



Scientific Research

Comparison of the stability of the nanoliposome form of the citron (*Citrus medica* L.) peel essential oils obtained from different extraction methods during the storage period at 4°C and -18°C

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ABSTRACT

One of the critical methods to maintain the stability and functional properties of plant essential oils as a useful source of bioactive compounds against environmental damage is their encapsulation in nanocarrier systems such as nanoliposomes. In this study, nanoliposome containing the citron peel essential oils were prepared without the use of toxic organic solvent and by employing health-giving compounds such as sesame oil in addition to lecithin for the first time in the formulation. The stability of the samples during 30 days of storage at temperatures of 4°C and -18°C was determined by investigating the retention amount of phenolic compounds, pH changes, antioxidant and antimicrobial performance. The nanoliposomal samples of essential oils of hydrodistillation and supercritical CO₂ of citron peel prepared with different concentrations of lecithin oil had different quantities of pH and phenol retention percentage, and their amount reduced with increasing storage time at both test temperatures. DPPH inhibitory ability and antimicrobial activity of both citron peel essential oils were improved after encapsulation in nanoliposome. But their amount in both storage temperatures decreased with the advancing of time. The nanoliposome of the supercritical fluid essential oil of citron peel respectively with the formulation containing the highest and lowest amount of lecithin oil at the storage temperature of 4°C showed the best result in this study. Therefore, the citron peel essential oil with encapsulation in the nanoliposome system prepared from lecithin-sesame oil, due to improvement of antioxidant and antimicrobial activity and its higher stability against storage temperature, can be used as an effective natural functional additive in the food industry.

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1- Introduction

Balang with a scientific name *Citrus medicine* L., a species of citrus belonging to the family Rutaceae is known as one of the sources of essential oil production. The essential oil of this fruit is a well-known oil with a pleasant aroma that is obtained from the epicarp (outer layer) and its skin [1 and 2]. In traditional medicine, it is used as a herbal medicine to treat diseases [3 and 4]. In previous studies, it was reported that balang skin essential oil is a rich source of bioactive compounds such as phenol and has significant antioxidant and antimicrobial activity [1, 4, 5]. Having favorable functional characteristics and a high amount of health-giving compounds has drawn attention to the use of this essential oil in the food and pharmaceutical industries [1, 6, 7]. However, the use of these valuable compounds freely has limitations due to sensitivity to environmental conditions such as temperature and oxygen during storage and processing, high volatility, low solubility in water, and interaction with food matrix components [8 and 9]. In the study of Rafiei et al. (2017) and Dehghan et al. (2020), it was reported that the phenolic compounds in plant extracts and essential oils are vulnerable to environmental conditions due to the presence of unsaturated bonds in their structure and may be. During food processing and storage, they are subjected to decomposition [10 and 11]. Therefore, the use of appropriate and efficient methods such as encapsulation can protect these valuable compounds from environmental factors, bring about their controlled release, and thus increase their functional properties such as antioxidant activity. [11]. Encapsulation of natural compounds such as plant essential oils in

nanocarrier systems such as nanoliposomes is one of these effective methods that improves their stability and bioavailability [12]. Liposomes are spherical vesicles consisting of phospholipid bilayer, whose structure is similar to cell membrane [13]. Despite the advantages of nanoliposomes, the stability of these nanocarrier systems may decrease due to the destruction of phospholipids in their membrane structure under the influence of environmental stress during storage, thus affecting the performance and stability of the encapsulated bioactive compounds. Storage temperature is one of the factors influencing the stability of nanoliposomes. This can limit the application of nanoliposomes in food and pharmaceutical industries. Therefore, the study of their stability has been the focus of researchers [14-17]. Factors such as the lipid composition of the nanovesicle membrane and the addition of components such as sterols in their formulation can affect the stability of the nanoliposome and the release and performance of the coated substance during storage at different temperatures [14 and 15]. Most of the lipid molecules in the nanoliposome structure are composed of lecithin, which contains various types of phospholipids of natural origin, and due to its high accessibility and safety, and having health-enhancing effects and antioxidant properties, it is a useful material for the production of nanoliposomes in the food industry. It is considered [10, 18 and 19]. Cholesterol has been the most widely used sterol used in membrane lipid composition to increase nanoliposome strength and decrease its permeability. But in recent years, the use of phytosterols has been considered as a potential substitute for

cholesterol due to its favorable nutritional properties and more beneficial effects for health [14, 15, 18, 20]. Therefore, in this study, sesame oil, which is a rich source of phytosterols, contains a high level of unsaturated fatty acids, linoleic acid, and has the antioxidant substances sesamulin and tocopherol, as a lipid component along with lecithin in the preparation of nanoliposomes. took [21 and 22]. Therefore, the aim of this study is to investigate the stability of the functional properties of essential oils distilled with water and the supercritical fluid of the skin of balang loaded in the nanoliposome system prepared with lecithin-sesame oil combination during storage at 4 and -18 degrees Celsius. The present study is the first report on the comparison of the functional characteristics of essential plant sample obtained from different methods of extraction in nanoliposome form with the aforementioned formulation under conditions of refrigerator and freezer storage.

