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Formulation of Grape Leaf Extract (*Vitis vinifera*) Phytoniosomes and Evaluation of Their Effects in Enriched Stirred Yogurt on Appetite Suppression and Weight Reduction in Rats on a Normal Diet

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ABSTRACT

Microencapsulation technology is a novel approach to enhancing bacterial resistance against environmental, chemical, and gastrointestinal stresses. In this study, milk protein concentrate (MPC) was used as a wall material due to its strong biological and protective properties. The aim was to evaluate the effect of this material, combined with transglutaminase enzyme, on the survival of *Lactobacillus rhamnosus* in the acidic environment of orange juice. Four samples were prepared: S1 (plain orange juice), S2 (protein + bacteria), S3 (protein + bacteria + enzyme), and S4 (protein + enzyme). During storage, samples were analysed for bacterial viability, antioxidant activity, pH, titratable acidity, vitamin C, reducing sugar, total phenolic content, color, FTIR spectra, and molecular weight. The results showed that sample S3 exhibited the best performance with a viability of 5.5×10^7 CFU/mL, antioxidant activity of 68.5%, and acidity of 4.4%, along with a pH of 3.6. In contrast, sample S4 showed the lowest viability (3.8×10^7 CFU/mL) and vitamin C content (32.3 mg/L). Sample S2 had the highest total phenolic content (14.7 mg GAE/mL) and the lowest reducing sugar (9.07 mg/mL). During storage, vitamin C, reducing sugars, and phenolics decreased, whereas acidity and antioxidant activity increased. Samples containing bacteria showed higher vitamin C and acidity, while those with the enzyme exhibited higher reducing sugar content. FTIR analysis confirmed the presence of carbonyl, methyl, and amine functional groups. Sample S2 showed decreased molecular weight and increased solubility, indicating effective interactions between bacteria and protein.

1- INTRODUCTION

Grape leaves (*Vitis vinifera*) have long been consumed in the Mediterranean diet and Iran, primarily in traditional foods and herbal teas. These leaves are rich in polyphenolic compounds, flavonoids, and anthocyanins, offering valuable nutrients and bioactive properties. While their antioxidant, anti-inflammatory, and antimicrobial effects are well-documented, their potential role in weight reduction has received less attention [1]. Concurrently, obesity—a chronic disease characterized by abnormal fat accumulation—has emerged as a major global health threat driven by sedentary lifestyles and poor dietary habits. In Iran, nearly 50% of adults aged 15 to 65 are affected by overweight or obesity, with a notably higher prevalence among women [1].

Various approaches, including medications, surgery, and lifestyle modifications, are available for obesity management. However, many pharmaceutical interventions are associated with significant side effects, dependency issues, and weight regain following discontinuation [2,3]. These limitations have spurred interest in natural herbal supplements. Medicinal plants such as *Trigonella foenum-graecum* (fenugreek), cinnamon, soy, and black cumin have demonstrated weight-reducing effects through mechanisms like appetite suppression, increased fat metabolism, and hypolipidemic activity. Despite these benefits, the application of herbal extracts in the food industry is severely hindered by their instability, sensitivity to light and oxygen, poor bioavailability, and undesirable off-flavors [4].

Nanotechnology, particularly the development of lipid nanocarriers like phyto-niosomes, offers a promising solution to overcome these challenges. Phyto-niosomes are amphiphilic, spherical vesicles ranging from 10 nm to 10 μ m, capable of encapsulating both hydrophilic and lipophilic compounds. By entrapping herbal polyphenols within their bilayer structure, these systems enhance the stability, gastrointestinal absorption, and cellular penetration of bioactive compounds while masking their unpleasant taste and protecting them from degradation [5-7]. Furthermore, stirred yogurt serves as an excellent functional food matrix to deliver these nanocarriers due to its high consumer acceptance.

In this study, grape leaf extract was obtained using a green extraction method and encapsulated into a phyto-niosomal carrier

formulated with Span 60, Tween 80, and phytosterols via thin-film hydration. The physicochemical characteristics of the synthesized phyto-niosomes were evaluated, and their incorporation into stirred yogurt was investigated to assess the functional effects on appetite suppression and weight management in healthy mice.

2-Materials and Methods

1. Extraction and preparation of grape leaf extract

Fresh, healthy grape leaves (*Vitis vinifera* L. cv. Asgari) were collected in June 2023 from vineyards in the Gehro region, Chaharmahal and Bakhtiari Province, Iran. The leaves were thoroughly washed with distilled water, shade-dried at room temperature under adequate ventilation, and subsequently ground into a fine powder using a laboratory mill. The powder was passed through a 60-mesh sieve (approximately 250 μ m) and stored in an airtight container at 4 °C in the dark until further use.

The polyphenolic extract of grape leaves was obtained using a green extraction method combining maceration, ultrasonic treatment, and microwave treatment, as described by Jakpa et al. (2018) with slight modifications [8]. Briefly, 10 g of plant powder was mixed with a solvent consisting of deionized water and polyethylene glycol-400 (PEG-400) at a ratio of 7:3 (v/v), using a solid-to-liquid ratio of 1:10 (g/mL). The mixture was transferred into sealed glass containers and degassed with argon gas before the extraction began in order to remove oxygen and prevent oxidation of phenolic compounds. The degassed mixture was magnetically stirred at 130 rpm at room temperature for 72 h. After maceration, the sample was subjected to ultrasonic treatment for 25 min at a frequency of 40 kHz and a power of 120 W while the temperature was maintained at 4 °C. The sample was then allowed to reach room temperature before microwave treatment was applied for 2 min at 180 W. This ultrasonic–microwave treatment cycle was repeated once to maximize the extraction yield.

The resulting extract was initially filtered through Whatman No. 1 filter paper and subsequently centrifuged at 8000 rpm for 20 min at 4 °C to remove any remaining solid particles. The clarified supernatant was passed through Whatman No. 1 filter paper once more and transferred to a freeze-dryer (lyophilizer) to obtain a lyophilized powder. The dried powdered

extract was stored at 4 °C in the dark until further use.



