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Storage stability, heat stability, controlled release and antifungal activity of liposomes as alternative fungal preservation agents

Mine Aslan, Nilgün Ertaş *, M. Kürşat Demïr

Department of Food Engineering, Engineering Faculty, Necmettin Erbakan University, Konya, 42050, Turkey

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ABSTRACT

The aim of this study was to investigate the characteristics of liposomes encapsulated ethanolic *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum* and *Laurus nobilis* extract prepared using lipid phase containing phosphatidylcholine/extract mixture of 10:1. The antifungal activity, size, zeta (ζ) potential, morphology, physical stability, heat stability, release in in-vitro digestion, color and differential scanning calorimetry (DSC) were conducted to determine the characteristics of liposomes. All extract-loaded liposomes showed prominently antifungal activity against *Aspergillus* sp. and *Penicillium* sp. The encapsulation efficiency of all samples was 86.60%–92.87%. *Curcuma longa* loaded liposome had high encapsulation efficiency after freeze dry-rehydration (FD-RH), long-term and freeze-thaw (FT) storage treatment. The thermal stability of *Syzygium aromaticum* loaded liposomes was higher than the others. The highest release values in the *in-vitro* gastric and intestinal condition were detected in *Laurus nobilis* loaded liposome. DSC data of liposomes demonstrated to be the endothermic phase at three different temperature ranges: lipid bilayers transition from gel state to liquid crystal form (1.69–5.31 °C), the pre-phase transition temperature (102.16–133.10 °C) and the main phase transition temperature (116.47–165.22 °C).

1. Introduction

Fungal contamination is an important problem for foods and leads to lots of negative effect as enormous economic losses, food quality degradation, deterioration of nutritional content and mycotoxins formation (Garcia et al., 2019; Sun et al., 2020). Chemical-based preservatives are often used in the food industry to inhibit and destroy fungal growth. However, the use of synthetic preservatives above acceptable limits and with long consumption causes many health problems and also most importantly, being accepted generally safe (GRAS) of natural preservatives made a very popular search for natural materials for new alternative preservatives (Batiha et al., 2021; Lucera et al., 2012). Bioactive components evaluated as natural components are the most important components used as fungal growth inhibitors.

Bioactive compounds have extra nutritional properties and provide a beneficial effect on specific functions (Teodoro, 2019). In food systems are used to improve various properties like anti-fungal, anti-bacterial, anti-oxidant and the natural bioactive compounds usually have superior safety profiles to synthetic compounds and high consumer acceptability (Gupta et al., 2010). However, the sensitive nature, poor solubility, low stability and low bioavailability of bioactive compounds decrease application options in the food industry (Bao et al., 2019). For bioactive compounds, different delivery and protection systems have been developed to improve water solubility, to protect against adverse environmental conditions, to improve permeability through the intestinal mucus layer and epithelial cells, as well as to masked bad taste and odor.

Liposomes are self-assembled spherical vesicles with one or multi phospholipid bilayers or lamellae encompassing an internal aqueous phase. The amphiphilic nature of liposomes provides the encapsulation of hydrophilic and lipophilic active ingredient (Mozafari et al., 2008). This unique feature is due to the two head groups of phospholipid molecules. A hydrophilic interaction occurs with the polar head group and the inner/outer aqueous phases, and hydrophobic with the aliphatic chains (Bozzuto & Molinari, 2015; Torchilin, 2005). Liposomes are an important technique for the release of bioactive compounds to targeted area under specific conditions (Chen et al., 2017).

In the literature, Pinilla et al. (2019) were investigated *in-vitro* antifungal activity against the selected fungal strains *Penicillium expansum*, *Aspergillus niger*, *Penicillium herquei*, *Fusarium graminearum* and *Aspergillus flavus* of liposomal encapsulated garlic extract and stated that

* Corresponding author. Food Engineering Department, Engineering and Architecture Faculty, Necmettin Erbakan University, 42050, Konya, Turkey.
E-mail address: nertas@erbakan.edu.tr (N. Ertaş).

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liposomal encapsulation is potential application for microbiological stability of food products. To our knowledge, no studies have been conducted on the antifungal activity and characterization quality for *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum*, *Laurus nobilis* encapsulated with liposomal technique. *Cinnamomum verum, Curcuma longa, Zingiber officinale, Syzygium aromaticum* and *Laurus nobilis* extract obtained by different solvent extraction reported to possess an important source in terms of bioactive compounds and also antimicrobial properties. For instance in literature, *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum* and *Laurus nobilis* had shown significant antifungal activity against *Aspergillus* and *Penicillium* sp. strains (Aly & Gumgumjee, 2011; Mahmoud, 2012; Pundir & Jain, 2010; Singh et al., 2008). Some of the properly investigated extracts demonstrated remarkable antifungal activity against *Aspergillus* and *Penicillium* sp. strains thanks to high bioactive compounds and so included in the present study. Also, the main objectives in the selection of the liposomal system for encapsulation of these ethanolic extracts; improve the solubility by increasing the surface area of the bioactive components, increasing the bioavailability, providing a controlled release to the target point, masking the negative taste and odor, and most importantly, no adversely affect the quality of the final product.

Therefore, the present study aimed to evaluate the *in-vitro* antifungal activity of liposomes containing *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale, Syzygium aromaticum, Laurus nobilis* against some *Aspergillus* and *Penicillium* strains. Also, liposomes were investigated in terms of the physical and morphological quality.

2. Material and methods

2.1. Materials

Cinnamon (*Cinnamomum verum*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), clove (*Syzygium aromaticum*) and laurel (*Laurus nobilis*) were obtained from a local company in Konya (Turkey). *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* were obtained from Tokat Gaziosmanpaşa University Plant Protection Department and Yıldız Technical University Food Engineering Department. Soy lecithin was purchased from Tito (Istanbul, Turkey).

2.2. Methods

2.2.1. Preparation of ethanolic extracts

Medicinal aromatic plant extracts production was carried out by modifying the method of Grigoras et al. (2013). 100 g of grounded sample in 600 ml solvent was agitated in a shaking water bath (Daihan Wisebath WSB-30, Gangwon, South Korea) for 3 h at 170 rpm at 25 ± 1 °C and was filtered. Then, agitated adding 400 ml ethanol for 6 h and kept at room temperature (25 ± 1 °C) for 12 h. The filtered extracts were evaporated using a rotary evaporator (Büchi R20, Switzerland) at 40 °C. Extracts were stored at 4 °C until analysis.

2.2.2. Purification of phosphatidylcholine from soy lecithin

Phosphatidylcholine was purified following the procedure described by Mertins et al. (2008) and Machado et al. (2014). Soy lecithin (25 g) was dissolved in ethyl acetate (125 mL) and added deionized water (5 mL). In the formed phase separation, the liquid part was removed and to obtain pure phosphatidylcholine the lower phase was washed with acetone (75 mL), being the liquid part was separated by decanting. Then the same process was repeated and then dried at 40 °C in vacuum-oven. The partially purified phosphatidylcholine were stored at 4 °C until use.

