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RESEARCH

Effect of Spray Drying on Physicochemical Stability and Antioxidant Capacity of *Rosa pimpinellifolia* Fruit Extract-Loaded Liposomes Conjugated with Chitosan or Whey Protein During In Vitro Digestion

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Abstract

Spray drying is a well-established, energy efficient, and scalable process widely used in the food industry, however it may lead to thermal degradation of susceptible compounds, such as (poly)phenols, resulting in biological activity loss to some extent. In this study, we aimed to improve the physicochemical stability and bioaccessibility of (poly)phenols from *Rosa pimpinellifolia* fruit extract (Rosa extract) loaded in liposomes by generating solid particles via spray drying. Liposomes were conjugated with chitosan (Ch) and whey protein (Wp) to optimize the biopolymer concentrations by monitoring mean particle diameter, polydispersity index, and surface charge. The mean diameter of liposomes ranged between 135 and 210 nm upon optimal addition of Ch (0.4%, w/v) and Wp (4.0%, w/v) which also increased the entrapment efficiency of (poly)phenols from 74.2 to 77.8% and 79.1%, respectively. After spray drying, about 65–76% of the antioxidant capacity were retained in biopolymer-conjugated liposomes (Ch or Wp) while the retention rate was 48% in only spray-dried extract (Rosa extract powder). Compared to unencapsulated Rosa extract, spray drying (Rosa extract powder) and conjugation with Ch (Ch-Lip powder) or Wp (Wp-Lip powder) significantly increased the bioaccessibility of (poly)phenols and preserved their antioxidant capacity. Based on the findings of this study, Ch- or Wp-conjugation of liposomes prior to spray drying could improve physicochemical stability and protect (poly)phenols loaded in liposomes against processing stress and passage through the digestive tract. Further in vitro and in vivo investigations on a variety of bioactive compounds may draw more attention to their potential as functional foods.

Keywords Black rosehip · (Poly)phenols · Ethanol injection · Encapsulation efficiency · Bioaccessibility

Introduction

The application of additives from natural sources, as alternative to synthetic sources, in food products has gained increasing attention from consumers and researchers in the field (Kalajahi et al., 2022). However, the use of natural antioxidants such as polyphenolic compounds, (poly)phenols, is limited due to their low solubility and sensitivity under food processing and storage conditions (temperature, oxygen, light), or in the gastrointestinal tract (pH, digestive enzymes, presence of other nutrients) (Fang & Bhandari, 2010). Liposomes are extensively used encapsulation systems to protect and release (poly)phenols, as they can be manufactured using simple methods and food grade materials (McClements, 2015). Nonetheless, thermodynamic instability, phospholipid oxidation, and membrane fusion can lead to losses of entrapped material (Tan et al., 2014). Hence, in comparison with dispersion form, solid

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liposomes offer a greater stability of (poly)phenols during storage (Yu et al., 2021). Spray drying is an easily scalable and highly efficient dehydration technique which is widely preferred in food and pharmaceutical industries (Lu et al., 2020). The cost of drying is 30 to 50 times lesser in spray drying compared to freeze drying (Desobry et al., 1997). In order to retain susceptible (poly)phenols during this hot drying process, suitable protectants, also known as carrier materials, are applied. Nevertheless, spray drying of liposomes with protectants could led to aggregation and creaming, probably resulting from the exclusion of vesicles (Karadag et al., 2013). Taken together, to overcome these limitations of liposomes for industrial applications, electrostatic deposition of biopolymers was suggested to facilitate the rigidity of liposomal membrane (Tan et al., 2021). In our previous work, we confirmed the occurrence of flocculation and phase separation in uncoated liposomes after maltodextrin addition to aid spray drying (Gültekin-Özgüven et al., 2016).

Through intermolecular electrostatic interactions, biopolymers can form conjugates (polyelectrolyte complexes) with oppositely charged molecules. In the case of liposomes, the biopolymer selection is restricted to water-soluble and biocompatible ones, which have generally recognized as safe (GRAS) status (Madrigal-Carballo et al., 2010). Biopolymers used to conjugate liposomes in previous studies include chitosan (Hasan et al., 2016; Sarabandi & Jafari, 2020a; Shin et al., 2013; Tan et al., 2016), whey protein (Dag et al., 2019; Frenzel & Steffen-Heins, 2015; Pan et al., 2020), alginate (Gómez-Estaca et al., 2021; Liu et al., 2016; Wang et al., 2015), pectin (W. Zhou et al., 2014), and others (Alemán et al., 2016; Dadashzadeh et al., 2010; Dag et al., 2019). Although the majority of polysaccharides are either neutral or negatively charged in an acidic condition, chitosan is a polycationic linear aminopolysaccharide. It is derived from partial deacetylation of chitin, which is the most abundant polysaccharide biopolymer occurring in nature, from exoskeletons of crustaceans, insects, and the cell walls of fungi (Cheung et al., 2015). On the other hand, whey protein isolate, a by-product from the dairy industry, contains globular proteins including β-lactoglobulin, α-lactalbumin, and bovine serum albumin with a net positive charge under an acidic environment (Kilara, 2015). The significant potential of liposomes loaded with natural-derived antioxidant or antimicrobial extracts from broccoli sprout (Azarashkan et al., 2022), brown algae (Savaghebi et al., 2021), garlic (Pinilla et al., 2020), green tea (Jara-Quijada et al., 2023), mulberry waste (Gültekin-Özgüven et al., 2016), myrtle leaf (Gorjian et al., 2022), and thyme (Aziz & Almasi, 2018) in the manufacture of functional foods were well-established by the previous studies in aqueous, spray dried, or freeze-dried form.

In this study, the pseudo-fruits of the genus *Rosa* L. were selected as source of (poly)phenols because of their high content of bioactive compounds (Zhou et al., 2023). *Rosa pimpinellifolia* L. is a deciduous shrub with purple-black fruits, rich in particularly anthocyanins. *Rosa pimpinellifolia* fruit extract (Rosa extract) was obtained using aqueous-ethanol-modified supercritical CO_2 extraction which provides extracts that are safe for human consumption (Kasapoğlu et al., 2023). To the best of our knowledge, liposomal encapsulation of Rosa extract through ethanol injection method combined by microfluidization has not been evaluated. To develop a food-grade formulation that is stable over time and appropriate for industrial processes, the aim of this study was to compare the conjugation of liposomes with two frequently used polysaccharide- and protein-based biopolymers, i.e., chitosan and whey protein, based on their retention of the antioxidant features during spray drying and simulated gastrointestinal digestion. It should be noted that spray drying is also a widely preferred encapsulation technique in the food and pharmaceutical sector to protect bioactive compounds as it is a cost-effective one-step process (Murugesan & Orsat, 2012). Therefore, Rosa extract was encapsulated using both liposomes and spray drying. The objectives of this study were (i) conjugation of liposomes with varying concentrations of chitosan (Ch) and whey protein (Wp) and selecting the optimal treatment and (ii) to investigate the impact of spray drying on retention and bioaccessibility of the (poly) phenols content and antioxidant capacity of Rosa extract loaded in biopolymer-conjugated liposomal powders.