2- Materials and methods

2-1- Raw materials

Food-grade soy liquid lecithin from Amitex (India), sesame oil from Laden (Iran), glycerol, dialysis bag with a molecular weight of 12-14 kilodaltons, DPPH, potassium ferricyanide, sodium phosphate buffer and ferric chloride were purchased from Sigma Aldrich (USA). Folin Ciocalteu reagent, trichloroacetic acid and Mueller Hinton agar culture medium manufactured by Merck (Germany) were used. Bacterial strains including *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27883. They were prepared from Pasteur Institute of Iran.

2-2- Extracting the essential oil of balang skin using different methods of

distillation with water and supercritical fluid

Balang fruit was obtained fresh from the local market of Amol city and its skin was dried and powdered after separation to prepare essential oil. Then the essential oil was extracted by distillation with water using a Cloninger machine for 3 hours. In the supercritical fluid method, extraction of essential oil at a temperature of 35 degrees Celsius and a pressure of 100 bar by a supercritical carbon dioxide device (Suprex MPS/225 Multipurpose system) was done for 30 minutes [5].

2-3- Preparation of nanoliposomes carrying essential oils of balang skin

Nanoliposomes of both types of balang skin essential oil were produced based on thermal method without using organic solvent. First, different concentrations of lecithin-sesame oil (40:60, 50:50 and 60:40% by weight) were prepared and then in order to dissolve these compounds, they were heated on a magnetic hot plate at 30 degrees Celsius with Speedrpm 600 was used for 10 minutes. In the next step, add 0.4 grams of the essential oil of balang skin drop by drop to the lecithin-oil combination at a temperature of 30 degrees Celsius withrpm 1000 was added on the hot plate. Then this mixture was hydrated by adding the aqueous phase containing 1.1 g of glycerol and stirred on a hot plate for 45 to 60 minutes until the mixture was completely smooth and uniform. In the next step, the sample is homogenized using an ultrathorax at a temperature of 30 degrees Celsius in two steps, first with speedrpm 12,000 for 15 minutes (5 minutes on and 5 minutes off) and then at speedrpm 18,000 was homogenized for 10 min (1 min on and 1 min off). Then, liposomal dispersion using a probe-type ultrasound generator (modelKS-250F, made in China) with an intensity of 80% and a frequency of 20 kHz at a temperature of 25 degrees Celsius, for 7 minutes

(1 second on and 1 second off) was subjected to sonication [18, 23, 24].

2- 4- Determination of particle size and zeta potential of nanoliposomes

To evaluate the particle size and zeta potential, first the samples Nanoliposomes were diluted with distilled water at a ratio of 1:001 and then measured Using the Zeta Sizer device (CompanyMalvern, England) in temperaturethC 25 With the help of dynamic light scattering method was done [18].

2- 5- Storage of nanoliposome samples at refrigerator temperature (thC4) and Fraser (thC18-)

Transfer 2 ml of each sample into a microtube and refrigerate for 30 days at two temperatures(thC4) and Fraser(thC18-) They were kept without using cold protection. The samples were examined every 15 days to perform stability tests, which are described in detail below. The tests of the samples kept at freezer temperature were done after they were defrosted at room temperature and vortexed.]25 and 26[.

2- 6- Evaluation of the stability of nanoliposomes during the storage period

2- 6- 1- Determining the retention percentage of phenol

The amount of trapped phenol was determined as a difference between the total amount of essential oil phenol added to the nanoliposome system and the amount of free essential oil phenol. To measure the amount of free phenol, first the free essential oil (unencapsulated essential oil) using an Amicon filter (molecular weight,KDa 100) and centrifuge at high speed rpm 2000 was separated from nanovesicles for 5 minutes. Then, the amount of free phenol using the colorimetric method of Folin Ciocalteu and absorbance readingat a wavelength of 760 nm by a spectrophotometer Obtained. Next, after calculating the amount of trapped phenol, percentageThe shelf life of phenol

was determined according to the following formula[18, 27 and 28].

Percent shelf life phenol

$$= \frac{t \text{ time in the sample trapped in the amount of phenol}}{\text{The sample was trapped in the initial amount of phenol}} \times 100$$

2- 6- 2- measurementpH

In this test,pH Samples by devicepH Meter So Calibrate the device with standard buffers 4 and 7 became [18].

2- 6- 3- Evaluation of free radical inhibition activityDPPH

In this method, 0.5 ml of free and loaded samples of balang skin essential oil are mixed with 2.5 ml of 60 micromolar solution.DPPH was combined in methanol. Then this mixture was kept in a dark place for 1 hour and the absorbance of the sample was read at a wavelength of 517 nm using a spectrophotometer. Finally, the percentage of inhibition of free radicalsDPPH It was calculated according to the following formula [5 and 26].

$$\begin{aligned} & \text{DPPH free radical inhibition percentage} \\ &= \frac{\text{DPPH absorption} - \text{sample absorption}}{\text{DPPH adsorption}} \\ & \times 100 \end{aligned}$$

2- 6- 4- Assessment of antimicrobial activity

In this test, 100 microliters of microbial suspension (CFU/mL $10^8 \times 1.5$) on Mueller Hinton Agar culture medium It was poured and spread in all its parts using a sterile swab. Then, 10 microliters of the sample was poured on sterile blank paper discs (diameter 6.4 mm) and the discs were placed at a suitable distance from each other on the culture medium containing bacteria. After incubation for 24 hours at 37°C, the diameter of the non-growth halo was measured using a ruler and the corresponding average was reported [5, 29].