2.2.3. Preparation of liposomes

The multilamellar liposomes were produced with modification of the thin film hydration method described by Szoka and Papahadjopoulos (1978) and Lu et al. (2011). Briefly, phosphatidylcholine (0.5 g) and extract samples (0.1 g) was dissolved with chloroform:methanol (3:1) in a round-bottomed flask. The solvents were completely evaporated under a vacuum at 40 °C until a thin film (JSVO-60 T, Korea). The lipid film was suspended in 10 ml of deionized water in a water bath at 40 °C and 120 rpm for 2 h. Finally, the liposome suspensions (50 mg/ml phosphatidylcholine and 10 mg/ml extract at final concentration) were sonicated 3 times for 30 s in a bath-type sonicator to reduce the size and obtain a homogeneous size. The prepared liposomes were placed in bottles and were allowed to hydrate for 24 h at 4 °C. The liposome suspensions were freshly prepared for each analysis.

2.2.4. Antifungal assay

The antifungal assay was carried out with *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum* and *Laurus nobilis* extract samples.

2.2.4.1. Fungal cultures. *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* strain were incubated 26.5 °C for 7 days on Potato Dextrose Agar (PDA) plates. Then, 10 mL of 0.01% Tween 80 (Sawai & Yoshikawa, 2004) was added and were adjusted to equal the 0.5 McFarland standards with absorbance 0.400–0.450 at 400 nm (Kızılkeçili, 2007).

2.2.4.2. Disc diffusion methods. According to agar disc diffusion, 0.1 ml of mold suspension were inoculated over agar with a sterile pipette (Research Plus, Eppendorf, Germany) and spread uniformly using a glass spreader. Firstly, 100 and 200 mg/mL concentration of extract samples were prepared with ethanol. Then, 20 μL from 100 mg/mL extract samples, 200 mg/mL extract samples, negative controls (ethanol) and positive controls (2 mg/ml calcium propionate) were impregnated on 4 discs on plate and were incubated at 25 °C for 72 h. The diameter of growth inhibition was determined as millimeters.

2.2.4.3. Minimum inhibition concentration (MIC) and minimum fungicidal concentration (MFC). The MIC values of the extract samples was determined by the agar dilution method. Stock solutions of dissolved extract in ethanol were added to sterile melted PDA at 50 °C to give final concentration of 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156 and 0.078 mg/mL with PDA at 50 °C. The resultant dilutions was poured into petri plate in the amount of 12–15 ml and waited at 10 min. Then, 0.1 ml mold suspension prepared separately for *Aspergillus flavus*, *Aspergillus oryzae*, *Aspergillus niger*, *Penicillium digitatum* and *Penicillium camemberti* was transferred in petri dishes and were spread homogeneously on the petri plate with a glass spreader. Finally, the petri dishes were incubated at 25 °C for 48–72 h and the lowest concentration value at which no growth was determined as the MIC value (mg/mL). For MFC, the samples in the petri plate no growth were transferred onto fresh PDA medium by streaking with a loop. Then, these plates were incubated at 25 °C for 48–72 h, and the lowest concentrations without visible growth were recorded as MFCs.

2.2.5. Particle size distribution and zeta (ζ) potential

The particle size distribution profile of liposome suspensions was determined using a static light scattering instrument (Malvern Mastersizer 3000, Malvern Instruments Ltd, UK). For the particle size distributions was used a refractive index of 1.44 and 1.33 for lecithin and deionized water, respectively in aqueous phase and the degree of obscuration up to 10%. The values of Sauter mean diameter ($D_{3,2}$) and De Brouker mean diameter ($D_{4,3}$), span and uniformity were reported with the five times measurements. For zeta (ζ) potential, liposomal suspensions were diluted 50-fold with deionized water and were determined with a Zetasizer (Zetasizer 2000; Malvern Instruments) at 25 °C with the scattering angle of 173°.

2.2.6. Encapsulation efficiency

The encapsulation efficiency (EE%) is stated as the percentage amount of entrapped TPC in the total amount during the encapsulation procedure (Marín et al., 2018). The encapsulation efficiency (EE) was calculated as follows:

$$\% \text{ EE} = \text{encapsulated TPC / total TPC} \times 100 \quad (1)$$

The liposomal suspensions were centrifuged at 10.000 rpm for 10 min to remove the free extract and then encapsulated extract in liposomes were separated by mixing with ethanol (1/1000 v/v). Free TPC in the aqueous phase were measured using spectrometer UV–Vis at 760 nm according to Maurya and Singh (2010) with modification, respectively.

2.2.7. Physical stability

The physical stability of liposome samples was investigated by recording the % EE and particle size distributions of liposomes after freeze-thaw (FT), freeze dry-rehydration (FD-RH) and long-term storage (4 °C and 20 °C) according to method described by Li et al. (2015). Storage conditions for physical stability were used following;

Freeze-thaw (FT) was carried out by storing at −20 °C for 4 weeks then before analysis, thaw at room temperature for 4 h. Freeze dry-rehydration (FD-RH) was performed that liposome suspensions were cooled from 25 to −40 °C and kept at −40 °C for 8 h. Then maintained at −40 °C for 48 h, heated from −40 to 25 °C, and dried at 25 °C for 10 h. Finally, samples were stored at 4 °C for 4 weeks. Long-term were assessed at 20 °C and 4 °C over a period of 4 weeks.

2.2.8. Heat stability

Freshly liposome suspensions were placed into glass tube and were exposed at 50, 60, 70 and 80 °C for period of 30 min heat treatment (Picot & Lacroix, 2003). All samples were analyzed in terms of the % EE at the end of the application.

2.2.9. In-vitro gastrointestinal digestion

The resistance of the liposomal encapsulated samples to digestive system conditions was determined as *in-vitro*. The *in-vitro* digestion was carried out by simulating the gastric and intestine conditions as described previously (Brinques & Ayub, 2011; Pedroso et al., 2012). Simulated gastric fluid (SGF) was prepared 9 g/l sodium chloride and 3 g/l pepsin at final concentration of 1890 U/ml was adjusted to pH 2.0 with 0.1 M HCl before the in vitro digestion. Simulated intestinal fluid (SIF) was prepared with 9 g/l sodium chloride, 10 g/l pacreatin at final concentration of 40 U/ml, 10 g/l trypsin at final concentration of 10.000–20.000 U/ml, 3 g/l bile salt and was adjusted to pH 8.0 with 0.1 M NaOH. Liposome suspensions were then mixed with the SGF and SIF at a volume ratio of 1:9 and separately incubated at 37 °C for 180 min with continuous stirring at 150 rpm in a temperature-controlled water bath. Sub-samples were taken periodically for analysis at different time intervals (0, 60, 90, 120 and 180 min). The collected supernatants were hold at −20 °C for further analyses. The free TPC of the liposome suspensions were determined initially and after each digestion phase. *In-vitro* gastric and intestinal digestion results are expressed as %.

2.2.10. Color properties

Lightness (L*), redness/greenness (a*), yellowness/blueness (b*) parameters of liposomes were measured using Minolta CR-400 (Konica Minolta, Inc. Osaka, Japan) (Francis, 1998). Hue angle, chroma values and the total color difference (ΔE) were calculated according to L*, a* and b* values.

$$Hue\ angle = arctan\frac{b*}{a*} \quad (2)$$

$$Chroma = \sqrt{a*^2 + b*^2} \quad (3)$$

$$\Delta E = \sqrt{\Delta L*^2 + \Delta a*^2 + \Delta b*^2} \quad (4)$$

2.2.11. Morphological characterization

The morphological attributes of liposomes was evaluated under optical microscope equipped with digital camera (Olympus C3 × 33, Tokyo, Japan). Images were obtained at a magnification of 40 × with liposomes suspension on a glass slide.