2. Determination of Total Phenolic Content (TPC) of the Plant Extract

The total phenolic content (TPC) of the grape leaf extract (*Vitis vinifera* L.) was determined using the standard Folin–Ciocalteu colorimetric method with slight modifications [10]. For this purpose, 100 µL of the extract solution (at a predetermined concentration) was mixed with 500 µL of Folin–Ciocalteu reagent that had been diluted 1:1 with distilled water. After 5 min of incubation at room temperature, 400 µL of sodium carbonate solution (Na₂CO₃, 20% w/v) was added, and the final mixture was thoroughly mixed. The reaction mixtures were then kept in the dark at room temperature (25 °C) for 60 min to allow complete color development. The absorbance of each solution was measured at a wavelength of 760 nm using an Ultraspec 2000 spectrophotometer.

4. Preparation and formulation of phyto-niosomes

The phyto-niosomes containing grape leaf extract were prepared according to the method described by Pandu et al. [9], with slight modifications. The purpose of encapsulation was to enhance the stability of phenolic compounds, mask the undesirable taste and odor of the extract, and improve its bioavailability in food formulations. In this method, amphiphilic components including Tween 80 (polysorbate 80), Span 60 (sorbitan monostearate), and a phytosterol component (gamma-oryzanol) were dissolved in 10 mL of absolute ethanol at optimized ratios selected within the suitable hydrophilic–lipophilic balance (HLB) range for vesicular systems (HLB 6–10). The resulting solution was transferred to a 250 mL round-bottom flask and subjected to rotary evaporation at 60 °C under vacuum to gradually remove the organic solvent. This step led to the formation of a thin, uniform lipid film on the inner wall of the flask.

Subsequently, 10 mL of deionized water containing predetermined concentrations of freeze-dried grape leaf extract (based on the optimization design) was added to the flask. The

flask was then placed in a rotary evaporator equipped with temperature control and maintained at 65 °C. The film was hydrated while rotating at 100 rpm for 60 min, allowing complete hydration of the lipid layer. This process resulted in the formation of multilamellar vesicles (MLVs) in the micrometer-size range.

To reduce the particle size and produce a uniform nanosuspension, the hydrated vesicle dispersion was transferred to a high-speed homogenizer and homogenized at 15,000 rpm for 15 min. This step led to the formation of nanometer-sized phyto-niosomes with a multilamellar structure. The final phyto-niosomal suspension was transferred into amber glass containers, stored at 4 °C, and protected from light to prevent degradation caused by light and heat. The samples required for physicochemical analyses, including particle size, zeta potential, and encapsulation efficiency, were prepared immediately after production.

5. Evaluation of Phyto-niosomes Characteristics

5.1. Determination of Encapsulation Efficiency (EE%)

The encapsulation efficiency (EE%) of grape leaf extract in phyto-niosomes was determined by separating the free, non-encapsulated extract from the encapsulated fraction, as described by Mohammadi et al. (2016) [9]. For this purpose, UV–Vis spectrophotometry was initially performed over a wavelength range of 200–400 nm to determine the maximum absorption wavelength (λ_{max}) of the polyphenolic extract. A calibration curve was then prepared at 280 nm using serial dilutions of grape leaf extract.

To separate the free extract from the encapsulated extract, 2 mL of the phyto-niosome suspension was diluted with distilled water and transferred to an Amicon centrifugal filter unit. The sample was centrifuged at 4000 × g for 20 min at 4 °C. During this process, the free, non-encapsulated phenolic

compounds passed through the membrane due to their lower molecular weight and were collected in the filtrate compartment, whereas the phyto-niosomes and encapsulated extract remained above the filter membrane. The concentration of phenolic compounds in the filtrate (C_{free}), corresponding to the free extract, was determined using the calibration curve and absorbance measurement at 280 nm.

The total concentration of phenolic compounds in the original sample (C_{total}) was determined after complete disruption of the phyto-niosomes using an organic solvent (methanol or ethanol; the exact solvent should be specified) and subsequent measurement of the released compounds using the Folin–Ciocalteu method.

Finally, the encapsulation efficiency was calculated using Equation 1:

$$\text{EE (\%)} = [(C_{\text{total}} - C_{\text{free}}) / C_{\text{total}}] \times 100 \quad (\text{Equation 1})$$

where C_{total} is the total concentration of phenolic compounds in the phyto-niosome suspension, and C_{free} is the concentration of free, non-encapsulated phenolic compounds.

5.2. Zeta potential measurement, particle size distribution, and Fourier-transform infrared spectroscopy (FTIR)

The zeta potential of phyto-niosomes containing grape leaf extract (VLE) at concentrations of 10%, 15%, 20%, and 25% w/w was measured to evaluate the colloidal stability and aggregation tendency of the nanocarrier system using electrophoretic light scattering (ELS). The particle size distribution and polydispersity index of the samples were also determined using dynamic light scattering (DLS). The measurements were performed using a Malvern Zetasizer Nano ZS instrument (Malvern Panalytical, Malvern, UK).

To identify and confirm possible molecular interactions between the phyto-niosomal carrier and the extract, Fourier-transform infrared (FTIR) spectroscopy was performed on the selected phyto-niosomal formulation. The samples analyzed included blank phyto-niosomes (without extract), pure grape leaf extract, and extract-loaded phyto-niosomes. Each dried sample was homogenized with KBr powder at a sample-to-KBr weight ratio of 1:100 and compressed into transparent pellets. The infrared spectra were recorded over the wavenumber range of 4000–400 cm^{-1} . The

spectra were then compared to identify shifts in absorption bands and the appearance or disappearance of specific functional-group bands, which may indicate molecular interactions or structural changes between the phyto-niosomal carrier and the extract components. In this experiment, the phyto-niosomal formulation containing 20% w/w grape leaf extract was selected for FTIR analysis.