Materials and Methods

Materials

Lecithin (Lipoid P75®) was a kind present from Lipoid GmbH (Ludwigshafen, Germany). Tween®80 (P1754) and cholesterol (C8667) were obtained from Sigma-Aldrich (Steinheim, Germany). Chitosan with 80% DDA (degree of deacetylation) was a gift from Primex (Siglufjordur, Iceland). Whey protein isolate (Wp) (89% protein) was purchased from Isopure Company, LLC (195 Engineers Road Hauppauge, NY 11788, USA). Maltodextrin with a dextrose equivalent of 16 (Glucidex®) was donated (Roquette, France). Triton X100 and other chemicals were obtained from Merck (Darmstadt, Germany). The enzymes used for the in vitro digestion, pepsin from porcine gastric mucosa (P7000; ≥ 250 units/mg), pancreatin from porcine pancreas (P7545; 8xUSP specification), porcine bile (B8631), bovine bile (B3883), and lipase from porcine pancreas Type II (L3126) were from Sigma-Aldrich (Steinheim, Germany). All the reagents were of analytical grade throughout the study.

Preparation of Rosa extract

The fruits of *Rosa pimpinellifolia* L. were collected from Gümüşhane Province in Blacksea region of Turkey. Fresh fruits were cleaned, milled (including seeds) using liquid nitrogen, and freeze-dried (Christ Alpha 1–2 LD plus, Buch & Holm, Denmark). (Poly)phenols were extracted from fruits according to our previous study (Kasapoğlu et al., 2023). Ethanol-modified supercritical carbon dioxide (CO_2) extraction of (poly)phenols was performed using a supercritical fluid extraction system (Waters SFE 1000, USA) applying hydrated ethanol (25%, v/v) as entrainer (10%, w/w). Freeze-dried powdered fruits were extracted at 60 °C and 280 bar. The CO_2 mass flow rate was 50 g min^{-1} during 60 min. Ethanol content in the obtained extract was evaporated (Buchi Rotavapor RII, Flawil, Switzerland), and the remained extract was lyophilized and stored at − 20 °C.

Preparation of Liposomes

Liposomes were prepared using an ethanol injection method followed by high-pressure microfluidization according to Zou et al. (2014) with minor modifications. Lecithin (Lipoid® P75), cholesterol, Tween 80, and Rosa extract were mixed in a mass ratio of 8:2:4:1. The mixture was dissolved in ethanol and injected into the same volume of phosphate buffer (pH 4.0, 0.01 M phosphate/0.1 M acetic acid) while stirring. After 1 h, the dispersion was transferred to a rotary evaporator, and ethanol was removed under reduced pressure at 60 °C. Then, liposomal dispersions were passed through a high-pressure homogenizer (Microfluidizer Processor M-110L, Microfluidics, Newton, USA) 5 times at 25.000 psi.

Conjugation of Liposomes with Chitosan and Whey Protein

Negatively charged liposomes were conjugated with positively charged biopolymers as previously described (Kasapoğlu et al., 2022). To determine the optimum biopolymer concentrations for conjugation, empty liposomes and Rosa extract-loaded liposomes were added to chitosan (Ch) (0.2–1.0%, w/v) and whey protein (Wp) solutions (2.0–10.0%, w/v) dissolved in distilled water containing acetic acid (pH 3.0) with 1:1 weight ratio and stirred overnight at room temperature. The experimental design to estimate the effect of spray drying on physicochemical stability and antioxidant capacity of Rosa extract-loaded liposomes conjugated with Ch or Wp is displayed in Fig. 1.

Spray Drying of Liposomes

Spray drying of conjugated liposomes was performed after addition of pre-hydrated maltodextrin (20%, w/v) in phosphate buffer (pH 4.0). The resulting liposomal feed consisted of 20% (w/v) maltodextrin, 0.05% (w/v) Rosa extract, 0.5% (w/v) lecithin, and 0.2% (w/v) Ch or 2.0% (w/v) Wp and dried at a feed rate of 2.5 cm^3/min and 0.67 m^3/min air flow using mini spray dryer B-290 equipped with a 1.5-mm nozzle atomizer (BUCHI, Switzerland). The inlet air temperature was set at 150 ± 5 °C resulting in an outlet temperature of 85 ± 5 °C. Rosa extract (0.05%, w/v) dissolved in ethanol-maltodextrin solution was also spray dried to represent the process control. All spray-dried powders were collected, packed in airtight bags, and placed in a desiccator at 4 °C.

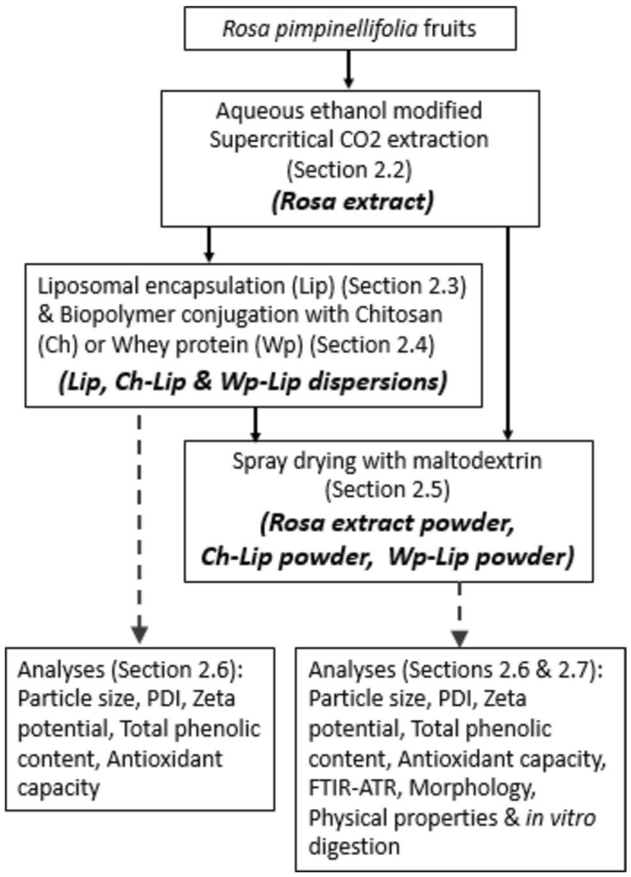

Fig. 1 Experimental design to determine the effect of spray drying on physicochemical and in vitro antioxidant stability of Rosa extract-loaded liposomes conjugated with chitosan or whey protein

Physicochemical Characterization of Particles

Measurement of Particle Diameter and Zeta Potential

The particle diameter, polydispersity index, and zeta (ζ) potential of liposomes were determined by static light scattering using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Liposome dispersions were diluted to 1:5 with phosphate buffer solution (pH 4.0) prior to all

measurements. The refractive index used for lecithin and aqueous phase was 1.44 and 1.33, respectively. Mean particle diameters were reported using the volume mean diameter ($d_{4,3}$). The powders were redispersed in phosphate buffer with a ratio of 1:5 (w/v) for measurement of the particle diameter, PDI, and ζ-potential after spray drying.

Estimation of Total (Poly)phenols Content and Antioxidant Capacity

Folin-Ciocalteu reagent was used to determine the total (poly)phenols content (TPC) (Altin et al., 2018). For the determination of antioxidant capacity (AOC), cupric ion reducing antioxidant capacity (CUPRAC) and DPPH (2,2-diphenyl-1-picrylhydrazyl free radical scavenging) assays were performed (Kasapoğlu et al., 2022). Empty liposomes without extract were taken as blank in the calculations. TPC and AOC were expressed as μg gallic acid equivalents (GAE) mL^{-1} and μg trolox equivalents (TE) mL^{-1}, respectively.