2.2.12. Differential scanning calorimetry

Differential scanning calorimetry (DSC) device (DSC 25, TA Instruments, New Castle, DE, USA) was used to examine the thermal properties of liposomes. Liposome suspensions (≈5–10 mg) in hermetic in aluminum pans were heated from −30 °C to 250 °C at a heating rate of 10 °C/min under dry nitrogen purge (flow rate 50 mL/min). Endothermic peak temperatures (T_{peak}, °C) and enthalpies (ΔH, J/g) were calculated using the equipment software.

2.2.13. Statistical analysis

Statistical analysis was performed using the JMP statistical program, version 5.01 (SAS Institute Inc. Cary, NC, USA). The average values of the main variation sources were reported as mean ± SD and were compared at $p < 0.05$ significance levels.

3. Results and discussion

3.1. In-vitro antifungal activity of liposomes

3.1.1. Inhibition zone diameter

Inhibition zone diameter of liposomes against *Aspergillus* sp. and *Penicillium* sp. were carried out after 72 of incubation using the disc diffusion method for *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum* and *Laurus nobilis* loaded liposomes. Inhibition zone diameter values are presented in Table 1. According to Table 1, *Penicillium* sp. Strains were more sensitive than *Aspergillus* sp. strains against liposomes at different concentrations. *Penicillium camemberti* was found to be the most susceptible, followed by *Penicillium digitatium*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus oryzae*. The empty liposomes lead to no fungal growth inhibition and also all liposome samples (all concentration) demonstrated a greater zone of inhibition against *Aspergillus* sp. and *Penicillium* sp. strains than the positive control calcium propionate (2 mg/mL concentration) as shown in Table 1. All extracts were found antifungal activity against fungal strains in different intensities. The inhibition zone for fungal strains ranges from 7.8 to 23.0 mm and with *Cinnamomum verum* liposome was generally observed higher inhibition zone. According to the results of the previous study (not data shown), the liposome samples formed a very high inhibition zone diameter despite the ten-fold decrease in the extract concentration and this revealed that encapsulation efficiency had an important place in the antifungal activity. As stated by Pagnussatt et al. (2016), integration of the extracts into the liposome increases the hydrophobicity of the phenolic compounds, thus leading to strong interaction with the microbial cell membrane and deeper penetration into the hydrophobic region of the membrane. Moreover, liposome structures can interface with microbial cells in different ways, including intermembrane transfer, contact release, absorption, fusion, and phagocytosis, and can enhance cell permeability to antimicrobial agents (Ge & Ge, 2015; Liolios et al., 2009). All these properties allow the potency and duration of the pure extract to increase even more.

3.1.2. Minumum inhibition and minimum fungusidial concentration

Table 2 show MICs and MFCs value of the extracts of *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum* and *Laurus nobilis* against *Aspergillus* sp. and *Penicillium* sp. strains respectively. The MIC and MFC values of fungal strains ranged between <0.625 and >20 mg/ml and 2.5 mg/ml to >20 μg/ml respectively. The lowest MIC value of <0.625 mg/ml of liposomes were exhibited in following way, *Cinnamomum verum* and *Syzygium aromaticum* loaded

Table 1
Inhibition zone diameter values against *Aspergillus* sp. and *Penicillium* sp. of extract loaded liposomes (mm)[1].

Liposome type	*Aspergillus oryzae*		*Penicillium digitatum*		*Aspergillus flavus*		*Penicillum camemberti*		*Aspergillus niger*	
	100 mg/ml	200 mg/ml	100 mg/ml	200 mg/ml	100 mg/ml	200 mg/ml	100 mg/ml	200 mg/ml	100 mg/ml	200 mg/ml
Cinnamomum verum	16.4 ± 0.57^{a}	16.3 ± 0.42^{a}	18.0 ± 0.71^{a}	22.4 ± 0.57^{a}	12.2 ± 0.28^{a}	17.1 ± 0.85^{a}	16.1 ± 0.42^{a}	23.0 ± 0.14^{a}	15.6 ± 0.57^{b}	20.6 ± 0.85^{a}
Curcuma longa	13.4 ± 0.99^{b}	15.3 ± 0.99^{a}	16.7 ± 0.57^{ab}	19.3 ± 0.57^{b}	10.6 ± 0.42^{ab}	12.0 ± 0.42^{b}	15.5 ± 0.71^{a}	17.7 ± 1.13^{b}	12.2 ± 0.28^{c}	16.8 ± 0.57^{bc}
Zingiber officinale	11.4 ± 0.85^{b}	15.2 ± 0.71^{a}	13.9 ± 0.57^{c}	16.5 ± 0.28^{c}	8.9 ± 0.71^{b}	11.9 ± 0.57^{b}	11.3 ± 0.42^{b}	17.1 ± 0.85^{b}	14.8 ± 0.28^{b}	15.5 ± 0.42^{cd}
Syzygium aromaticum	7.8 ± 0.14^{c}	16.0 ± 0.85^{a}	11.1 ± 0.42^{d}	16.3 ± 0.42^{c}	9.6 ± 0.28^{b}	14.8 ± 0.85^{a}	10.3 ± 0.42^{b}	18.5 ± 0.42^{b}	17.2 ± 0.28^{a}	18.2 ± 0.57^{ab}
Laurus nobilis	12.6 ± 0.42^{b}	14.6 ± 0.71^{a}	15.8 ± 0.28^{bc}	17.4 ± 0.99^{bc}	11.1 ± 0.85^{ab}	16.9 ± 0.57^{a}	15.4 ± 0.85^{a}	16.9 ± 0.71^{b}	11.8 ± 0.42^{c}	14.1 ± 0.71^{d}
Empty liposome	–		6.1 ± 0.20		–		5.7 ± 0.30		–	
Negative control	–		–		–		–		–	
Positive control	6.8 ± 0.80		7.6 ± 0.00		6.4 ± 0.40		–		6.0 ± 0.00	

[1]Means followed by the same letter within a column are not significantly different ($p > 0.05$). [2]Negative control: ethanol. [3]Positive control: 2 mg/ml calcium propionate.

Table 2
Minimum inhibition concentration (MIC) and minumum fungusidial concentration (MFC) values extract loaded liposomes (mg/ml)[1].