4-3. Evaluation of grape leaf extract release from phyto-niosomes under simulated gastrointestinal conditions

To assess the controlled release behavior and storage stability of the phyto-niosomal nanostructures, an *in vitro* release study was performed using grape leaf extract-loaded phyto-niosomes. The formulation containing 20% w/w grape leaf extract was selected as the optimal sample. For this purpose, 150 μL of the phyto-niosome suspension was placed inside a dialysis bag (MWCO: specify kDa) and immersed in a release medium consisting of simulated gastric fluid (SGF, pH 3.5), prepared with HCl and pepsin (specify enzyme concentration). In parallel, simulated intestinal fluid (SIF, pH 7.0), containing phosphate buffer and pancreatin (specify enzyme concentration), was used to evaluate extract release under neutral conditions. The release system was incubated at 37 $^{\circ}\text{C}$ with continuous shaking at 100 rpm. At predetermined time intervals of 0, 0.5, 1, 2, 4, 8, 12, and 24 h, 2 mL aliquots were withdrawn from the external release medium and immediately replaced with 2 mL of fresh pre-warmed medium. The collected samples were kept at room temperature for 30 min under static conditions at the corresponding pH before further analysis. To remove any residual nanocarrier particles, each sample was passed through an Amicon centrifugal filter (10 kDa molecular weight cutoff) and centrifuged at $4000 \times g$ for 15 min. The filtrate, containing the released extract, was collected for analysis, while the phyto-niosomes retained on the filter were considered the retentate. To determine the amount of extract remaining in the nanocarrier, the retentate was disrupted and extracted using chloroform (specify solvent volume or ratio). The concentration of grape leaf extract in each fraction was measured using a UV–Vis spectrophotometer at 228 nm. This release procedure was repeated on days 1, 7, 15, and 21 to evaluate the release behavior during storage.

5. Formulation and preparation of phyto-niosome-enriched yogurt containing grape leaf extract

Stirred yogurt containing 2.5% (w/w) fat was

obtained from Shimbar Dairy Products Company (specify city, Iran). To evaluate the applicability of grape leaf extract-loaded phyto-niosomes in a food matrix, yogurt samples enriched with phyto-niosomal formulations containing 10%, 15%, 20%, and 25% w/w polyphenolic extract were prepared. For this purpose, fifteen grams of each phyto-niosomal formulation were added separately to 100 g of stirred yogurt, corresponding to 15 g of phyto-niosomal formulation per 100 g of yogurt. As a comparison, a sample containing 1.5% w/w free (unencapsulated) grape leaf extract was also prepared. Stirred yogurt without any extract or phyto-niosomes was considered the control group. The samples were mixed gently until homogeneous.

5-1. Evaluation of physicochemical properties of stirred yogurt containing grape leaf extract-loaded phyto-niosomes

To evaluate the storage stability and quality of phyto-niosome-enriched yogurt formulations containing grape leaf extract at four different concentrations, compared with the control sample (plain yogurt without extract or phyto-niosomes), physicochemical analyses were performed at four time points (days 0, 7, 15, and 21) during storage at 4 ± 1 °C. The pH of the samples was measured using a digital pH meter (specify model and manufacturer). Titratable acidity was determined as percent lactic acid (w/v) by volumetric titration with 0.1 N sodium hydroxide using phenolphthalein as the indicator. Color characteristics of the samples were evaluated using a colorimeter (specify model and manufacturer) in the CIE-Lab color system, and the L* (lightness), a* (redness–greenness), and b* (yellowness–blueness) values were recorded. Measurements were taken at three different points of each sample using illuminant D65. Syneresis (water separation) was determined as an index of the colloidal stability of the yogurt. After preparation, the samples were placed in standard containers and stored at 4 °C. At each sampling time, the water separated from the surface of the sample was carefully collected using a pipette and weighed. The percentage of syneresis was calculated as follows:

Syneresis (%) = (weight of separated water / total weight of yogurt sample) $\times$ 100

Emulsion stability was also evaluated using a centrifugation test ($3000 \times g$ for 10 min), and any phase separation, layering, or sedimentation was recorded. In addition, the antioxidant activity of the yogurt samples was measured using the DPPH radical scavenging assay to assess the retention of active phenolic compounds during storage. All measurements were performed in three independent replicates ($n = 3$), and the results are reported as mean $\pm$ standard deviation.

6. Animal study design, feeding, and evaluation of nutritional parameters

In this study, 20 male Wistar rats with an initial body weight of 230–250 g were randomly divided into four groups ($n = 5$ per group): (1) the control group, receiving standard rodent diet and drinking water without yogurt; (2) the plain yogurt group, receiving stirred yogurt containing 2.5% (w/w) fat; (3) the free extract group, receiving yogurt containing 1.5% (w/w) free (unencapsulated) grape leaf extract; and (4) the phyto-niosome group, receiving yogurt enriched with grape leaf extract-loaded phyto-niosomes (specify whether the formulation contained 20% w/w grape leaf extract or 20% w/w phyto-niosomes in the yogurt). After a 1-week acclimatization period under controlled environmental conditions (22 ± 2 °C, $55 \pm 3\%$ relative humidity, and a 12:12-h light/dark cycle), the animals received their assigned yogurt treatments twice daily at fixed times for 6 weeks. The animals had free access to drinking water and a standard diet throughout the experiment.

To assess the effects of the dietary treatments on feeding behavior, food intake was recorded at 30, 60, 120, and 240 min after yogurt administration. In addition, the body weight of the animals was measured weekly using a digital balance with an accuracy of 0.1 g.

All experimental protocols were conducted in accordance with the ethical guidelines of the Ethics Committee of Golestan University of Medical Sciences and were approved under the code IR.GOUMS.REC.1404.110.

Table 1. Experimental Groups and Treatments

Description of treatment received	Number of Rats	Group
Stirred yogurt without any additives	5	Control Group
Stirred yogurt + Unencapsulated <i>Vitis vinifera</i> L. leaves, (VLE)	5	First Treatment

Stirred yogurt + Phytoniosomal VLE20%	5	Second Treatment
Only food and water without yogurt	5	Negative Control

7. Statistical Analysis

All data were analyzed using SPSS statistical software. The results are presented as mean $\pm$ standard deviation. One-way ANOVA was used for comparing the means between multiple groups. A significance level of $p < 0.05$ was considered for all tests.