Entrapment Efficiency of Liposomes

Liposome entrapment efficiency was evaluated using the dialysis method for separating the non-entrapped compounds. Briefly, a 5 mL aliquot of liposomal dispersion was placed into cellulose dialysis bag (molecular weight cutoff, 10 kDa, Sigma Aldrich, Germany) and immersed in 100 mL of phosphate buffer (pH 4) with mild stirring. The buffer was refreshed every 2 h. After 6 h, liposomes were destabilized with Triton X-100 (0.15%, w/v) to unbound the encapsulated (poly)phenols. EE was interpreted in terms of the ratio of the TPC or AOC before and after dialysis in percentage as given in Eq. (1):

$$\textit{Entrapment efficiency}\ (\%) = \frac{\text{TPC or AOC in dialyzed fraction}}{\text{TPC or AOC before dialysis}} \times 100 \quad (1)$$

Retention Rate of TPC and AOC After Spray Drying

In order to estimate the effect of the spray drying on Rosa extract encapsulated in conjugated liposomes, retention rate was calculated by means of the TPC and AOC in powders after redispersion in dry weight as given in Eq. (2):

$$\textit{Retention rate}\ (\%) = \frac{\text{TPC or AOC in spray dried powder}}{\text{TPC or AOC in feed solution}} \times 100 \quad (2)$$

FTIR-ATR Analysis

Fourier transform infrared spectroscopic method with attenuated total reflectance (FTIR-ATR) spectra of samples were examined in the mid-IR interval (400–4000 cm^{-1}) using a Bruker Alpha-P FTIR spectrometer with a diamond ATR crystal module (Bruker Optik GmbH, Ettlingen, Germany) and OPUS v7.5 software.

Morphology

The particle morphology was observed for the resultant spray-dried powders based on micrographs taken by ZEISS EVO LS 10 scanning electron microscope (SEM; Carl Zeiss, Oberkochen, Germany).

Other Physical Properties

Powder recovery after spray drying was computed by the mass ratio of the resultant powder to the initial total solid content in the feed solution. The moisture content and solubility of the obtained powders were analyzed as described previously (Rehman et al., 2021). The CIELAB color space parameters (L^*, a^*, b^* values) were measured using a Minolta colorimeter (CR 400, Japan). Whiteness index (WI), chroma, and hue angle were computed accordingly (Granato & Masson, 2010).

Simulated Gastrointestinal Digestion

The in vitro digestion was carried out as previously described (Kasapoğlu et al., 2022). The final digests were centrifuged (4,700 × g, 60 min, 4 °C) and then filtered through a 200-nm membrane filter (Filtropur S, Sarstedt, Germany) to obtain the bioaccessible fraction. The bioaccessibility (%) refers to the TPC or AOC measured after the gastrointestinal digestion by comparison with the value for the undigested sample.

Statistical Analysis

Minitab Statistical Software v.16 (Minitab Inc., State College, PA, USA) was used for statistical analysis of the results. Unless stated otherwise, all analyses were conducted at least in triplicate, and the results were given as mean ± standard deviation (SD). Differences were analyzed by Turkey's post-hoc test comparisons and considered significant at a $p < 0.05$.

Results and Discussion

Liposome Characteristics

The PDI, mean particle diameter, and ζ-potential of unconjugated loaded liposomes with Rosa extract and empty (unloaded) liposomes are given in Table 1. The polydispersity index (PDI) values represent the heterogeneity of the particle size distribution and can range between 0 and 1, where values ≤ 0.1 are considered highly monodisperse, or polydisperse with moderate (0.1–0.4), broad (0.4–0.7),

and very broad distribution (> 0.7) (Estrada-Fernández et al., 2018). Liposomes conjugated with Ch or Wp exhibited a moderate polydisperse classification with PDI values ranging between 0.25 and 0.41 (Table 1). Small unilamellar liposomes with a mean diameter of 86.1 nm were obtained which increased slightly to 95 nm ($p < 0.05$) upon loading with the Rosa extract. The surface charge of the empty (− 5.9 ± 0.5 mV) and Rosa extract-loaded liposomes (− 6.8 ± 0.5 mV; $p > 0.05$: Table 1) were similar to previously reported liposomes loaded with tea polyphenols obtained by ethanol injection combined with dynamic high-pressure microfluidization as in our current study (Zou et al., 2014).

To select the optimal biopolymer concentration, the effect of different concentrations of Ch and Wp on the particle diameter and ζ-potential of liposomes was evaluated. The colloidal stability of liposomes is highly dependent on the biopolymer concentration used in the surface modification. In other words, inadequate amounts of biopolymers could result in incomplete conjugation of the liposomal membrane with the biopolymer molecules, whereas excessive biopolymer concentrations can lead to depletion flocculation (Jo et al., 2020). The surface charge of the anionic unconjugated liposomes (− 6.8 mV) increased (11.4–16.8 mV) when cationic Ch (0.1 to 0.5% (w/v) was added, suggesting successful conjugation (Table 1). The greatest ζ-potential was 16.8 ± 1.4 mV for a Ch concentration of 0.4% (w/v). The more positively or negatively charged a particle is, the more stable it will be as the particles tend to repel one another electrostatically, thereby reducing or eliminating agglomeration. In another study employing ethanol injection method, curcumin-loaded nanoliposomes had a surface charge of − 14.1 mV while the ζ-potential increased to 24.3 mV after conjugation with Ch (Shin et al., 2013). Furthermore, the liposomal dispersions were reported to remain stable after 3 months of storage at 4 °C. The addition of 4.0% w/v whey protein increased the particle diameter from 95.4 ± 0.8 to 207.3 ± 5.0 nm with a ζ-potential of 5.2 ± 1.0 mV (Table 1). However, a further increase in whey protein concentration (5.0%, w/v) exhibited a significant rise in the particle diameter (341.7 ± 8.1 nm) but not a significant increase in the ζ-potential ($p > 0.05$). Therefore, we chose a Ch concentration of 0.4% (w/v) or Wp concentration of 4.0% (w/v) to fabricate conjugated liposomes for all subsequent experiments as these samples had the highest ζ-potential, without substantial increases in mean particle diameter. These concentrations are in agreement with an earlier report (Gültekin-Özgüven et al., 2016; Pan et al., 2020). The incorporation of thyme extract-loaded liposomes into Wp (5, 10, and 15% w/w) film to provide antioxidant and antimicrobial properties was studied earlier (Aziz & Almasi, 2018). However, to our knowledge, no other group employed optimization using Ch and Wp for conjugation of liposomes in the same experimental setting.

Maltodextrin is widely preferred as a carrier to trap the core materials during spray drying process against heat and can protect the vesicular membrane without a substantial loss of the loaded compounds after redispersion (Wang et al., 2015). To assess whether particle properties were affected by addition of maltodextrin, the vesicle characteristics of the liposomal dispersions mixed with maltodextrin were also analyzed prior to spray drying (Table 2). As expected, the particle diameter, PDI, and ζ-potential of both Ch- and Wp-conjugated liposomes did not change after addition of maltodextrin ($p > 0.05$), as it is not a surface-active polysaccharide (Fang & Bhandari, 2012).