Liposome type	*Aspergillus oryzae*		*Penicillum camemberti*		*Penicillium digitatum*		*Aspergillus niger*		*Aspergillus flavus*	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Cinnamomum verum	<0.625	20	2.5	2.5	5	5	1.25	10	<0.625	10
Curcuma longa	1.25	>20	2.5	>20	1.25	>20	<0.625	>20	<0.625	20
Zingiber officinale	0.625	>20	5	>20	2.5	>20	10	>20	2.5	20
Syzygium aromaticum	<0.625	20	<0.625	>20	>20	>20	5	20	<0.625	20
Laurus nobilis	1.25	>20	5	>20	>20	>20	5	>20	0.625	20

[1]Means followed by the same letter within a column are not significantly different ($p > 0.05$). MIC: Minumum inhibition concentration. MFC: Minumum fungusidial concentration.

liposome against *Aspergillus oryzae*; *Syzygium aromaticum* loaded liposome against *Penicillum camemberti*; *Curcuma longa* against *Aspergillus niger* and *Cinnamomum verum*, *Curcuma longa* and *Syzygium aromaticum* loaded liposome against *Aspergillus flavus*. Whereas, the least MFC value was recorded at 2.5 mg/ml, 5 mg/ml, 10 mg/ml and 10 mg/ml with *Cinnamomum verum* loaded liposomes against *Penicillium camemberti*, *Penicillium digitatum*, *Aspergillus niger* and *Aspergillus flavus*, respectively. For *Aspergillus oryzae* was determined with *Cinnamomum verum* and *Syzygium aromaticum* loaded liposome as 20 mg/ml. The results of the present study indicated that MIC values of liposomes were lower than compared with pure extract samples (not data shown). The liposomal application increased the inhibition efficiency against fungal strains, especially in *Curcuma longa*, *Zingiber officinale* and *Laurus nobilis* extract. The efficiency can be related to the release into cell membranes or the microorganisms' interior of the trapped active ingredient by allowing fusion with mold strains membranes thanks to the lipid bilayer structure of cell-mimicking vesicles (Risaliti et al., 2020).

3.2. Particle size distribution, ζ –potential and morphological characterization

The particle size in the liposomal system is an important quality criterion for bioavailability, solubility and stability of the active ingredient (Talebi et al., 2021). Particle size and ζ -potential of liposome suspensions with and without extract are presented in Table 2. The recorded $D_{3,2}$ and $D_{4,3}$ values were 0.765–9.95 μm and 16.5–143.0 μm for extract loaded liposomes, respectively. The loading of extract lead to a decrease of the $D_{3,2}$ values and an increase of the $D_{4,3}$ values compared to free liposome. This demonstrated that liposome suspensions had an increase in aggregation and smaller particle population compared to empty liposomes. The highest $D_{3,2}$ and $D_{4,3}$ values were obtained with *Laurus nobilis* and *Cinnamomum verum* loaded liposomes. The results can be related that phenolic compounds in extract samples with many hydroxyl groups are hydrophilic, and therefore can interact with the phosphatidylcholine head, form complexes, and even increase the particle size by causing aggregation (Pimentel-Moral et al., 2018). As seen in Table 3, the particle size distribution of the liposome samples has a wide range. The differences in values of empty and extract-loaded liposomes may be due to the diversity in the hydrophobic and hydrophilic components affected the solubility properties of the extracts. The span value specifies the distribution width and the uniformity of liposome and the small span value refers to the narrow size distribution (Didar, 2021). The span and uniformity values demonstrated a change between 2.048-29.828 and 0.804–7.167, respectively. Integration of extract in liposomes had an important effect on span and uniformity as shown in Table 3. The results showed that *Curcuma longa* and *Zingiber officinale* loaded liposomes had larger size distribution values and significant differences in the characteristics of other liposomes ($p < 0.05$).

ζ-Potential is important for a satisfactory level of the physical stability of liposomes, and the higher ζ-potential, the higher stability (Wu et al., 2011). The zeta potential values of all liposome suspensions demonstrated highly negative values. As presented in Table 3, the zeta potential values changed between −57.8 and −70.9 mV and this change was determined to be statistically insignificant. These values revealed good stability of liposomes in suspensions and integration of extract in liposomes showed no adverse effect on surface charge. Because the zeta potential values above 30 mV or below −30 mV express physically stable liposome suspensions (Müller et al., 2001).

Morphological characterizations of the obtained extract loaded liposomes were examined with an optical microscope equipped with a digital camera. Microscopic images of liposome samples are given in Fig. 1 (1a-b, 2a-b, 3a-b, 4a-b, 4a-b and 6a-b) are representative images of empty, *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale*, *Syzygium aromaticum* and *Laurus nobilis* loaded liposomes, respectively. The optical microscope images of extract-loaded liposomes demonstrated the spherical shape formation with micrometer-size and the empty,

Table 3
Particle size distribution, zeta (ζ) potential and encapsulation efficiency values of extract loaded liposomes.[1].

Liposome type	D3,2 (μm)	D4,3 (μm)	Span	Uniformity	Zeta Potential (mV)	EE (%)
Cinnamomum verum	2.01 ± 0.06^{bc}	143.0 ± 3.54^{a}	14.29 ± 1.47^{b}	4.158 ± 0.40^{b}	−55.6 ± 11.0^{a}	92.87 ± 0.18^{a}
Curcuma longa	1.01 ± 0.08^{bc}	108.0 ± 4.10^{b}	28.213 ± 0.40^{a}	7.167 ± 0.60^{a}	−66.9 ± 12.1^{a}	91.45 ± 0.91^{ab}
Zingiber officinale	2.02 ± 0.10^{b}	112.0 ± 3.96^{b}	29.828 ± 2.33^{a}	7.051 ± 0.30^{a}	−61.3 ± 9.86^{a}	90.87 ± 0.27^{abc}
Syzygium aromaticum	0.765 ± 0.03^{c}	23.0 ± 1.27^{c}	9.053 ± 0.72^{c}	4.11 ± 0.13^{b}	−57.8 ± 7.94^{a}	86.60 ± 0.69^{c}
Laurus nobilis	9.95 ± 0.68^{a}	16.5 ± 1.84^{c}	2.048 ± 0.12^{d}	0.804 ± 0.12^{c}	−70.9 ± 12.2^{a}	87.75 ± 2.07^{bc}
Empty liposome	13.6 ± 0.15	16.0 ± 0.19	1.16 ± 0.54	0.36 ± 0.08	−61.4 ± 10.9	–

[1]Means followed by the same letter within a column are not significantly different ($p > 0.05$). EE: Encapsulation efficiency.