3- Results and Discussion

3-1. Total Phenolic Content in grape leaf extract and phyto-niosomes

The total phenolic content (TPC) was determined spectrophotometrically using the Folin–Ciocalteu reagent, with gallic acid as the standard. The results showed that increasing the concentration of grape leaf extract (VLE) from 10% to 25% led to a significant increase in the total phenolic content. This increase was directly correlated with the enhanced ability of the extract to scavenge DPPH free radicals, with the percentage of inhibition showing a concentration-dependent upward trend. These findings are consistent with established chemical principles, confirming that the hydroxyl groups present in phenolic compounds play a key role in the antioxidant activity of plant extracts.

The results of this study are in agreement with findings reported by other researchers. Gambuti et al. reported that extracts from grape leaves and stems with higher phenolic concentrations exhibited lower IC_{50} values in the DPPH assay, indicating stronger antioxidant capacity [21]. Additionally, a comprehensive review by Kang et al. confirmed that compounds such as quercetin,

chlorogenic acid, and other phenolics are key contributors to the inhibition of lipid oxidation [22].

As shown in Figures 2 and 3, the encapsulation of grape leaf extract within phyto-niosomes resulted in a significant increase ($p < 0.05$) in the measured total phenolic content compared to the control group (blank phyto-niosomes without extract). The upward trend observed from 10% to 25% extract concentration indicated that the phenolic content increased progressively with the percentage of extract incorporated. The highest total phenolic content was observed in the 25% treatment, suggesting that this level provided the greatest phenolic loading capacity under the conditions tested.

The results of the DPPH radical scavenging assay were also consistent with the total phenolic content values, demonstrating that the extract-loaded phyto-niosomes exhibited significantly higher antioxidant activity compared to blank phyto-niosomes. The increasing trend in antioxidant capacity was more pronounced at higher extract concentrations, particularly at the 20% and 25% levels, with the highest DPPH radical inhibition observed at the 25% extract concentration. Other studies have also supported the role of phenolic compounds in grape leaf extracts, including flavonoids and tannins, in neutralizing free radicals and preventing lipid peroxidation [23, 24]. Furthermore, the encapsulation of these bioactive compounds within the phyto-niosomal structure may contribute to their improved stability and controlled release.

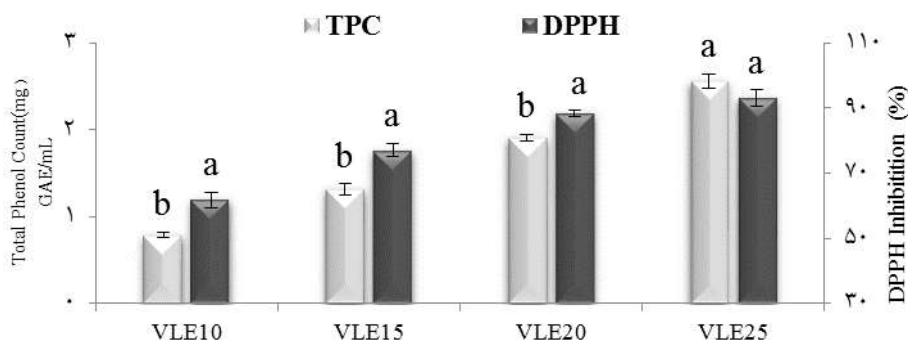


Figure 2. Phenolic content and antioxidant activity of VLE. Different letters denote significant differences ($p < 0.05$).

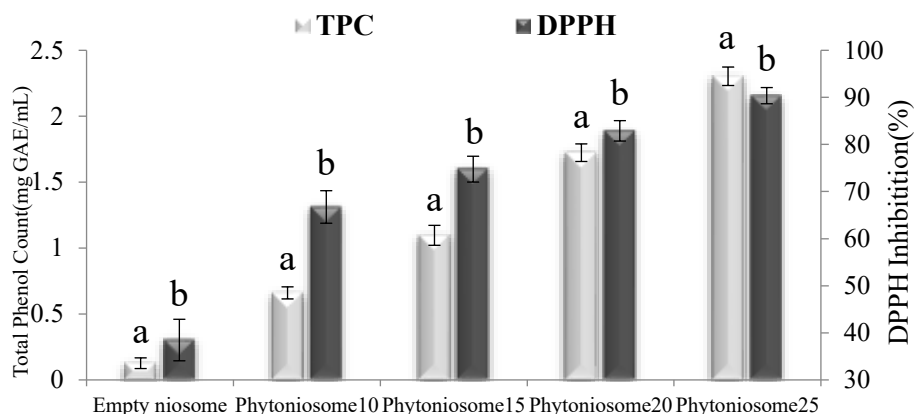


Figure 3. Amount of phenolic compounds and percentage of free radical inhibition of nanocarriers and phytoniosomes at different concentrations. Same letters indicate no significant difference at the confidence level of 5%.

3-2. Encapsulation efficiency and average particle size of phyto-niosomes containing grape leaf extract

As shown in Figures 4 and 5, the average particle size of phyto-niosomes increased from approximately 120 nm to 190 nm as the grape leaf extract concentration increased from 10% to 25% at the initial measurement temperature. This increase in particle size can be attributed to the higher content load within the vesicular structure, as greater amounts of encapsulated extract lead to an expansion of the vesicle volume. At 4 °C, the increase in particle size was less pronounced compared to the initial temperature, suggesting that lower storage temperatures provide greater structural stability to the phyto-niosomes. At 30 °C, the largest particle size was observed in phyto-niosomes containing 25% extract, indicating that elevated temperatures combined with high extract concentrations promote particle enlargement and potential instability. In contrast, formulations containing 10% to 20% extract exhibited better stability under the different storage conditions tested.