Total (Poly)phenols Content, Antioxidant Capacity, and Entrapment Efficiency of Liposomal Dispersions Before and After Spray Drying

The Rosa extract-loaded (0.2%, w/v)-unconjugated liposomes (Lip) contained 230.9 ± 1.3 μg GAE mL^{-1} total (poly)phenols content (TPC) and had a cupric ion reducing antioxidant capacity of 380.1 ± 27.7 μg TE mL^{-1} (Table 3). As expected, when the concentration of extract was reduced (0.1 and 0.05%), so was the TPC and the antioxidant capacity (AOC) ($p < 0.05$). The TPC of the Rosa extract was positively related to its respective antioxidant potential, as previously demonstrated in fruits from different *Rosa pimpinellifolia* L. cultivars (Liaudanskas et al., 2021). The entrapment efficiency is a major parameter for the applications of liposomal delivery systems, reflecting the incorporation ability of the vesicles. The entrapment efficiency of the TPC in the unconjugated liposomes was 74.2 ± 2.8% while 64.9 ± 3.2% of the AOC was maintained (Table 3). In our work, Ch or Wp conjugation did not result in remarkable increases in entrapment efficiencies ($p > 0.05$), which was probably due to the adsorption of biopolymers onto the surface of the liposomal membrane (Nguyen et al., 2014). The same was also observed by Zhou et al. where no significant change in the entrapment efficiency (~ 49%) of vitamin C-loaded liposomes was reported after conjugation with various concentrations of pectin (W. Zhou et al., 2014). On the other hand, Gorjian et al. (2022) studied two lipid-based nanovesicles encapsulated myrtle leaf extract and reported a stronger antioxidant activity in nanoliposomal extract compared to nanoniosome formulation and free extract by means of DPPH assay due to rapid release and interaction of (poly)phenols with lipid layers (Gorjian et al., 2022). However, the authors did not investigate the effect of biopolymer conjugation or drying on antioxidant properties of nanoliposomal extract unlike our study. As the shelf-life of aqueous liposomes is limited, the development of more stable liposomal surface through the conjugation of biopolymers and dehydration is important to ensure a prolonged

Table 1 The changes in mean particle diameter, polydispersity index (PDI), and ζ-potential of empty liposomal dispersions (Empty Lip) and Rosa extract-loaded liposomal dispersions (Lip) after conjugation with 0–0.5% (w/v) chitosan (Ch) and 0–5.0% (w/v) whey protein (Wp)

Chitosan (Ch) concentration added to liposomal dispersions (Lip)												
	0.00%		0.10%		0.20%		0.30%		0.40%		0.50%	
	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip
Particle diameter (nm)	86.1 ± 1.1^{f}	95.4 ± 0.8^{ef}	102.3 ± 2.3^{de}	101.3 ± 2.1^{de}	118.1 ± 2.2^{c}	122.4 ± 1.7^{bc}	141.4 ± 16.3^{a}	141.5 ± 0.9^{a}	111.2 ± 1.4^{cd}	133.1 ± 1.6^{ab}	119.2 ± 3.6^{bc}	124.7 ± 1.6^{bc}
PDI	0.256 ± 0.005^{a}	0.293 ± 0.036^{a}	0.280 ± 0.004^{a}	0.298 ± 0.001^{a}	0.273 ± 0.007^{a}	0.287 ± 0.016^{a}	0.291 ± 0.038^{a}	0.300 ± 0.062^{a}	0.283 ± 0.008^{a}	0.250 ± 0.007^{a}	0.302 ± 0.044^{a}	0.319 ± 0.060^{a}
ζ-potential (mV)	-5.9 ± 0.5^{d}	-6.8 ± 0.5^{d}	13.7 ± 0.5^{bc}	13.4 ± 1.0^{bc}	13.1 ± 0.4^{bc}	15.3 ± 0.9^{ab}	15.4 ± 0.6^{ab}	12.6 ± 1.1^{c}	11.4 ± 0.4^{c}	16.8 ± 1.4^{a}	12.5 ± 0.2^{c}	11.7 ± 0.5^{c}
Whey protein (Wp) concentration added to liposomal dispersions (Lip)												
	0.00%		1.00%		2.00%		3.00%		4.00%		5.00%	
	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip	Empty Lip	Lip
Particle diameter (nm)	86.0 ± 1.1^{h}	95.4 ± 0.8^{ef}	92.2 ± 0.4^{gh}	94.7 ± 0.3^{gh}	134.1 ± 1.3^{f}	136.3 ± 2.3^{f}	150.8 ± 1.4^{e}	147.8 ± 1.1^{e}	169.3 ± 1.0^{d}	207.3 ± 5.0^{c}	322.4 ± 3.0^{b}	341.7 ± 8.1^{a}
PDI	0.256 ± 0.005^{d}	0.293 ± 0.036^{a}	0.271 ± 0.009^{d}	0.363 ± 0.010^{bc}	0.298 ± 0.007^{cd}	0.298 ± 0.002^{cd}	0.276 ± 0.009^{d}	0.275 ± 0.010^{d}	0.284 ± 0.009^{d}	0.275 ± 0.010^{d}	0.309 ± 0.013^{cd}	0.411 ± 0.079^{ab}
ζ-potential (mV)	-5.9 ± 0.5^{e}	-6.8 ± 0.5^{d}	-0.7 ± 0.8^{d}	-5.0 ± 0.9^{e}	1.9 ± 0.1^{c}	2.7 ± 0.9^{bc}	2.7 ± 0.5^{bc}	3.6 ± 0.9^{bc}	4.2 ± 0.4^{bc}	5.2 ± 1.0^{ab}	6.8 ± 0.8^{a}	6.8 ± 0.8^{a}

Values with different superscript lowercase letters within the same row are significantly different ($p < 0.05$)

Table 2 Mean particle diameter, PDI, and ζ-potential of empty liposomal dispersions (Empty Lip) and Rosa extract-loaded liposomal dispersions (Lip) conjugated with 0.4%(w/v) chitosan (Ch) and 4.0% (w/v) whey protein (Wp) before and after spray drying with 20% (w/v) maltodextrin (MD)