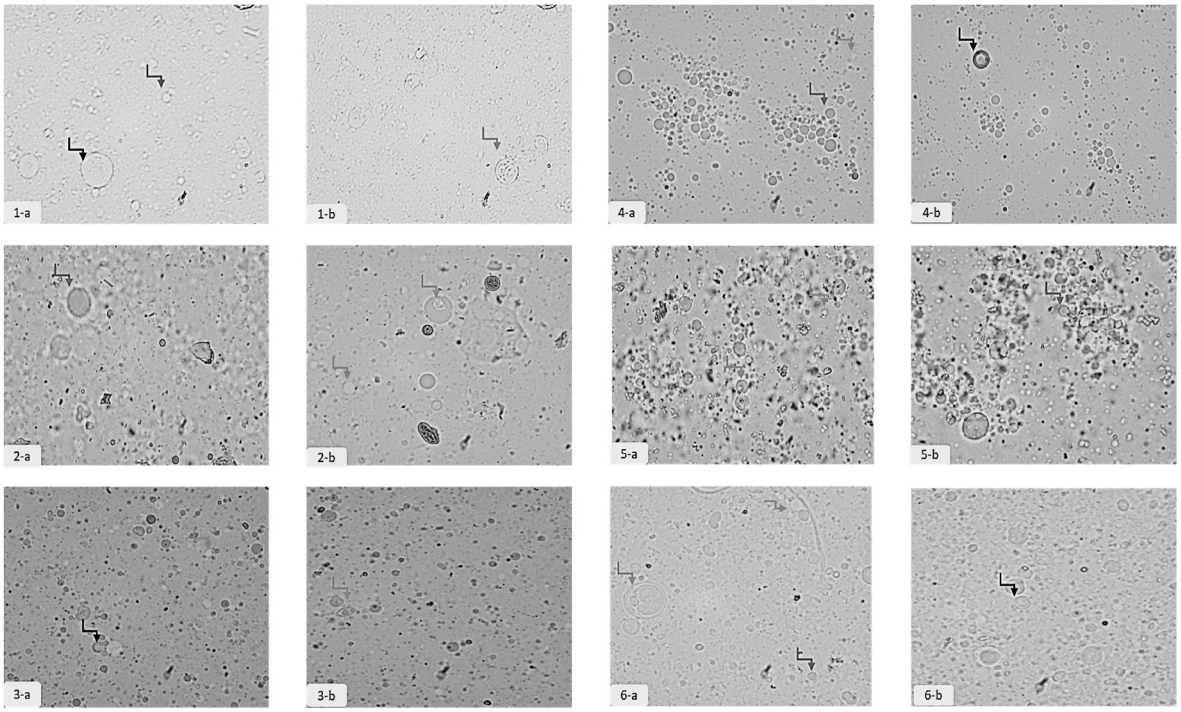

Fig. 1. Microscope images of liposomes. (1a–b: Empty liposom; 2a–b: *Cinnamomum verum*; 3a–b: *Curcuma longa*; 4a–b: *Zingiber officinale*; 5a–b: *Syzygium aromaticum*; 6a–b: *Laurus nobilis*).

unilamellar, multilamellar and multiple liposome vesicles were observed in liposome suspensions.

3.3. Encapsulation efficiency

Encapsulation efficiency is the most important parameter in terms of the encapsulation process success as an indicator of coated and free extract amount. In the case of liposomal structures, encapsulation efficiency depends on various factors such as the lipophilic and hydrophilic structure of coating material, the structure and concentration of phospholipids, preparation methods and environmental factors (pH and temperature) (Siyar et al., 2021). Encapsulation efficiency was stated as % in terms of TPC in liposomes and shown in Table 3. The highest EE of 92.87% was obtained with *Cinnamomum verum* loaded liposome, lower for *Curcuma longa* loaded liposome (91.45%), *Zingiber officinale* loaded liposome (90.87%), *Laurus nobilis* loaded liposome (87.75%) and the lowest of 86.60% for *Syzygium aromaticum* loaded liposome. The high encapsulation efficiency of *Cinnamomum verum* is associated with a non-polar structure of cinnamaldehyde component embedding easier in hydrophobic region of the liposome bilayer with hydrophobic group interaction (Chen et al., 2019). In addition, the specific surface areas and size of the liposome structures affected the encapsulation efficiency of *Cinnamomum verum* loaded liposome the active compounds, being in agreement with the results obtained in particle size distribution (Rasti et al., 2012).

3.4. Physical stability

3.4.1. Encapsulation efficiency during storage

The physical stability of liposomes was investigated by measuring encapsulation efficiency and particle size distribution at the end of

Table 4
Encapsulation efficiency values during storage of extract loaded liposomes [1].

Liposome type	Physical stability conditions			
	FD-RH	4 °C	20 °C	FT
Cinnamomum verum	87.26 ± 1.59^{abA}	89.57 ± 1.22^{aA}	89.70 ± 1.13^{abA}	90.78 ± 0.23^{aA}
Curcuma longa	89.70 ± 1.25^{aA}	90.89 ± 0.45^{aA}	91.02 ± 1.09^{aA}	92.12 ± 0.12^{aA}
Zingiber officinale	86.68 ± 1.27^{abA}	77.74 ± 0.86^{bB}	87.03 ± 1.24^{bA}	83.88 ± 1.99^{bA}
Syzygium aromaticum	75.69 ± 0.90^{cC}	82.61 ± 0.79^{bB}	86.00 ± 0.38^{bA}	84.05 ± 0.38^{bAB}
Laurus nobilis	83.91 ± 0.37^{bA}	79.63 ± 0.75b^{cB}	74.96 ± 0.81^{cB}	83.09 ± 0.53^{bA}

[1]Means followed by the same small letter within a column and the same capital letter within a row are not significantly different ($p > 0.05$). EE: Encapsulation efficiency. FD-RH: Freeze dry-rehydration. FT: Freezee-thaw.

storage under different conditions (Table 4 and Table 5). Liposomes showed significant differences in encapsulation efficiency during storage. During freeze dry-rehydration (FD-RH) storage and freeze-thaw (FT), liposomes showed significant encapsulation efficiency change after 4 weeks of storage and the highest average encapsulation efficiency loss after storage was measured to be 10.90% for *Syzygium aromaticum* loaded liposome and 6.99% for *Zingiber officinale* loaded liposome. In view of results in Table 4, the highest encapsulation efficiency loss during long-term storage at both 4 and 20 °C were detected with *Laurus nobilis* loaded liposome (8.12% and 12.79% loss), on the other hand *Curcuma longa* loaded liposome (0.56% and 0.43% loss) had the highest physical stability with lower loss ratio. This important loss can be originated diffusion of extract from the phospholipid bilayer during storage. In general, liposomes were maintained physical stability the least under FD-RH and FT. The active substance leakage in the liposome structures may be caused by the chemical destruction of liposomes due to a strong increase in mechanical stress and the solute substance as stated by Nakhla et al. (2002). Similarly, Li et al. (2015) associated with particle destabilization and core material leakage with the mechanical stress caused by the penetration of the formed ice crystals into the membrane of the liposomes.

Table 5
Particle size distribution values during storage of extract loaded liposomes [1].

