires the highest desorption energy. The surface hydrophobicity increases the adhesion to packaging films.

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