Liposomal dispersions (Lip)						
	Empty Lip	Lip	Empty Ch-Lip	Ch-Lip	Empty Wp-Lip	Wp-Lip
Particle diameter (nm)	86.1 ± 1.1^{e}	95.4 ± 0.8^{e}	106.7 ± 1.4^{de}	107.8 ± 2.2^{de}	191.1 ± 1.4^{c}	183.7 ± 0.7^{c}
PDI	0.256 ± 0.005^{cd}	0.293 ± 0.036^{cd}	0.458 ± 0.008^{abcd}	0.470 ± 0.026^{abcd}	0.414 ± 0.041^{abcd}	0.499 ± 0.019^{abc}
ζ-potential (mV)	-5.9 ± 0.5^{f}	-6.8 ± 0.5^{f}	21.0 ± 0.7^{ab}	24.0 ± 0.5^{a}	6.2 ± 0.8^{c}	5.8 ± 0.2^{cd}
Dispersions mixed with maltodextrin before spray drying						
	Rosa extract + MD		Empty Ch-Lip + MD	Ch-Lip + MD	Empty Wp-Lip + MD	Wp-Lip + MD
Particle diameter (nm)	47.0 ± 21.6^{e}		101.2 ± 1.3^{de}	108.1 ± 2.6^{de}	197.7 ± 0.8^{c}	188.4 ± 2.7^{c}
PDI	0.531 ± 0.256^{ab}		0.526 ± 0.042^{ab}	0.548 ± 0.047^{ab}	0.409 ± 0.021^{abcd}	0.439 ± 0.032^{abcd}
ζ-potential (mV)	-3.7 ± 0.8^{f}		22.4 ± 0.4^{ab}	22.3 ± 0.6^{ab}	3.1 ± 0.2^{cde}	5.0 ± 0.2^{cd}
Redispersion of powders after spray drying in water						
	Rosa extract powder*		Empty Ch-Lip powder*	Ch-Lip powder*	Empty Wp-Lip powder*	Wp-Lip powder*
Particle diameter (nm)	162.6 ± 25.9^{cd}		345.1 ± 37.9^{ab}	406.7 ± 47.6^{a}	356.8 ± 22.2^{ab}	339.6 ± 38.3^{b}
PDI	0.253 ± 0.090^{d}		0.599 ± 0.041^{a}	0.588 ± 0.015^{a}	0.410 ± 0.101^{abcd}	0.341 ± 0.040^{bcd}
ζ-potential (mV)	0.5 ± 0.4^{e}		21.2 ± 3.8^{a}	19.5 ± 1.2^{b}	1.5 ± 0.1^{e}	2.8 ± 0.2^{de}

Values are mean ± standard deviation ($n = 3$). Values with different superscript lowercase letters within the same row are significantly different ($p < 0.05$)

*Denotes powder obtained after spray drying with maltodextrin (MD)

Table 3 Total (poly)phenols content (TPC), antioxidant capacity (AOC), entrapment efficiency, and retention rate of Rosa extract-loaded liposomes before and after spray drying

	Liposomal dispersions (Lip)	
	TPC (μg GAE mL^{-1}), [Entrapment efficiency, %]	AOC (μg TE mL^{-1}), [Entrapment efficiency, %]
Lip (0.2%, w/v)	230.9 ± 1.3^{a} [74.2 ± 2.8^{a}]	380.1 ± 27.7^{a} [64.9 ± 3.2^{a}]
Ch-Lip (0.1%, w/v)	125.0 ± 7.5^{b} [77.8 ± 0.2^{a}]	211.1 ± 29.9^{b} [65.1 ± 2.1^{a}]
Wp-Lip (0.1%, w/v)	154.6 ± 21.0^{b} [79.1 ± 7.8^{a}]	280.0 ± 27.4^{b} [69.5 ± 0.6^{a}]
	Redispersion of powders after spray drying with maltodextrin	
	TPC (μg GAE mL^{-1}), [Retention rate, %]	AOC (μg TE mL^{-1}), [Retention rate, %]
Rosa extract powder (0.05%, w/v)	52.2 ± 0.5^{b} [53.5 ± 1.0^{b}]	78.8 ± 5.6^{a} [47.6 ± 2.3^{c}]
Ch-Lip powder (0.05%, w/v)	50.0 ± 0.6^{c} [64.7 ± 8.5^{a}]	94.4 ± 10.0^{a} [76.0 ± 0.9^{a}]
Wp-Lip powder (0.05%, w/v)	65.9 ± 0.8^{a} [62.5 ± 2.8^{a}]	86.8 ± 9.8^{a} [65.1 ± 4.8^{b}]

Means in the same column with the different superscripts (a, b, c) are statistically differrent ($p < 0.05$)

AOC was assessed by CUPRAC assay. Entrapment efficiency refers to the ratio of TPC or AOC recovered after dialysis. Retention rate refers to the ratio ot TPC or AOC retained after spray drying process

Lip unconjugated liposome, *Ch-Lip* chitosan-conjugated liposome, *Wp-Lip* whey protein-conjugated liposome

shelf-life product and to avoid the use of the "cold chain" during distribution (Yu et al., 2021).

After spray drying, the lowest amount of TPC (~53.5%) retained in Rosa extract (powder), while significantly higher retention was observed in liposomal powders conjugated with both Ch (64.7%) and Wp (62.5%) (Table 3). As discussed, electrostatic conjugation of liposomes should increase the stability of liposomal membrane, thus, improving retention of the encapsulated TPC. These rates confirmed our previous work in which liposomes prepared using a different technique and conjugated with Ch (62.9%) and Wp (65.6%) ($p > 0.05$) (Kasapoğlu et al., 2022). In another study, when Wp:Ch coacervates containing garlic extract were spray dried, the range of the TPC retention was 51.0 to 61.4% (Tavares & Zapata Noreña, 2019). Ch-Lip and Wp-Lip powders had high retention rates of $76.0 \pm 0.9\%$ and $65.1 \pm 4.8\%$ in terms of AOC ($p < 0.05$), respectively, where spray-dried Rosa extract only retained $47.6 \pm 2.3\%$. The higher retention of the AOC in the Ch-conjugated liposomes may indicate that the Ch layer protects the thermo-sensitive antioxidant compounds better than the Wp ($p < 0.05$).

FTIR-ATR Results

FTIR-ATR spectroscopy was performed to examine the subtle changes in the configuration of spray-dried Rosa extract and conjugated liposome powders. The peaks in frequencies between 2800 and 3700 cm^{-1} were related to the hydrogen bonds (O−H stretch), carboxylic acids, and residual water in all samples (Fig. 2) (Sarabandi et al., 2019a, b). As shown in (Fig. 2a) freeze-dried Rosa extract, the peaks around 1604, 1397 to 1100 cm^{-1} are ascribed to stretching C=C aromatic ring and the carbonyl group (C=O), C–OH stretching vibrations, respectively, due to the phenolic and anthocyanin content in Rosa extract (Edelmann et al., 2001; Li et al., 2018). Furthermore, the peaks at 2933, 1604, 1522, 1397, 1268, 1198, 1102, 1047, 865, and 818 cm^{-1} were observed could be attributed to proanthocyanidins in accordance with the bands exhibited by berries from *Vaccinium* species (Esquivel-Alvarado et al., 2020). The presence of proanthocyanidins in *Rosa spinosissima* fruits was reported previously (Dabić Zagorac et al., 2020). The predominant peak at 1604 cm^{-1} disappeared in all Rosa extract containing powder samples implying that the extract was covered by the carrier agent (maltodextrin) during spray drying. Moreover, pure maltodextrin presented its characteristic fingerprint region of 1637 (C=O stretching), 1360 (O−H bending), 1148 (C−O stretching and C−O−H bending), 1077, 1015 (angular deformation of =CH and $=CH_2$ bonds), 760, and 705 cm^{-1} (vibrations of the pyranoid ring), in accordance with earlier investigations (Sarabandi et al., 2018). The slightly shifted peaks in domain of pure maltodextrin appeared in FTIR spectra of all spray-dried samples, suggesting the formation of some hydrogen bonds, hydrophobic and ionic interactions between the wall materials (Sarabandi & Jafari, 2020a).