	Liposome type	D3,2 (μm)	D4,3 (μm)	Span	Uniformity
FD-RH	*Cinnamomum verum*	2.34 ± 0.28^{c}	76.6 ± 2.83^{a}	7.138 ± 0.13^{a}	2.174 ± 0.24^{a}
	Curcuma longa	26.6 ± 2.12^{ab}	38.2 ± 1.56^{c}	1.684 ± 0.59^{c}	0.536 ± 0.07^{b}
	Zingiber officinale	29.0 ± 1.98^{a}	48.0 ± 2.97^{b}	1.965 ± 0.31^{bc}	0.733 ± 0.14^{b}
	Syzygium aromaticum	1.34 ± 0.08^{c}	23.5 ± 1.41^{d}	3.10 ± 0.16^{b}	0.976 ± 0.33^{b}
	Laurus nobilis	23.2 ± 0.57^{b}	39.7 ± 1.84^{bc}	2.19 ± 0.25^{bc}	0.718 ± 0.12^{b}
	Empty liposome	20.7 ± 0.47	30.7 ± 1.54	1.735 ± 0.25	0.591 ± 0.10
4 °C	*Cinnamomum verum*	1.47 ± 0.10^{b}	68.7 ± 3.96^{b}	7.357 ± 0.30^{b}	2.42 ± 0.07^{b}
	Curcuma longa	1.06 ± 0.04^{b}	99.9 ± 3.54^{a}	26.729 ± 1.77^{a}	6.691 ± 0.63^{a}
	Zingiber officinale	12.9 ± 2.40^{a}	34.3 ± 1.70^{c}	1.834 ± 0.10^{c}	1.783 ± 0.22^{bc}
	Syzygium aromaticum	1.34 ± 0.07^{b}	18.2 ± 0.85^{d}	2.85 ± 0.20^{c}	1.093 ± 0.11^{c}
	Laurus nobilis	1.05 ± 0.07^{b}	12.3 ± 0.71^{d}	2.483 ± 0.12^{c}	0.854 ± 0.20^{c}
	Empty liposome	15.3 ± 0.54	20.3 ± 1.15	1.652 ± 0.16	0.514 ± 0.11
20 °C	*Cinnamomum verum*	1.24 ± 0.23^{b}	51.0 ± 4.53^{c}	6.253 ± 0.18^{c}	2.171 ± 0.19^{c}
	Curcuma longa	0.953 ± 0.06^{b}	87.0 ± 1.98^{b}	27.89 ± 2.86^{b}	7.105 ± 0.08^{b}
	Zingiber officinale	0.911 ± 0.09^{b}	215 ± 7.07^{a}	40.37 ± 0.35^{a}	12.158 ± 0.40^{a}
	Syzygium aromaticum	1.24 ± 0.27^{b}	15.2 ± 1.27^{d}	2.608 ± 0.65^{c}	1.04 ± 0.07^{d}
	Laurus nobilis	11.1 ± 0.42^{a}	42.15 ± 2.64^{c}	6.466 ± 0.41^{c}	2.719 ± 0.34^{c}
	Empty liposome	10.5 ± 0.37	17.5 ± 0.41	24.4 ± 2.15	6.68 ± 0.05
FT	*Cinnamomum verum*	1.49 ± 0.14^{c}	55.5 ± .1.13^{a}	6.171 ± 0.34^{b}	2.146 ± 0.13^{b}
	Curcuma longa	14.3 ± 0.71^{ab}	41.1 ± 1.27^{b}	1.387 ± 0.10^{c}	2.083 ± 0.15^{b}
	Zingiber officinale	14.7 ± 1.70^{a}	44.0 ± 0.28^{b}	1.454 ± 0.13^{c}	2.161 ± 0.22^{b}
	Syzygium aromaticum	0.712 ± 0.02^{c}	18.0 ± 0.85^{c}	8.355 ± 0.16^{a}	4.002 ± 0.15^{a}
	Laurus nobilis	10.9 ± 0.99^{b}	14.8 ± 1.56^{c}	1.308 ± 0.07^{c}	0.526 ± 0.12^{c}
	Empty liposome	1.68 ± 0.17	13.6 ± 1.41	1.94 ± 0.19	0.635 ± 0.14

[1]Means followed by the same letter within a column are not significantly different ($p > 0.05$). FD-RH: Freeze dry-rehydration. FT: Freezee-thaw.

3.4.2. Particle size distribution during storage

Particle size distribution of liposomes is an important parameter to show changes in physical stability. Liposomes had significantly different particle size distribution ratios compared to before storage. This indicated major aggregation and fusion of liposomes lead to the destabilization and the shift toward a higher value in particle size distribution. The $D_{3,2}$ values were found to an increase of 0.16, 25.33, 27.71, 0.75 and 1.33-fold for *Cinnamomum verum, Curcuma longa, Zingiber officinale, Syzygium aromaticum* and *Laurus nobilis* loaded liposomes during FD-RH. It was revealed that $D_{4,3}$ values did not show a regular change like $D_{3,2}$ values at the end of storage. A similar situation in terms of physical stability was observed for the liposomes having different extracts during FD-RH storage and generally span, and uniformity values detected increment values including being the different situations. During long storage at 4 °C and 20 °C, extract-loaded liposomes turned out to be the most diverse and unstable in terms of particle size distribution among all analyzed samples. The difference in the solubility character of extract and liposome structure and the solubility or association of component-lipid complexes may cause these irregular changes. Especially, the decreases in $D_{4,3}$ values can be explained by the fact that the liposome aggregation in the initial liposome suspension is less stable and aggregation decreases with dissolution late (Volodkin et al., 2007). During FT, the $D_{3,2}$ values of the extract-loaded liposome samples varied between 0.712 and 14.7 μm, and the $D_{4,3}$ values between 14.8 and 55.5 μm. Similarly, for FT storage, reductions in $D_{4,3}$ values indicated that the liposomes remained in the small size region and the collection of only a small fraction of droplets after each freeze-thaw cycle (Mao et la. 2015). We found a decrease in span and uniformity of liposomes as a result of the freeze-thaw process (Table 5).

3.5. Heat stability

For heat stability, encapsulation efficiency and the encapsulation efficiency % loss according to initial values was observed after heat-treatment at 50, 60, 70 or 80 °C for 30 min and presented in Table 6. After 30 min of heat treatment at 50 °C, the encapsulation efficiency loss from liposomes changed between 0.84% and 3.44%, whereas were occurred a change between 7.28% and 14.87% with heat treatment at 80 °C. Results revealed that a deterioration of the lipid layers with high heat treatment leads to active substance loss. Stability of *Syzygium aromaticum* loaded liposomes at all heat temperature was 3.46%, 3.49%, 8.21% and 7.59% higher than the stability of *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale* and *Laurus nobilis* loaded liposomes (Table 5). The phenolic compounds created different hardening effects on the liposome structure may cause to be variable responses of liposome vesicles against different temperatures. In a similar manner, Niu vet al. (2012) reported that the phospholipid bilayer structure is largely decomposed at high temperatures above 70 °C and leads to very close relative density values for liposomal and free active substance at 70–80 °C. This was associated with the transition of the phospholipid bilayer of the liposome from the solid gel phase to the liquid-liquid crystal phase with the increased temperature. Also, the water solubility properties of the substance enable to be present in the lipid layer inside or on the surface, which can affect stability, especially at high temperatures (Khan et al., 2008). That's way; the substance embedded between the double lipid layer can be released more easily than that of the liposome core structure.

Table 6
Encapsulation efficiency values during heat treatment of extract loaded liposomes [1].

Liposome type	Heat stability conditions							
	50 °C	% EE Loss	60 °C	% EE Loss	70 °C	% EE Loss	80 °C	% EE Loss
Cinnamomum verum	89.95 ± 1.27^{aA}	3.14	87.92 ± 0.34^{aAB}	5.33	86.54 ± 0.57^{aB}	6.81	82.9 ± 0.18^{aC}	10.74
Curcuma longa	89.46 ± 0.71^{aA}	2.17	82.77 ± 1.01^{cB}	9.49	83.30 ± 0.04^{bB}	8.91	81.6 ± 0.75^{abB}	10.77
Zingiber officinale	89.21 ± 0.78^{abA}	1.83	86.08 ± 0.21^{abB}	5.27	78.98 ± 0.12^{cC}	13.08	76.8 ± 0.83^{cC}	15.38
Syzygium aromaticum	85.88 ± 0.18^{bcA}	0.83	84.46 ± 0.04^{bcB}	2.47	83.72 ± 0.04^{bB}	3.33	80.3 ± 0.54^{bC}	7.28
Laurus nobilis	84.73 ± 0.86^{cA}	3.44	82.70 ± 0.28^{cA}	5.75	75.97 ± 1.29^{dB}	12.05	74.7 ± 0.46^{cB}	14.87

[1]Means followed by the same small letter within a column and the same capital letter within a row are not significantly different ($p > 0.05$). EE: Encapsulation efficiency.