2- 7- Statistical Analysis

Data analysis using statistical software 9.1 SAS and by completely random design and analysis of variance table (ANOVA) Was performed. Duncan's test was used to compare means at the 5% level and graphs were drawn using the software Excel 12.0 Was performed.

3. Results and Discussion

3- 1- Particle size and zeta potential of nanoliposomes

The amount of particle size and zeta potential of nanoliposomes prepared with different concentrations of lecithin-sesame oil containing essential oils distilled with water and the supercritical fluid of balang skin in Figure 1 (respectively A and B) it has been shown. The results of particle size and zeta potential of the samples were in the range between 252 and 311 nm and -42.5 to -55.9 mV respectively. The lowest amount of particle size and the highest amount of zeta potential were obtained for nanoliposome of supercritical fluid essential oil with the formulation containing the highest amount of lecithin and the lowest amount of oil. The particle size of nanoliposomes decreased with increasing lecithin concentration, which is consistent with the results of Gerjian et al. (2021). These researchers attributed the reason to the decrease in the resistance of the nanoliposome membrane to the shear force and the breaking of the particles under the effect of sonication with an increase in the concentration of lecithin [18]. In the study of Khatib et al. (2019), it was reported that the negative value of the zeta potential of nanoliposomes is due to the negative charge of polar head groups (such as phosphate) in the two layers of lecithin [30]. Therefore, in our study, the increase in zeta potential by increasing the amount of lecithin in the formulation may also be the reason. The

difference in particle size and zeta potential of nanoliposomes containing the essential oil of the supercritical fluid of balang skin compared to the samples distilled with water can be due to the difference in the amount of phenolic compounds in the two types of essential oils [5]. In the study of Rafiei et al. (2017), it was reported that the amount of phenolic compounds affected the size of nanoliposome particles, which is consistent with the results of this study [10]. These researchers stated that probably the interaction between the phenolic compounds with the acyl chains of the liposome lipid bilayer has reduced the size of the liposome. In the study of Rezaei Ermi et al. (2019) and Sawaqabi et al. (2019), it was reported that the concentration of phenolic compounds and their interaction with the liposome lipid membrane can change the value of zeta potential [31 and 32]. In previous researches, it was stated that the presence of negatively charged carboxyl group in the structure of phenolic compounds can affect the value of zeta potential [10 and 31]. In addition, the hydroxyl group of phenolic compounds can form a hydrogen bond with the choline group (with a positive charge) in the structure of the lipid membrane, causing the choline group to be pulled to the inner parts of the membrane and increasing the phosphate groups (with a negative charge) on the surface of the membrane. be [10 and 31].

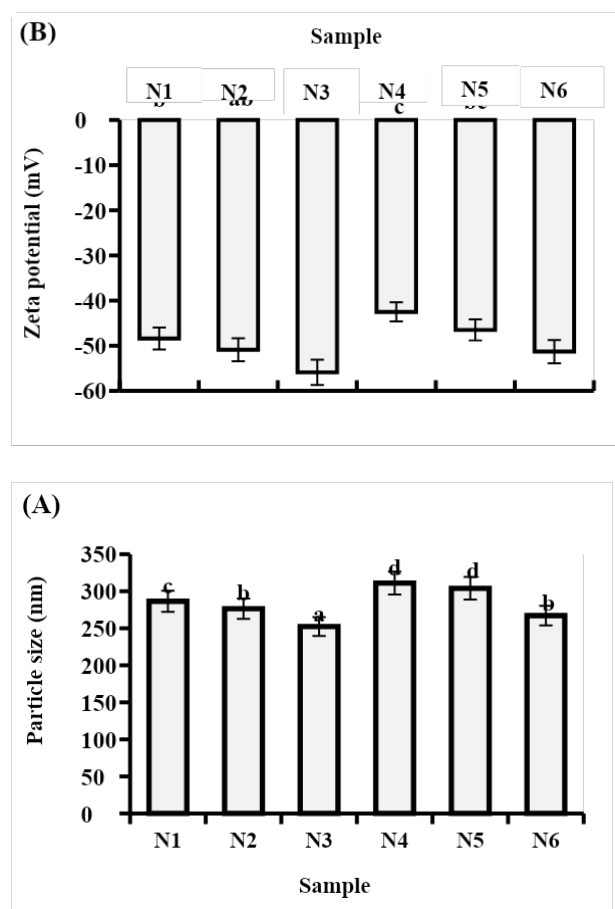


Figure 1. Particle size (A) and zeta potential (B) of nanoliposome samples containing essential oils of supercritical fluid (N1, N2 and N3) and hydrodistillation (N4, N5 and N6) of citron peel. Nanoliposomes formulation (W/W %): N1 and N4 (lecithin 40: oil 60), N2 and N5 (lecithin 50: oil 50) and N3 and N6 (lecithin 60: oil 40). Different letters indicate significant differences ($P < 0.05$).