The blank vesicles (without extract) showed relatively less membrane stability, which may be

related to the absence of stabilizing interactions between the encapsulated compounds and the vesicular membrane. Conversely, phyto-niosomes containing 25% extract showed the greatest increase in particle size at elevated temperatures, likely due to the saturation of the vesicular membrane with the encapsulated compounds, increased internal osmotic pressure, and intermolecular interactions among phytochemical constituents that may alter the vesicular structure. At the initial measurement temperature, the change in particle size was relatively limited across all concentrations.

Increasing temperature generally enhances molecular mobility and reduces the stability of the phyto-niosomal membrane, which can lead to particle fusion, aggregation, and subsequent enlargement of the average particle size. At 30 °C, the particle size increased more markedly as the extract concentration increased. However, the change in particle size with temperature variation from 4 °C to 30 °C was less significant for phyto-niosomes containing 15% and 20% extract compared to other concentrations, suggesting that these intermediate formulations may offer a more favorable balance between loading capacity and structural stability.

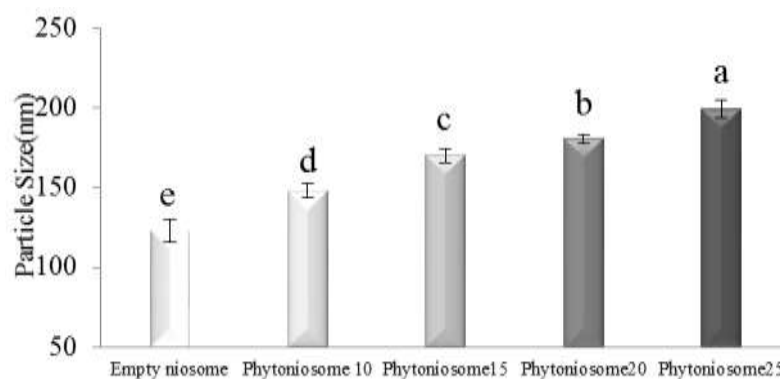


Figure 4. Average Particle Size (nm). Same letters indicate no significant difference at the confidence level of 5%.

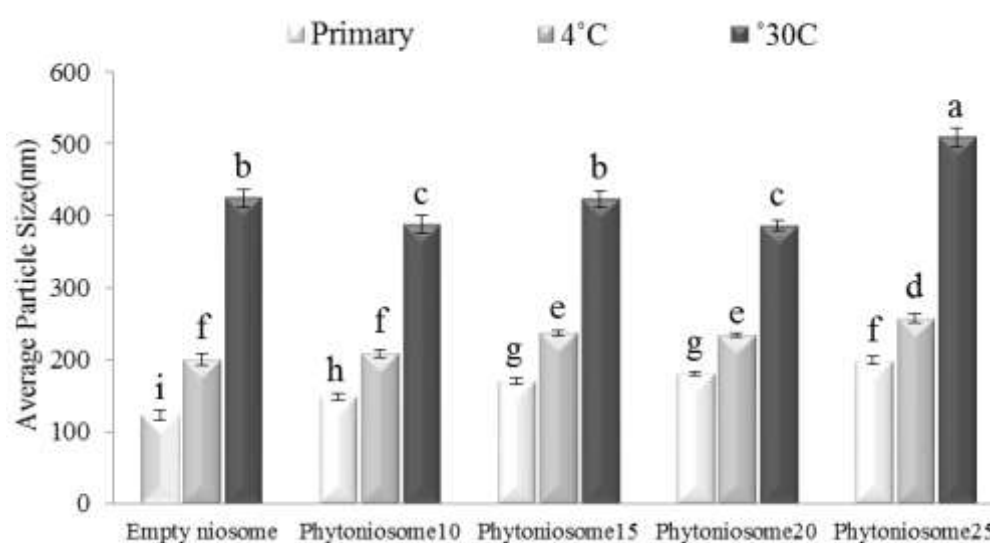


Figure 5. Average particle size (nm) at different storage temperatures. Same letters indicate no significant difference at the confidence level of 5%.

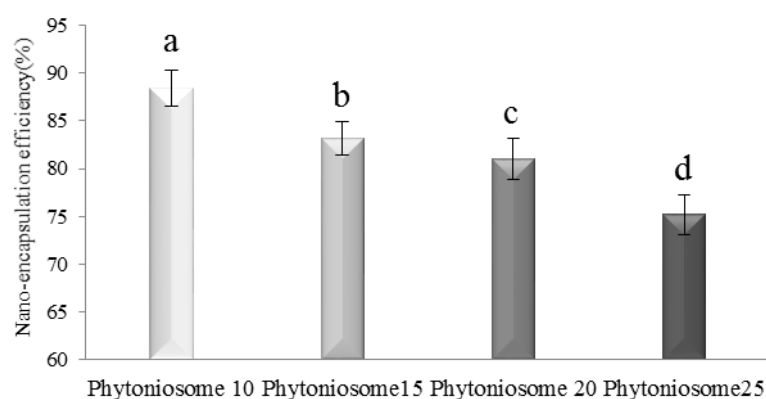


Figure 6. Nano-Encapsulation efficiency (%) of phyto-niosomes at different concentrations, Same letters indicate no significant difference at the confidence level of 5%.

3-3. Relationship between active compound concentration, zeta potential changes, and physical stability of phyto-niosomes

In this study, it was observed that as the percentage of grape leaf extract in the phytoniosomal formulation increased from 10% to 25%, the zeta potential (ζ) became significantly

more negative (Figure 7). This trend may indicate the adsorption or arrangement of molecules bearing ionizable functional groups, such as carboxyl or hydroxyl groups derived from phenolic compounds, at the surface of the particles, thereby increasing the negative surface charge. From a functional perspective, the increase in negative surface charge could contribute to improved short-term physical stability by enhancing electrostatic repulsion between adjacent particles, which reduces the likelihood of aggregation. Additionally, a more negative surface charge may reduce non-specific interactions with cellular membranes, which are also predominantly negatively charged, potentially influencing the biological behavior of the nanocarriers. Generally, a higher absolute zeta potential value (typically $|\zeta| > 30$ mV) is associated with greater colloidal stability due to stronger electrostatic repulsion between particles. However, in the present study, the zeta potential values remained moderate, which may suggest that steric stabilization provided by the surfactant and phytosterol components also

contributes to the overall physical stability of the system.