3.6. *In-vitro gastrointestinal digestion*

The release result from extract-loaded liposomes under *in-vitro* stimulation digestion condition was evaluated immediately by measuring the concentration of free TPC amount and are presented in Fig. 2. With the increment of digestion time in gastrointestinal conditions, the extract release ratio from liposomes increased distinctly. In simulated gastric fluid, all extract-loaded liposomes except to *Laurus nobilis* loaded liposome (45.13%) showed a low TPC release ratio as 26.79%, 24.41%, 19.56% and 12.14% for *Cinnamomum verum*, *Curcuma longa*, *Zingiber officinale* and *Syzygium aromaticum* loaded liposomes. The integrity of the liposomal structure may be maintained in the simulated gastric digestion condition because the absence of specific enzymes that disrupt the phospholipid bilayers leads to low extract release at this stage (Wickham et al., 2009). The highest release of *Laurus nobilis* extract in liposome vesicles was measured as 71.18% of its initial concentration after 180 min in simulated gastric fluid consistent with poor vesicular size stability. In contrast, the effects of simulated intestinal fluid containing pancreatin and bile salts on the liposomes were proved with a more enormous release ratio (Fig. 2). The release trend was dramatically different in the simulated intestinal fluid and over 50% of the extracts were released from the liposomes. The release trend was dramatically different in the simulated intestinal fluid and over 50% of the extracts were released from the liposomes. The highest release ratio was found as 82.78% with *Laurus nobilis* loaded liposome, following 79.60% with *Curcuma longa* loaded liposome, 58.85% with *Zingiber officinale* loaded liposome, 51.79% with *Cinnamomum verum* loaded liposome and 27.80% with *Syzygium aromaticum* loaded liposome. As expected, the greatest release happened during the simulated intestinal fluid thanks to containing lipolytic enzymes such as lipase, phospholipase, and cholesterol esterase. Therefore, liposomal bilayers can be deteriorated by lipolytic enzymes, resulting in fusion and aggregation, and the release of the active components in vesicles. According to Liu et al. (2020), this high release may be due to three different reasons. (1)Bile salts can penetrate the phospholipid bilayers, causing swelling of the liposome vesicles, (2) the increase of membrane fluidity due to the permeability of bile salts, resulting in a higher level of lipase adsorption, and (3)penetration of bile salt and enzymatic hydrolysis of lipase disrupt the structural organization of liposomes, resulting in rapid leakage of the bioactive component.

3.7. *Color properties*

The lightness (L^*), redness (a^*) and yellowness (b^*) values of the liposomal samples were measured and the results are presented in Table 7. As seen in Table 7, the L^* values of the *Zingiber officinale* loaded liposomes were found to be the highest. The integration of extract in liposome vesicle might have provided the improvement of lightness value by masking the pure extract color. It was found that the *Cinnamomum verum* loaded liposomes was higher redness in color, while *Curcuma longa* loaded liposomes had higher yellowish color properties. The color quality of liposomes is affected by the phospholipid outer layer because of localized as the vesicle wall of phosphatidylcholine (Olatunde et al., 2019). The highest Chroma and Hue angle value was recorded in the *Curcuma longa* loaded liposomes and *Laurus nobilis* loaded liposomes. A similar observation as an increase in L^* values was reported by Olatunde et al. (2019) and Tagrida et al. (2021) and stated that the retention of the extract might be contributed to the color properties of the liposomal structures, and consequently, improve to be the color properties by masking the color. The total color difference (ΔE)

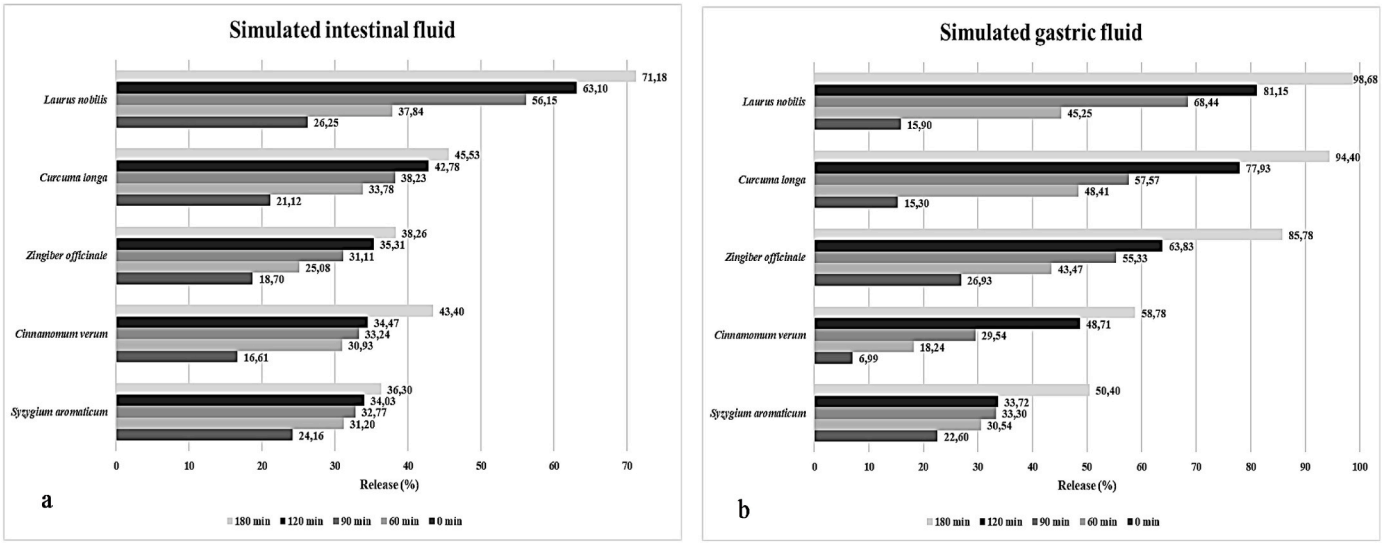

Fig. 2. *In-vitro* simulated gastric fluid (a) ve simulated intestinal fluid (b) stage % release values.

Table 7
Color properties of extract loaded liposomes [1].