2-3- Percent shelf life of phenol

The percentage of phenol retention in nanoliposome samples containing essential oil distilled with water and essential oil of the supercritical fluid of the skin of the bell during storage at temperatures of -4 and -18 °C in Figure 2 (respectively A and B) it has been shown. Based on the obtained results, the percentage of phenol retention in all samples decreased with time at both temperatures, and the amount of this decrease was greater during storage of the

samples at freezer temperature. More suitable temperature -4°C To preserve the samples and protect the phenolic compounds more, it may be due to the greater stability of nanoliposomes due to the less destruction of the components of the double-layer membrane and the lack of accumulation of nanoliposome particles at this temperature compared to freezer conditions [33]. The decrease in the content of phenolic compounds of the encapsulated samples during 30 days of storage at the tested temperatures is consistent with the results obtained from the study of Barto et al. (2020) [34]. The nanoliposome sample containing supercritical fluid essential oil with the formulation with the highest amount of lecithin and the lowest amount of oil showed the best result and there was a statistically significant difference between the tested samples. Thus, the aforementioned formulation was an ideal composition for the nanoliposome wall material, which played a more effective role in its stability and maintaining more phenolic compounds.

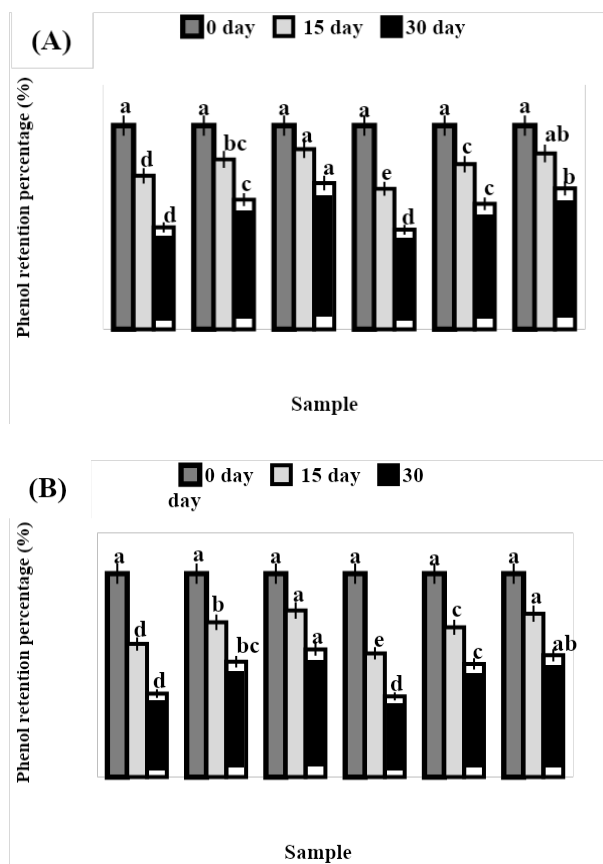


Figure 2. Comparison of the phenol retention percentage in nanoliposome samples containing essential oils of supercritical fluid (N1, N2 and N3) and hydrodistillation (N4, N5 and N6) of citron peel during storage at 4thC (A) and -18thC (B).

Nanoliposomes formulation (W/W %): N1 and N4 (lecithin 40: oil 60), N2 and N5 (lecithin 50: oil 50) and N3 and N6 (lecithin 60: oil 40). Different letters between test samples on the same storage day indicate significant differences ($P < 0.05$).

3-3- AmountpH

the amount ofpH Nanoliposome samples containing essential oil distilled with water and supercritical fluid essential oil of balang skin during storage at temperatures of -4 and -18 degrees Celsius in Figure 3 (respectivelyA AndB) it has been shown. Nanoliposomes prepared with different concentrations of lecithin-oil have amountpH were different in region 6 to 7, and with increasing lecithin amount,pH It was slightly higher. This result shows that the existence of ammonium ionic groups(⁺[N(CH₃)₃]) and phosphate (⁻[OP(O)OO]), especially the

phosphate group of membrane phospholipid molecules in interaction with aqueous environment,pH has affected nanoliposomes. Nanoliposomes containing supercritical fluid essential oil have very slight changes in the amount compared to samples distilled with water.pH were that the lack of effect of the type of essential oil obtained from the skin of Balang with the above two methods on the amountpH shows. In the present study, the amountpH All the samples decreased with time at both temperatures and the amount of this decrease was higher for the samples kept at freezer temperature. These results are consistent with the observations from the research of Gurjian et al. (2021) [18]. reduce the amountpH During the storage period (at both temperatures) and also its lower temperature in the freezer compared to the refrigerator temperature may be due to the destruction of the phospholipid structure of the membrane and the presence of more free fatty acids over time. This result shows the decrease in the stability of nanoliposomes during storage.