The comparison of FTIR spectra presented in Figure 8 reveals that the loaded phyto-niosomes exhibited shifts in characteristic absorption peaks and changes in their intensities compared to both the blank vesicles and the free extract. These spectral changes suggest the presence of molecular interactions, such as hydrogen bonding and possible electrostatic interactions, between the grape leaf extract constituents and the phyto-niosomal matrix, which supports the successful encapsulation of the extract within the vesicular structure. Most of the characteristic peaks observed in the spectra of the free extract and blank vesicles were also present in the spectrum of the extract-loaded phyto-niosomes, although with altered intensities. Notably, certain peaks associated with the free extract were diminished or broadened in the phyto-niosomal spectrum, indicating the entrapment of the extract within the vesicular matrix and the partial masking of its functional groups by the surrounding carrier structure.

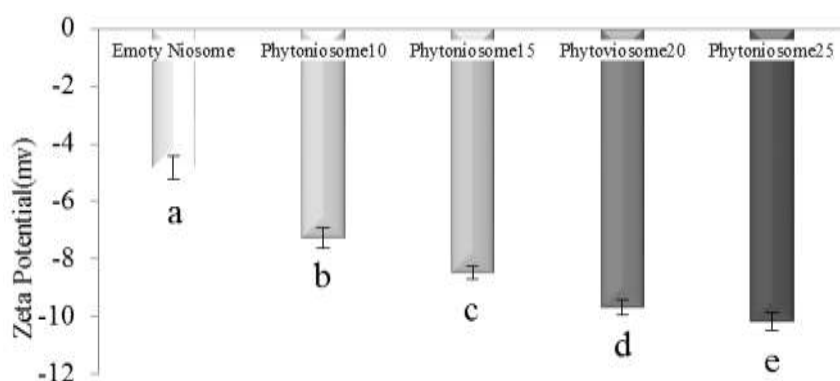


Figure 7. Comparison of Zeta Potential of Empty Niosome and Phytoniosomes with Different Concentration. Different letters

denote significant difference at the confidence level of 5%.

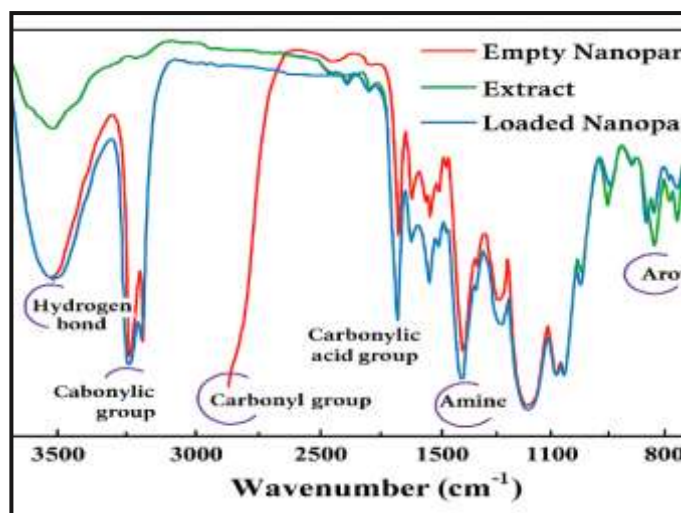


Figure 8. Fourier-transform infrared spectroscopy

3-4. Results of grape leaf extract release from phyto-niosomal nanocarriers

As shown in Figure 8, the release profile of grape leaf extract from phyto-niosomal nanocarriers was evaluated under simulated gastrointestinal conditions. In the simulated gastric fluid (SGF, pH 3.5), the release of the extract followed a gradual and relatively slow pattern. During the first hour, only approximately $10.3\% \pm 1.1$ of the encapsulated compound was released. After 8 hours, this value increased to $28.7\% \pm 2.5$, and by 24 hours, the cumulative release reached $36.4\% \pm 3.0$. This limited release under acidic gastric conditions suggests that the phyto-niosomal carrier provides a degree of protection to the encapsulated bioactive compounds, potentially reducing their premature degradation in the stomach.

In contrast, in the simulated intestinal fluid (SIF, pH 7.0), the release process was notably faster and more extensive. During the first hour, approximately $25.8\% \pm 2.2$ of the extract was

released. This value increased to $63.9\% \pm 3.3$ after 8 hours and ultimately reached $79.6\% \pm 2.9$ after 24 hours. The significantly higher release observed under intestinal conditions suggests a more efficient and nearly complete release of the encapsulated compounds in the neutral pH environment of the intestine, facilitated by the presence of pancreatic enzymes and the altered physicochemical conditions of the intestinal medium.

The differential release behavior observed between SGF and SIF indicates that the phyto-niosomal carrier can provide pH-responsive release characteristics, offering partial protection of the bioactive compounds in the acidic gastric environment while enabling enhanced release under intestinal conditions. This controlled release profile is particularly advantageous for food applications, as it may help preserve the stability and bioavailability of phenolic compounds during gastric transit and promote their absorption in the intestinal tract.

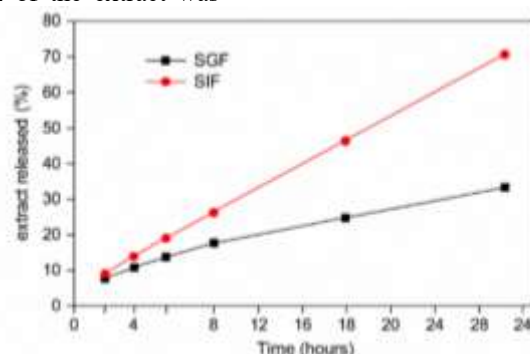


Figure 9. The release profile of VLE extract under simulated gastric and intestinal conditions at different time intervals.