Characteristic bands of soy phospholipids were confirmed (Fig. 2b) by the absorptions at about 1735 cm^{-1} (due to the C=O vibration of ester group), 1172 cm^{-1} (due to the PO_2 vibration), 1058 cm^{-1} (due to P− O−C νsym vibration), and 969 cm^{-1} (due to both P−O−C and PO_2 vibrations) (Vergara & Shene, 2019). Moreover, asymmetric and symmetric stretching of PO_2^- in the phosphatidylcholine group was detected at 1235 cm^{-1} and 1086 cm^{-1},

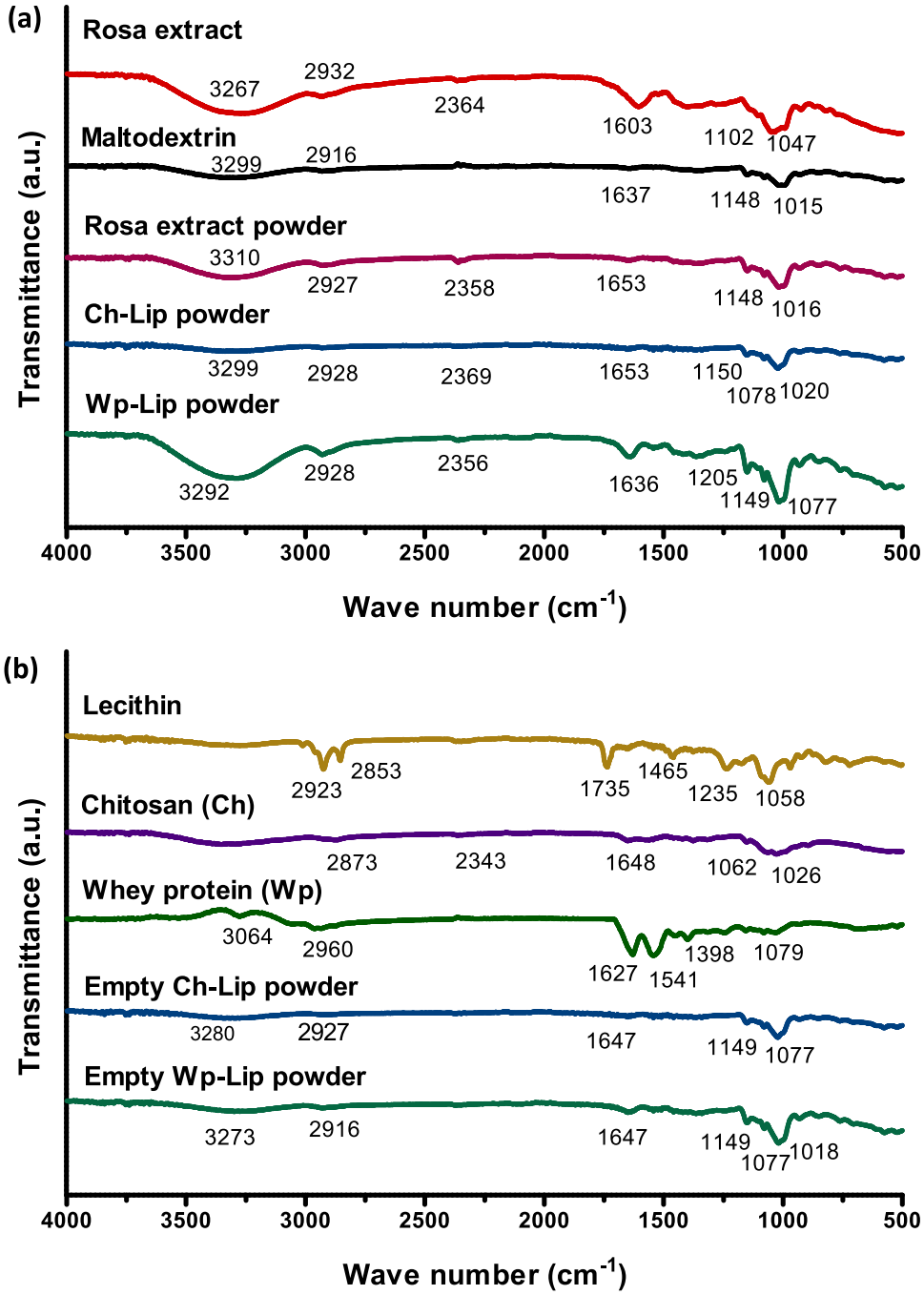

Fig. 2 FTIR-ATR spectra of **a** Rosa extract, Rosa extract powder, Ch-Lip powder, Wp-Lip powder, **b** lecithin, pure biopolymers, and empty liposomal powders. The samples spray dried with maltodextrin are defined as powder

respectively. In conjugated liposomes, the characteristic peak of phospholipids at 1465 cm^{-1} had a weakened intensity, because of the electrostatic bonding between biopolymers and liposomes. Phosphate groups are among the functional groups present in the structure of liposomes being able to form bonds with Ch (amino polysaccharide) through electrostatic interactions, as can be deducted from the shift of the absorption band of phosphate groups of lecithin to a higher frequency (from 1235 to 1243 cm^{-1}) (Hasan et al., 2016). The specific absorption peaks of pure Wp were observed between 1600 and 1700 cm^{-1} owing to the amide type I vibratory stretching (C=O and C–N) and between 1400 and 1550 cm^{-1} owing to the amide type II bending (N–H) (Aziz & Almasi, 2018). The amide type I bond shifted from 1627 to 1636 cm^{-1} in Wp-Lip powder, representing a linkage between Wp-conjugated liposome with maltodextrin. Overall, small variations between stretching of these functional groups indicated the incorporation of conjugated liposomes in the carrier matrix upon spray drying.

Characterization of Spray-Dried Liposomal Powders

A considerable ($p < 0.05$) increase in the particle diameter and PDI of unloaded and Rosa extract-loaded-conjugated liposomes was observed after redispersion. Possible explanations are the reduction of hydration capability of liposomes during particle reconstruction (Wang et al., 2015) and the sticking of some nanoparticles together due to damage of the liposomal membrane (Sarabandi et al., 2019a, b). In a similar study, the mean diameter of redispersed Ch-conjugated liposomes raised from 132 to 233 nm after spray drying (Sarabandi & Jafari, 2020a).

The SEM micrographs revealed concave shape of dried particles with shrinkage and dents on the particle surface (Fig. 3). During the spray drying process, the liquid feed was atomized into droplets that shrink upon solvent removal in hot drying medium, and subsequently form particles (Lu et al., 2020). Most of the particles had average diameter of less than 10 µm. When Rosa extract-loaded-conjugated liposome powders (Fig. 3a, b) compared to spray-dried Rosa extract powder (Fig. 3c), the mean diameter of liposomal formulations was generally bigger than Rosa extract powder. This finding agrees with the particle diameter data (Table 2). The absence of cracks or holes on the particle surfaces indicates continuous biopolymer coating and stability of conjugated liposomes during spray drying process. No considerable difference was observed among Ch- and Wp-conjugated liposome powders.

The moisture contents of the powders were ~ 5%, and the production recovery exceeded 70% with no significant differences among formulations ($p > 0.05$; Table 4), which were consistent with our earlier work (Gültekin-Özgüven et al., 2016). The residual water content of the resultant powder was an indicator of a long shelf-life of powders and remarkably affected by the operating conditions such as feed rate and inlet/outlet temperature in spray drying process (Ding et al., 2020). A high water dispersibility rate (> 90%) found for all spray-dried formulations ($p > 0.05$) using maltodextrin as carrier agent could be attributed to the superior water solubility of maltodextrin (Fazaeli et al., 2012). The dispersibility of spray-dried powders is a crucial parameter indicating ease of redispersion (reconstruction). When alginate was used as a carrier biopolymer during spray drying of liposomes, approximately 81% water dispersibility was reported (Gómez-Estaca et al., 2021).