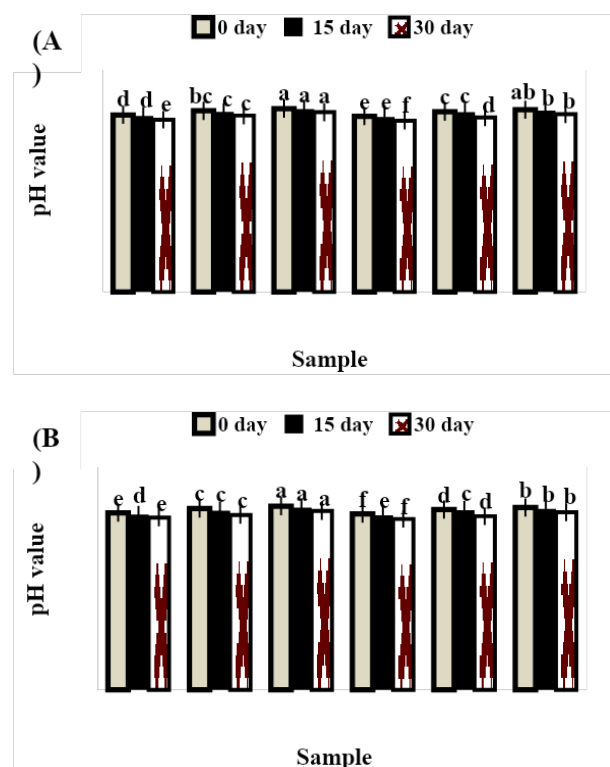


Figure 3. pH value of nanoliposome samples containing essential oils of supercritical fluid (N1, N2 and N3) and hydrodistillation (N4, N5 and N6) of citron peel during storage at 4 °C (A) and -18 °C (B). Nanoliposomes formulation (W/W %): N1 and N4 (lecithin 40: oil 60), N2 and N5 (lecithin 50: oil 50) and N3 and N6 (lecithin 60: oil 40). Different letters between test samples on the same storage day indicate significant differences ($P < 0.05$).

3- 4- The ability to inhibit free radicals DPPH

Antioxidant activity of essential oils distilled with water and supercritical fluid of the skin of the bell in the free form and encapsulated in the nanoliposome system during the storage period at temperatures of -4 and -18 degrees Celsius in Figure 4 (respectively A and B) it has been shown. Based on the obtained results, the ability to inhibit free radicals DPPH The samples decreased over time at both temperatures and the amount of this decrease was higher for the samples kept at freezer temperature. Lower antioxidant activity of the samples at temperature °C 18- Relative to °C 4 during storage can further decrease in shelf life and. The preservation of antioxidant compounds such as phenols can be attributed to this temperature. Nanoliposome samples of both types of essential oils showed better antioxidant activity than the free form of essential oils, and there was a statistically significant difference between the samples. This result is consistent with the observations from the research of Khatib et al. (2019) [30]. The higher antioxidant capacity of nanoliposome samples compared to the non-encapsulated state is probably due to the presence of more antioxidant compounds such as phenols in a higher surface-to-volume ratio. The results of the study conducted by Spigno et al. (2013) showed that the encapsulation process improves the antioxidant activity of these compounds through better distribution of phenolic compounds in the environment and making more of them available [35]. In the

present study, the ability to inhibit free radicals in nanoliposomes containing supercritical fluid essential oil was higher than the samples of essential oil distilled with water in nanoliposomal form, and nanoliposomes prepared with a formulation with a higher amount of lecithin and a lower amount of oil showed the best results. They gave. The better inhibitory performance of nanoliposomal samples of supercritical fluid essential oil compared to essential oil distilled with water can be due to the difference in the amount of bioactive compounds with antioxidant properties such as phenols in two types of essential oils [5]. The difference in antioxidant activity between nanoliposomes formed with different concentrations of the wall composition in this study can show the effect of the amount of nanoliposome wall constituents on the antioxidant activity, which probably caused the difference in the stability of nanoliposomes and encapsulated antioxidant compounds. In the research conducted by Gerjian et al. (2022), it was observed that nanoliposomes formed with different amounts of wall materials and containing different concentrations of phenolic compounds have the ability to inhibit free radicals DPPH were different, which is consistent with the results of the present study [29]. The improvement of the antioxidant activity of the nanoliposomal form of the essential oil compared to the free form may be due to the synergistic effect of the ingredients with antioxidant properties of the nanoliposome wall, including lecithin and sesame oil with the antioxidant compounds of the essential oil such as phenols [10, 19, 21]. Raushi et al. (2017) reported that lecithin has a synergistic effect with phenolic compounds due to hydrogen atom donation by aminophospholipid groups and reduction of oxidized phenolic compounds [19]. In the study of Rafiei et al. (2017), it was stated that the antioxidant

activity of phenolic compounds is influenced by various factors such as concentration, interaction with the two layers of the membrane and their position in the liposome [10].