Liposome type	L*	a*	b*	Chroma	Hue angle	ΔE
Cinnamomum verum	41.11 ± 0.64^{c}	14.50 ± 0.60^{a}	17.16 ± 0.39^{c}	22.46 ± 0.68^{b}	49.81 ± 0.53^{e}	22.10 ± 0.94^{b}
Curcuma longa	50.80 ± 0.12^{b}	5.15 ± 0.16^{b}	60.34 ± 0.04^{a}	60.56 ± 0.06^{a}	85.13 ± 0.15^{c}	53.74 ± 0.07^{a}
Zingiber officinale	53.48 ± 0.17^{a}	−2.49 ± 0.17^{d}	22.32 ± 0.78^{b}	22.45 ± 0.76^{b}	96.38 ± 0.65^{b}	15.33 ± 0.79^{c}
Syzygium aromaticum	49.65 ± 0.14^{b}	2.59 ± 0.06^{c}	6.87 ± 0.11^{d}	7.34 ± 0.13^{c}	69.38 ± 0.15^{d}	5.11 ± 0.00^{d}
Laurus nobilis	33.17 ± 0.05^{d}	−3.90 ± 0.01^{e}	21.10 ± 0.06^{b}	21.46 ± 0.06^{b}	100.45 ± 0.01^{a}	23.29 ± 0.00^{b}
Empty liposome	51.69 ± 0.76	−2.09 ± 0.13	7.1 ± 0.15	7.40 ± 0.19	106.4 ± 0.05	–

[1]Means followed by the same letter within a column are not significantly different ($p > 0.05$). ΔE: total color difference.

was found to be the highest in the *Curcuma longa* loaded liposome compared to the empty liposome sample. Considering all ΔE values, the ΔE of all liposome samples are noticeable to the human eye (Table 7). The biggest effect on the ΔE in the *Curcuma longa* loaded liposome is due to the rather high b* value.

3.8. Differential scanning calorimetry

DSC is a reliable method to detect possible interactions and to predict the effect on the on phase transitions of liposome structures of the phenolic extracts. The phase transition temperatures and enthalpy energy of fresh liposomal suspensions are demonstrated in Table 8. The extract-loaded liposome suspensions indicated three main endothermic peaks and many small peaks. The peaks had positive enthalpy variation associated with energy absorption of liposome vesicles by heating (Yokota et al., 2012). DSC analysis results showed melting transitions, the pre-transition, and liquid-crystalline phase transition in the range of −30 °C–250 °C temperatures. Liposome suspensions were characterized by a highly similar endothermic transition in the range of 1 °C–5 °C temperatures (T_{peak1}). The T_{peak1} value of the liposomes is stated as the Tc temperature at which the lipid bilayers change from gel state to liquid crystal form. The empty liposomes showed a major endothermic at 5.31 °C (199.88 J/g) and T_{peak1} values decreased with the integration of extract in liposomes compared to the empty liposomes. The decrease in the T_{peak1} of liposomes suspensions was ascribed structural changes in the bilayer membrane and a decrease of intercalation in the bilayer membrane of extract-loaded liposomes due to interactions with polar head groups (Pinilla et al., 2019). Considering T_{peak1}, endothermic peak results indicated the liposomes containing extract had a heterogeneous structure, and increase the ΔH values by inducing the interconnected gel phase and causing harder bilayers. The pre-phase transition peaks (T_{peak2}) changed between 102.16 and 133.10 °C and 1.06–3.78 J/g enthalpy values. However, the T_{peak2} was not observed in the empty liposome samples. The pre-phase transition temperatures are generally 5–10 °C lower than the main phase transition temperature due to the co-movement of the hydrocarbon chain or the rotation of the polar groups (Hinz & Sturtevant, 1972). The main phase transition (T_{peak3}) maximum temperatures and enthalpy of the liposome samples ranged from 116.47 °C to 841.29 J/g to 165.22 °C and 1106.7 J/g and the empty liposome T_{peak3} value was determined as 105.65 °C and 519.5 J/g. The difference in main phase transition can be associated with multi-crystalline form structures and liposoluble compounds in the extract. Furthermore, the extract-loaded liposomes showed higher enthalpy than the corresponding empty liposome and this is probably related to with alternation of van der Waals interactions between lipid acyl chains and phenolics (De Lima et al., 2010; Manrique -Moreno et al., 2010). Also, the difference in T_{peak3} can be explained by a significant variation in the particle size distribution. So, *Laurus nobilis* loaded liposome samples with smaller average particle sizes than other liposomes showed lower phase transition temperatures.

4. Conclusion

Liposomes are an effective technique between conventional delivery systems and novel delivery systems. MIC values were significantly reduced by liposome encapsulation and so the antifungal activity of extract increased significantly against *Aspergillus* sp. And *Penicillium* sp.

The extract-loaded liposomes were characterized by conducting particle size distribution, stability, morphology, release ratio in digestion and DSC data. The results of the present study indicate that the particle size distribution, zeta potential, physical and heat stability of liposomes changed depending on various hydrophilic and hydrophobic substances in the extract. According to the zeta potential values of the liposome samples, the thermodynamic stability of the samples can be listed as *Laurus nobilis* > *Curcuma longa* > *Zingiber officinale* > *Syzygium aromaticum* > *Cinnamomum verum* ($p > 0.05$). The highest EE of 92.87% was achieved for *Cinnamomum verum* loaded liposome. The heat and physical stability of liposomes were found high thanks to vesicles with multiple lipid lamellae. In liposome samples except for *Syzygium aromaticum* loaded liposomes occurred release % generally less than 50% in the simulated gastric fluid and more than 50% in the simulated intestine fluid. Liposomes with phosphatidylcholine/extract can serve as a delivery-based system for the improvement of antifungal properties and can be used as a natural antioxidant and antifungal agent in different food products as an alternative to existing chemical food additives.

Author contribution

Mine Aslan: Investigation, Resources, Formal analysis, Writing – original draft. Nilgün Ertaş: Methodology, Project administration, Funding acquisition, Conceptualization, Supervision, Writing-review & editing. M. Kürşat Demir: Project administration, Supervision, Writing-review & editing.

Table 8
T_{peak} temperature and enthalpy energy variation (ΔH) obtained by DSC of extract loaded liposomes [1].

Liposome type	T_{peak1} (*Tc*)		T_{peak2}		T_{peak3}	
	Maximum peak (°C)	Enthalpy (J/g)	Maximum peak (°C)	Enthalpy (J/g)	Maximum peak (°C)	Enthalpy (J/g)
Cinnamomum verum	2.39 ± 0.04^{c}	249.18 ± 11.06^{a}	132.71 ± 1.07^{a}	2.60 ± 0.07^{b}	152.07 ± 1.19^{b}	1049.60 ± 35.38^{ab}
Curcuma longa	2.48 ± 0.04^{c}	257.87 ± 10.96^{a}	132.56 ± 1.85^{a}	1.06 ± 0.03^{c}	152.60 ± 2.09^{b}	1095.60 ± 68.22^{a}
Zingiber officinale	1.69 ± 0.03^{d}	210.55 ± 7.34^{b}	131.19 ± 0.45^{a}	1.11 ± 0.04^{c}	148.56 ± 1.60^{b}	916.84 ± 20.48^{bc}
Syzygium aromaticum	4.09 ± 0.03^{a}	198.99 ± 4.85^{b}	133.10 ± 0.28^{a}	3.78 ± 0.10^{a}	165.22 ± 0.91^{a}	1106.70 ± 16.22^{a}
Laurus nobilis	2.81 ± 0.06^{b}	225.87 ± 7.79^{ab}	102.16 ± 0.85^{b}	2.46 ± 0.06^{b}	116.47 ± 1.26^{c}	841.29 ± 37.86^{c}

[1]Means followed by the same letter within a column are not significantly different ($p > 0.05$).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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