As an indication of white color, the L^* values (luminosity, maximum 100) of the samples without Rosa extract were substantially greater ($p < 0.05$) than those containing Rosa extract (Table 4). Whiteness index values demonstrated similar results to L^* values, implying that the empty formulations were lighter than Rosa extract containing samples. The samples containing Rosa extract had high values of color parameter a^* due to the anthocyanin content with the following order: Wp-Lip powder > Rosa extract powder > Ch-Lip powder. Although all samples had the same concentration of the Rosa extract (0.05% w/w), it can be observed that the highest a^* value was obtained with Wp-Lip possibly due to the color enhancement and protection of anthocyanins in the presence of Wp (Ren & Giusti, 2021). Similarly, the chroma values of samples were also correlated to the strength of the red color, suggesting the higher stability of Rosa extract anthocyanins in Wp-Lip as confirmed by the higher values of TPC in Wp-Lip (Table 3). Chroma represents colorfulness quantitatively, so the higher the chroma value, the higher the perceived color intensity (Granato & Masson, 2010). In addition, the hue angle demonstrated the influence of the white carrier agents added to the formulations. As expected, there was significant ($p < 0.05$) difference in the proportion of hue angle values for Rosa extract containing liposomes than their empty counterparts. The visual appearance of the resultant powders is presented in Supplementary Fig. 1.

Effects of Simulated Gastrointestinal Digestion on Stability of Rosa Extract

To estimate the effect of the human gastrointestinal digestion on the stability of Rosa extract in the different formulations, the TPC and AOC of unencapsulated freeze-dried extract (Rosa extract), spray-dried extract (Rosa extract powder), and the extract loaded in conjugated liposomal powders (Ch- and Wp-Lip powder) (Fig. 4), after an in vitro digestion, were compared. The TPC and AOC of the unencapsulated Rosa extract were the most susceptible to degradation during the in vitro digestion, resulting the highest reduction rates (values). Spray drying of the Rosa extract improved the bioaccessibility of TPC approximately 2.3-fold ($p < 0.05$), probably due to encapsulation of sensitive (poly)phenols within maltodextrin. Montero et al. (2016) achieved a sixfold enhancement of astaxanthin bioaccessibility by spray drying using maltodextrin as encapsulating agent (Montero et al., 2016). The spray-dried Rosa extract powder had higher antioxidant capacity (40%) than the unencapsulated Rosa extract (11%) ($p < 0.05$).

On the other hand, biopolymer-conjugated liposomal powders; the Ch-Lip and Wp-Lip had 5.1- fold and 5.2-fold more TPC, respectively, after the in vitro digestion, compared to the Rosa extract (unencapsulated, 17%). The higher bioaccessibility of TPC observed in the case of liposomal powders was in line with the retention of AOC as discussed above ("Effects of Simulated Gastrointestinal Digestion on Stability of Rosa Extract"). After digestion, the AOC of the Ch-Lip powder (37.9–73.1%) and Wp-Lip

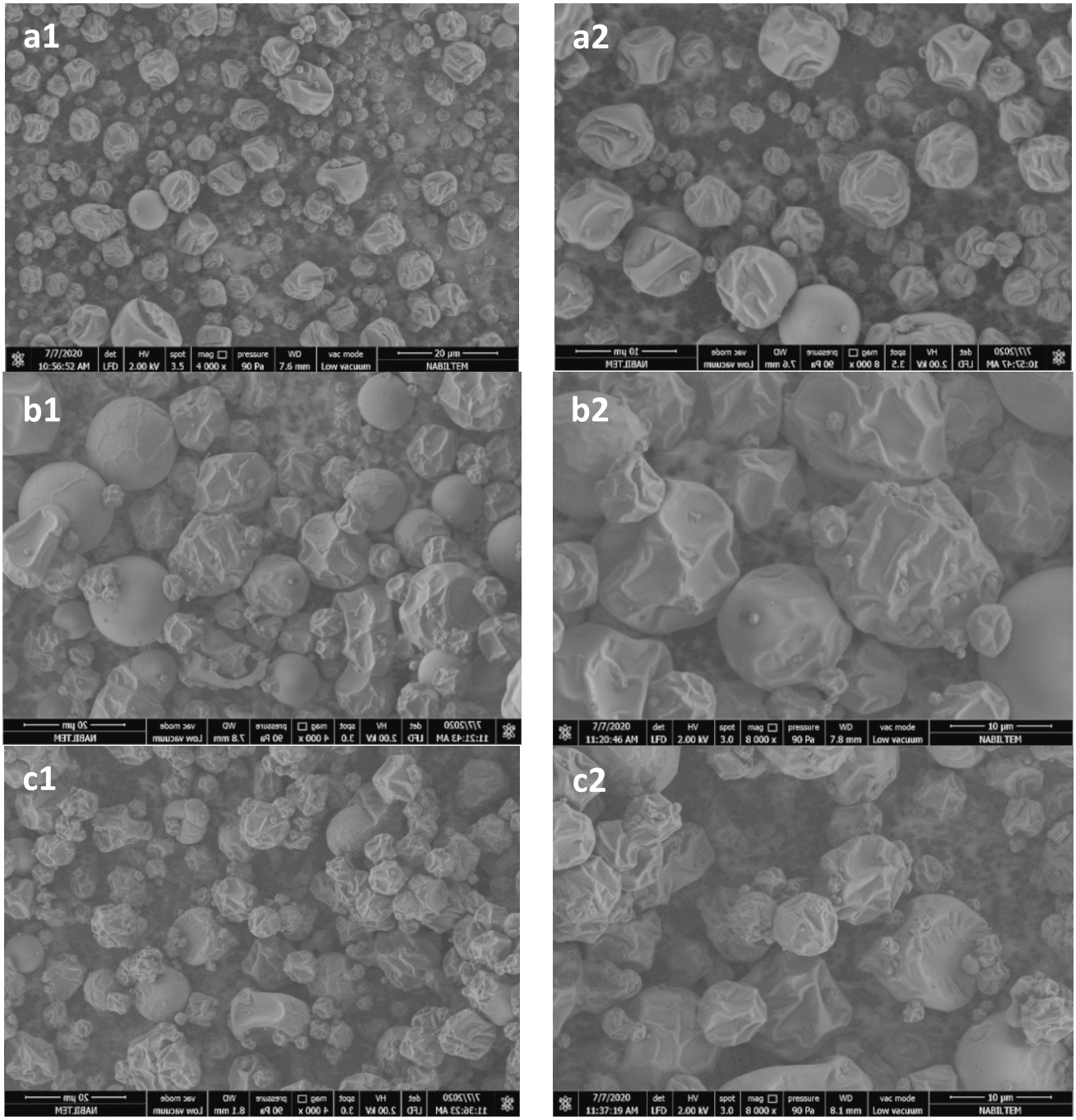

Fig. 3 SEM images of Rosa extract powder (**a**), Rosa extract-loaded Ch-Lip powder (**b**), and Rosa extract-loaded Wp-Lip powder (**c**). Pictures were taken at magnifications ×4000 (1) and ×8000 (2)

powder (51.5–63.4%) were much higher than the unencapsulated Rosa extract (6.1–13.7%), but rather similar to the spray-dried Rosa extract powder (34.9–44.9%) ($p < 0.05$). These results are in agreement with the values obtained for digested (poly)phenols encapsulated in liposomes combined with spray drying (Gómez-Estaca et al., 2021; Gültekin-Özgüven et al., 2016; Kasapoğlu et al., 2022) as well as in other biopolymer-based nanoparticle systems combined with spray drying (Hamad et al., 2020; Nalawade & Gajjar, 2015; Zhang et al., 2020). Recently, Jara-Quijada et al. (2023) reported that green tea (poly) phenols loaded in liposomal dispersions formed by ethanol injection had greater stability against thermal treatments when conjugated with Ch (Jara-Quijada et al.,