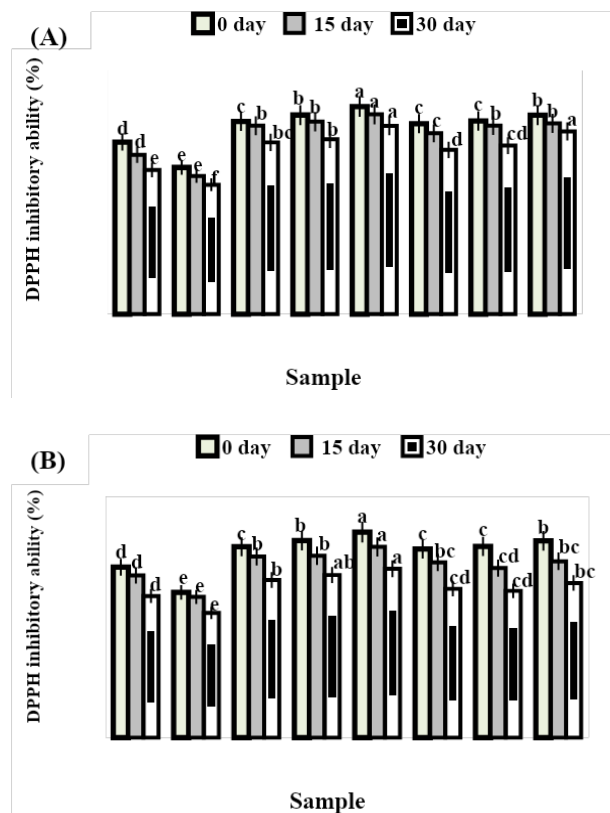


Figure 4. DPPH radical scavenging activity of free and nanoliposome samples of different essential oils of citron peel during storage at 4 °C (A) and -18 °C (B). Essential oils of supercritical fluid in free form (Es) and nanoliposome (N1, N2 and N3) and essential oils of hydrodistillation in free form (Eh) and nanoliposome (N4, N5 and N6). Nanoliposomes formulation (W/W %): N1 and N4 (lecithin 40: oil 60), N2 and N5 (lecithin 50: oil 50) and N3 and N6 (lecithin 60: oil 40). Different letters between test samples on the same storage day indicate significant differences ($P < 0.05$).

5-3- Antimicrobial activity

Antimicrobial activity of free and nanoliposomal forms of essential oils distilled with water and the supercritical fluid of the skin of Balang during storage at temperatures of -4 and -18 degrees Celsius in Figure 5 (respectively A and B) it has

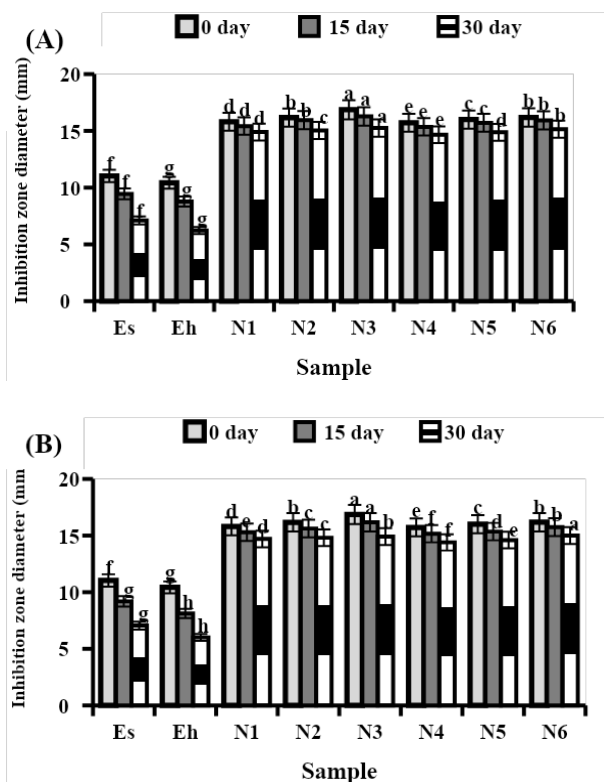
been shown. The diameter of the non-growth halo in nanoliposomal samples was higher than that of uncoated essential oils during storage, and both types of essential oils in free form and nanoliposome had more antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) than bacteria. Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*). The increase in antimicrobial activity after encapsulation of the essential oil in the nanoliposome system indicates the effectiveness of the process of nano-microencapsulation of the essential oil. The effect of inhibiting microbial growth of most antimicrobial compounds in nano dimensions can be due to their higher surface-to-volume ratio. Therefore, these compounds have more surfaces to bind to microorganisms and by creating more holes in the bacterial cell wall and disrupting the permeability of the membrane, they can be more effective in inhibiting the growth of microbes [36]. Our findings are consistent with the results of Wu et al.'s (2023) study. These researchers attributed the higher antibacterial ability of the encapsulated essential oil compared to the free form to the preservation of volatile components with antibacterial properties in the essential oil such as terpenes, alcohols, and aldehydes in an encapsulated state during the storage period [37]. The observation of the greater antimicrobial effect of the nanoliposome form of the samples against gram-positive bacteria compared to gram-negative bacteria is similar to the results of the research of Gerjian et al. (2022) [29]. These researchers explained the reason for this result, the greater sensitivity of gram-positive bacteria