Table 4. Comparison of Physicochemical and Sensory Parameters of Stirred Yogurt Samples under Various Treatments during the 21-day Storage.

blank	0.39± ۱۹	46±۱۴. ۷۳	3.69 ±۰. ۱۶	0.49±۰,۰۹	12.75 ±۱,۷۱	5± ۰,۰	4.9 ±۰. ۲۴	4.9 ±۰. ۲۸	4.9± ۰,۰۹	4.9±۰,۳
extract (10%)	0.54± ۱۷	68±۱۳. ۳۰	4.1 ±۰. ۱۷	0.59±۰,۰۷	15.33 ±۱,۷۳	4.8 ±۰. ۷	4.6 ±۰. ۲۱	4.8 ±۰. ۳	4.8± ۰,۶	4.9±۰,۳
extract (15%)	0.71± ۱۹	75±۱۴. ۱	4.15 ±۰. ۱۶	0.59±۰,۰۹	16.53 ±۱,۶۹	4.3 ±۰. ۷	4.7 ±۰. ۲۷	4.5 ±۰. ۲۰	4.4± ۰,۰۷	4.7±۰,۲۸
extract (20%)	0.89± ۱۹	91±۱۴. ۳۲	3.97 ±۰. ۱۶	0.61±۰,۰۷	17.43 ±۱,۷۳	3.6 ±۰. ۰۱	4.7 ±۰. ۲۴	4.5 ±۰. ۲۲	4±۰,۰	4.3±۰,۲۸
Extract (25%)	0.98± 0.22	94±۱۳	4.07 ±۰. ۱۶	0.73±۰,۰۷	15.93 ±۱,۷۰	3.6 ±۰. ۷	4.1 ±۰. ۲۳	4.3 ±۰. ۲۰	3.4± ۰,۰۰	4.1±۰,۲۰
nanopartic le loaded (10%)	0.55± ۱۸	63±۱۳. ۳۳	4.09 ±۰. ۱۷	0.43±۰,۰۸	16.32 ±۱,۷۴	4.8 ±۰. ۹	4.7 ±۰. ۲۱	4.7 ±۰. ۳	4.5± ۰,۰۳	4.6±۰,۲۷
nanopartic le loaded (15%)	0.69± ۱۷	70±۱۴	4.25 ±۰. ۱۷	0.56±۰,۰۸	17.33 ±۱,۶۰	4.5 ±۰. ۷	4.3 ±۰. ۲۷	4.5 ±۰. ۲۷	4.5± ۰,۰۹	4.2±۰,۳
nanopartic le loaded (20%)	0.78± ۲۱	79±۱۴. ۱	4.24 ±۰. ۱۰	0.48±۰,۰۹	16.11 ±۱,۶۹	4.3 ±۰. ۷۳	4.5 ±۰. ۲۳	4.2 ±۰. ۲۷	4±۰,۰	4.3±۰,۳
nanopartic le loaded (25%)	0.86± ۲	82±۰,۱ ۳	4.03 ±۰. ۱۶	0.5±۰,۰۹	18.91 ±۱,۷۴	4.1 ±۰. ۰۳	4.4 ±۰. ۲۱	4± ۰,۳	3.2± ۰,۶	3.9±۰,۲۸

6. Evaluation of the in vivo effect of stirred yogurt containing phyto-niosomes on appetite suppression and weight management

The in vivo efficacy of the formulated stirred yogurt was evaluated using an animal model, in which the subjects were divided into distinct dietary groups receiving yogurt fortified with specific concentrations of extract-loaded phyto-niosomes. As presented in Tables 5 and 6, which detail the body weight measurements and appetite assessments over the 6-week consumption period, the control group exhibited the highest daily food intake. Food intake progressively decreased in the treatment groups receiving the functional yogurt. Notably, the group receiving yogurt enriched with extract-loaded phyto-niosomes demonstrated a more significant reduction in both food intake and body weight gain compared to the group receiving yogurt with the free (unencapsulated) extract. This enhanced physiological response strongly suggests that encapsulation within the phyto-niosomal structure improves the gastrointestinal stability, intestinal absorption,

and overall bioavailability of the active phenolic compounds.

Furthermore, the groups receiving yogurt fortified with phyto-niosomes containing 20% and 25% grape leaf extract exhibited a significant attenuation in body weight gain compared to the control group ($p < 0.05$). This weight-modulating effect is likely mediated by the biological activity of the bioactive compounds in the grape leaf extract, which may influence the central and peripheral regulation of appetite-associated hormones such as ghrelin and leptin. Conversely, the groups receiving lower extract concentrations (10% and 15%) exhibited less pronounced changes in body weight and food intake, indicating a clear dose-dependent response to the encapsulated phytochemicals.

The observed suppression of appetite and subsequent attenuation of body weight gain in the groups receiving higher extract doses can be attributed to the rich profile of flavonoids and polyphenolic compounds present in grape leaves, which may include resveratrol, catechins, and quercetin. Beyond appetite regulation, the antioxidant and anti-inflammatory properties of these phytochemicals may contribute to the

amelioration of lipid and glucose metabolism. By mitigating oxidative stress and systemic inflammation, these compounds can enhance the basal metabolic rate and promote lipid oxidation,

thereby providing a multifaceted mechanism for weight management and metabolic support.