to phenolic compounds and the difference in the structure of the cell surface between the two types of bacteria. In the present study, the antimicrobial effect of all samples decreased with time in both temperatures, and a lower value of the diameter of the non-growth halo was recorded for the samples kept at freezer temperature. The largest diameter of the non-growth halo was obtained for the nanoliposome sample containing supercritical fluid essential oil with the formulation with the highest amount of lecithin and the lowest amount of oil, and the tested samples had statistically significant differences in terms of antimicrobial effect. Liolios et al. (2009) stated that liposomes depending on the physicochemical properties of their membrane (such as the composition of the membrane) and the composition of the bacterial membrane can be transported through different methods such as intermembrane transfer and fusion with the bacterial cell. interact and improve cell transport and release active compounds into the bacterial cell [38]. The results of the higher antimicrobial activity of the nanoliposome form of the sample compared to their free state are consistent with the observations obtained from the research of Khatib et al. (2012) [30]. These researchers attributed its cause to the addition of nanoliposomes to the bacterial outer membrane. In the present study, the growth inhibitory activity of different nanoliposomal form of supercritical fluid essential oils and distilled with water can be due to the difference in the amount of compounds with antimicrobial properties such as phenols and terpenes in two types of

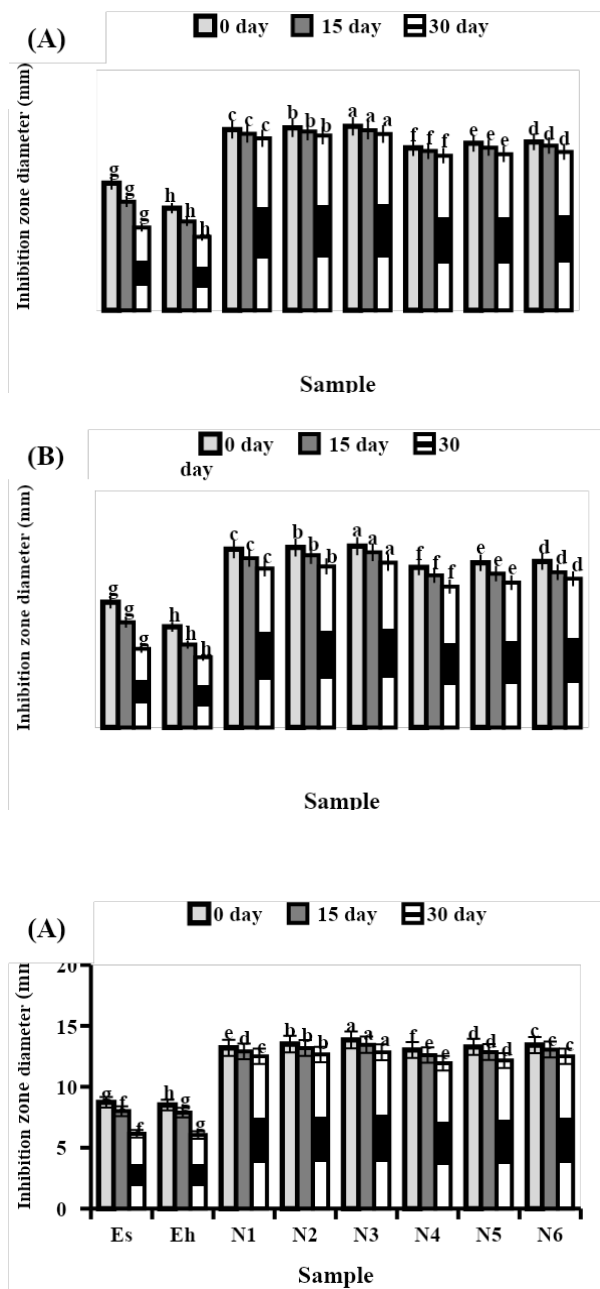
essential oils [5]. The different results obtained from the antimicrobial performance of nanoliposomes with different formulations are probably due to the different concentrations of wall components, different values of membrane strength.

provided the nanoliposome. As a result, the encapsulated antimicrobial compounds had different stability depending on the strength of the membrane in front of temperature degradation, and according to the results of this test, it can be said that the freezer temperature has a more destructive effect than the refrigerator temperature on the membrane of nanoliposomes and as a result of the antimicrobial compounds. had. Thus, in this study, the different amount of antimicrobial compounds retained by different nanoliposome formulations in storage conditions caused the difference in their antimicrobial activity.

(I)



(II)



(III)

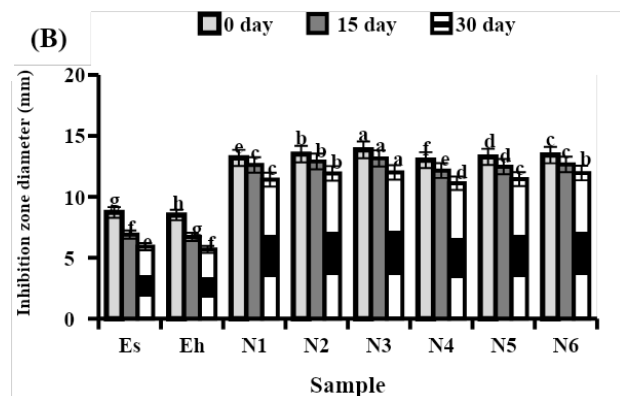


Figure 5. Antimicrobial activity of free and nanoliposome samples of different essential oils of citron peel against *Staphylococcus aureus* (I), *Escherichia coli* (II) and *Pseudomonas aeruginosa* (III) bacteria during storage at 4 °C (A) and -18 °C (B). Essential oils of supercritical fluid in free form (Es) and nanoliposome (N1, N2 and N3) and essential oils of hydrodistillation in free form (Eh) and nanoliposome (N4, N5 and N6). Nanoliposomes formulation (W/W %): N1 and N4 (lecithin 40: oil 60), N2 and N5 (lecithin 50: oil 50) and N3 and N6 (lecithin 60: oil 40). Different letters between test samples on the same storage day indicate significant differences ($P < 0.05$).

4- Conclusion

In this research, the preservation rate of bioactive compounds, antioxidant and antimicrobial performance of different essential oils distilled with water and supercritical fluid of the skin of the sea cucumber after being loaded into the nanoliposome system containing the health-promoting components of lecithin-sesame oil for 30 days storage in two refrigerator environments and Fraser were examined. The particle size and zeta potential of the produced nanoliposomes were in the range of 252 to 311 nm and -42.5 to -55.9 mV respectively. The results showed that the

nanoliposomes containing different essential oils of balang skin prepared with different concentrations of lecithin-oil have the retention percentage of phenolic compounds and the amount pH were different and their amount over time in storage temperatures °C 4 and °C 18- decreased. The antioxidant and antimicrobial activity of nanoliposomal samples of both types of essential oils was higher than the free form of essential oils, and the samples kept at refrigerator temperature had better performance than at freezer temperature. Nanoliposomes containing supercritical fluid essential oil showed more antioxidant and antimicrobial power than samples of essential oil distilled with water, and the antimicrobial activity of all samples was higher against Gram-positive bacteria than Gram-negative bacteria. Also, the concentration of components of the wall of nanoliposomes had an effect on their antioxidant and antimicrobial performance, and the best result was related to the formulation with the highest amount of lecithin and the lowest amount of oil. Therefore, the results of this study showed that the nanovesicles prepared using components with beneficial nutritional and functional properties in the formulation and without the presence of toxic organic solvents have strengthened the antioxidant and antimicrobial effect and improved the stability of the essential oil of the skin of the inside cover. It can provide its safe and effective use as a natural preservative in food products.

6- Resources

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مقایسه پایداری فرم نانولیپوزومی اسانس های پوست بالنگ به دست آمده از روش های مختلف استخراج در طول دوره نگهداری در دمای ۴ و ۱۸- درجه سانتی گراد

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یکی از روش های مهم برای حفظ پایداری و خصوصیات عملکردی اسانس های گیاهی به عنوان یک منبع مفید ترکیبات زیست فعال در مقابل آسیب های محیطی، درون پوشانی آن ها در سیستم های نانوحامل مانند نانولیپوزوم است. در این مطالعه نانولیپوزوم حاوی اسانس پوست بالنگ بدون استفاده از حلال آلی سمی و با به کار بردن ترکیبات سلامتی بخش مانند روغن کنجد علاوه بر لستین برای اولین بار در فرمولاسیون تهیه شدند. میزان پایداری نمونه ها در طول ۳۰ روز نگهداری در دماهای ۴°C و ۱۸°C-، با بررسی مقدار ماندگاری ترکیبات فنولی، تغییرات pH، عملکرد آنتی اکسیدانی و ضد میکروبی تعیین گردید. نمونه های نانولیپوزومی اسانس های تقطیر با آب و کربن دی اکسید فوق بحرانی پوست بالنگ تهیه شده با غلظت های مختلف لستین- روغن دارای مقدار متفاوت pH و درصد ماندگاری فنول بودند و میزان آن ها با افزایش مدت زمان نگهداری در هر دو دمای آزمون کاهش یافت. توانایی مهارکنندگی DPPH و فعالیت ضد میکروبی هر دو نوع اسانس پوست بالنگ بعد از درون پوشانی در نانولیپوزوم بهبود یافت. اما مقدار آن ها در هر دو دمای نگهداری با گذشت زمان کاهش یافت. نانولیپوزوم اسانس سیال فوق بحرانی پوست بالنگ به ترتیب با فرمولاسیون حاوی بالاترین و پایین ترین مقدار لستین- روغن در دمای نگهداری ۴°C، بهترین نتیجه را در این مطالعه نشان دادند. بنابراین اسانس پوست بالنگ می تواند با درون پوشانی در سیستم نانولیپوزوم تهیه شده از لستین- روغن کنجد به دلیل بهبود فعالیت آنتی اکسیدانی و ضد میکروبی و پایداری بالاتر آن در مقابل دمای نگهداری، به عنوان افزودنی عملگرایی طبیعی موثر در صنایع غذایی مورد استفاده قرار گیرد.