Table 5. Mean Food Intake $\pm$ Standard Deviation in Different Experimental Groups

Group	Mean pellete Food Intake by Wistar rats $\pm$ SD (g/day)
1. Control (Stirred Yogurt)	1.1 18.5 $\pm$
2. Stirred Yogurt + Unencapsulated VLE	15.5 $\pm$ 0.9
۳. Stirred Yogurt + Phytoniosomal VLE20%	13.3 $\pm$ 0.8

Table 6. Mean Body Weight Loss $\pm$ SD (g/week) in Experimental Groups

Group	Mean Body Weight Loss in Wistar rates $\pm$ SD(g/week)
1. Control (Stirred Yogurt)	1.2 $\pm$ 0.4
2. Stirred Yogurt + Unencapsulated VLE Extract	2.8 $\pm$ 0.6
۳. Stirred Yogurt + Phytoniosomal VLE20%	1.7 $\pm$ 0.5

7- Conclusion

Phyto-niosome technology can serve as an effective surfactant-based encapsulation system to enhance the bioavailability and efficacy of plant extracts in nutritional and pharmaceutical applications. This study demonstrated that phyto-niosomes prepared from grape leaf extract, particularly at the 20% concentration, exhibited favorable physicochemical properties, including optimal particle size, acceptable colloidal stability, and high encapsulation efficiency. The consumption of stirred yogurt enriched with these phyto-niosomes led to a significant reduction in food intake and body weight gain in laboratory rats, indicating the strong potential of

this delivery system to enhance the biological effects of plant extracts.

The slow and sustained release of encapsulated compounds under simulated gastrointestinal conditions supports the long-term efficacy of this system in appetite regulation and metabolic modulation. Stirred yogurt, as a food matrix for phyto-niosome delivery, offers several advantages, including favorable sensory properties, improved bioavailability, and a protective environment for nanoparticles during gastrointestinal transit. The fat and protein content of yogurt may interact with the phyto-niosomal structure to facilitate the controlled release of active compounds.

Although previous studies have implicated various phenolic compounds, such as flavonoids,

phenolic acids, and stilbenes, in the regulation of metabolism and body weight through multiple molecular pathways, the precise mechanisms underlying the anti-obesity effects of grape leaf extract within phyto-niosomes require further investigation. Since this study was conducted in an animal model on a normal diet, the results may not be directly extrapolatable to humans. Future studies should include comprehensive genetic, biochemical, and biopharmaceutical evaluations, as well as long-term safety assessments and clinical trials in human subjects to confirm the efficacy and safety of this functional food formulation.

1. Data Availability Statement

The data generated and analyzed during the current study, including the experimental results and data obtained from the analyses performed, are available from the corresponding author upon reasonable request. The relevant data may be made available to researchers and other qualified individuals upon appropriate request and subject to considerations regarding the appropriate use of the data.

2. Conflict of Interest Statement

The authors declare that there are no financial, professional, personal, or organizational conflicts of interest associated with the conduct of this research, the analysis and interpretation of the results, or the publication of this manuscript. The authors further declare that there are no relationships or interests that could reasonably be perceived as influencing their scientific judgment or the content of this work.

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4. Authors' Contributions

All authors contributed to the different stages of this research. Their contributions included conceptualization and study design, experimental work and data collection, data processing and

analysis, interpretation of the results, preparation of the initial manuscript draft, and scientific and editorial revision of the final manuscript. All authors participated in reviewing the scientific content of the manuscript, read and approved the final version, and accept shared responsibility for the accuracy and integrity of the scientific content presented in this article.

5. Ethics Statement

All experimental protocols were performed in accordance with the ethical guidelines approved by the Ethics Committee of Golestan University of Medical Sciences (IR.GOUMS.REC.1404.110).

6. Declaration of Generative AI Use

During the preparation and writing of this manuscript, generative artificial intelligence tools were used solely to assist with language editing, improving the structure, and enhancing the clarity and readability of the text. The scientific content, study design, experimental procedures, data analysis and interpretation, and scientific decision-making were carried out by the authors. The authors take full responsibility for the accuracy, originality, and integrity of the scientific content of the manuscript, and all final content was carefully reviewed and approved by the authors.

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فرمولاسیون فیتونیوزوم عصاره برگ انگور (*Vitis vinifera*) و ارزیابی اثر آن در ماست همزده غنی شده بر کاهش اشتها و کاهش وزن در موش های تحت رژیم طبیعی

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شناسایی ترکیبات مؤثر و پتانسیل ضدچاقی عصاره فیتونیوزومی برگ انگور (جنس *Vitis*) از طریق کاهش اشتها به طور کامل مطالعه نشده است. افزایش وزن به عنوان عامل خطر بسیاری از بیماری های مزمن شناخته می شود که مصرف مکمل های گیاهی یکی از راهکارهای مدیریت آن است. با این حال، محدودیت هایی نظیر جذب پایین، ناپایداری و اکسیداسیون ترکیبات فعال، کاربرد این عصاره ها را با چالش مواجه کرده است. استفاده از نانوحامل هایی مانند نیوزوم می تواند این محدودیت ها را کاهش دهد. در این مطالعه، فیتونیوزوم عصاره برگ انگور (VLE) تهیه و اثر آن بر کاهش اشتها ارزیابی شد. عصاره گیاهی از پودر خشک شده برگ انگور رقم عسگری به روش استخراج سبز تهیه شد و فیتونیوزوم آن به روش هیدراسیون لایه نازک تولید گردید. ویژگی های نانوحامل با دستگاه های DLS، پتانسیل زتا و FTIR مورد تحلیل قرار گرفت. محتوای پلی فنولی عصاره به روش فولین-سیوکالتی و در طول موج 750 نانومتر اندازه گیری شد. در نهایت، اثر ماست حاوی فیتونیوزوم بر اشتها و وزن 20 موش آزمایشگاهی در چهار گروه (شاهد، ماست ساده، ماست حاوی عصاره بدون پوشش و ماست غنی شده با نانوحامل حاوی 10 تا 25 درصد عصاره) طی شش هفته بررسی شد. فرمولاسیون بهینه فیتونیوزوم دارای میانگین اندازه ذرات 193.5 نانومتر، کارایی درون پوشانی حدود 80 درصد، شاخص پراکندگی 0.37 و پتانسیل زتای -10.16 میلی ولت بود. نتایج نشان داد مصرف ماست حاوی فیتونیوزوم منجر به کاهش معنی دار مصرف غذا و وزن بدن در موش ها شد.