Table 4 Characterization of Rosa extract powder and Rosa extract-loaded biopolymer-conjugated liposomal powders

	Rosa extract powder	Empty Ch-Lip powder	Ch-Lip powder	Empty Wp-Lip powder	Wp-Lip powder
Moisture content (%)	5.1 ± 0.4^{b}	4.8 ± 1.1^{a}	5.5 ± 0.9^{a}	5.2 ± 1.0^{a}	4.8 ± 1.7^{a}
Water dispersibility (%)	97.4 ± 1.3^{a}	94.5 ± 0.6^{b}	97.1 ± 2.1^{a}	94.5 ± 0.4^{b}	94.2 ± 0.9^{b}
Powder recovery (%)	71.5 ± 1.1^{a}	75.2 ± 0.9^{a}	74.7 ± 0.7^{a}	73.2 ± 1.5^{a}	73.1 ± 1.4^{a}
WI (%)	95.5 ± 0.34^{c}	98.0 ± 0.04^{a}	96.7 ± 0.15^{b}	97.9 ± 0.02^{a}	94.4 ± 0.13^{d}
L^*	97.0 ± 0.58^{bc}	99.4 ± 0.08^{a}	97.7 ± 0.23^{b}	99.7 ± 0.06^{a}	96.9 ± 0.06^{c}
a^*	3.31 ± 0.17^{b}	-0.27 ± 0.01^{d}	1.69 ± 0.01^{c}	-0.26 ± 0.01^{d}	4.28 ± 0.15^{a}
b^*	0.38 ± 0.18^{c}	1.90 ± 0.06^{ab}	1.73 ± 0.03^{b}	2.04 ± 0.03^{a}	1.70 ± 0.16^{b}
Chroma	3.33 ± 0.15^{b}	1.92 ± 0.06^{d}	2.42 ± 0.02^{c}	2.06 ± 0.03^{d}	4.61 ± 0.20^{a}
Hue angle	6.7 ± 3.38^{d}	98.0 ± 0.27^{a}	45.7 ± 0.54^{b}	97.2 ± 0.29^{a}	21.6 ± 1.04^{c}

Values with different superscript letters within the same row are significantly different ($p < 0.05$)

Ch chitosan, *Wp* whey protein, *Lip* liposome, *WI* whiteness index, L^* lightness, a^* red/green value, b^* blue/yellow value

2023). On the other hand, Ang et al. (2023) investigated the in vitro release profile of hydrophobic apigenin in liposomes and showed that Ch-coated liposomes spray dried with maltodextrin gave significantly slower release of apigenin ($77.0 \pm 6.2\%$) than that of uncoated liposomes ($94.0 \pm 5.3\%$) at 12 h (Ang et al., 2023). The authors did not report on the release of apigenin from only spray dried with maltodextrin. In the present study, we compared Rosa extract-loaded liposomal powders with Rosa extract powder (spray-dried extract with maltodextrin), and thus, it was also possible to compare micro- and nano-encapsulation techniques. According to the results, preparation of conjugated nanoliposomes prior to spray drying contributed to the stability of (poly)phenols by providing one more protection layer when compared to only spray-dried counterpart. Recently, researchers microencapsulated hydrolyzed flaxseed proteins using maltodextrin as carrier in spray dryer. The combination of surfactant (Polysorbate-80) was displayed as a good strategy to improve the retention of antioxidant properties during spray drying process (Sarabandi & Jafari, 2020b).

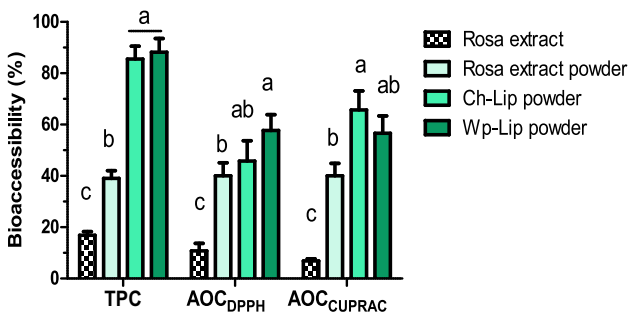

Fig. 4 Total (poly)phenols content (TPC) and antioxidant capacity (AOC) (evaluated by DPPH free radical scavenging activity assay and (CUPRAC) cupric ion reducing antioxidant capacity assay) of formulations in the bioaccessible fraction after in vitro gastrointestinal digestion

Conclusion

This study sought to optimize, characterize, and evaluate the processability and bioaccessibility of Ch- and Wp-conjugated liposome formulations loaded with *Rosa pimpinellifolia* (poly)phenols. The particle diameter, PDI, and ζ-potential of liposomal dispersions were optimal after conjugation with concentrations of 0.4% (w/v) Ch and 4.0% (w/v) Wp. Although Ch or Wp conjugation did not result in remarkable increases in entrapment efficiencies of TPC and AOC, greater retention rates were obtained after spray drying in conjugated liposomal powders. Microcapsules were formed through electrostatic interactions and hydrogen bonds between the (poly)phenols and encapsulating agents (lecithin, Ch, Wp, and maltodextrin) after spray drying, which was shown to appear in their IR spectrums with characteristic absorption peaks. Ch layer protected the thermo-sensitive antioxidant compounds better than the Wp during spray drying; however, no significant difference was found between two biopolymers by means of their stability during in vitro digestion. The bioaccessibility of (poly)phenols and their antioxidant capacity varied significantly according to the formulations followed a decreasing order of Ch-Lip powder ≈ Wp-Lip powder > Rosa extract powder > unencapsulated Rosa extract. Overall, these contribute to knowledge about the processability behavior of (poly)phenols from plant extracts and could be interesting for their incorporation into food and beverage formulations through loading into biopolymer-conjugated liposomes. Although our results revealed valuable information on the bioaccessibility of (poly)phenols encapsulated by spray drying technique, further in vitro intestinal cell model and in vivo studies are warranted to confirm the efficacy of these formulations. Future research investigating different food matrices during storage should be conducted to expand their practical applications.

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Author Contribution KNK: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft preparation; MG-Ö: conceptualization, methodology, validation, investigation, writing—review and editing; PB: investigation, formal analysis; AB-D; investigation, resources; JK: conceptualization, data curation, visualization, writing—review and editing; JF: resources, writing—review and editing; BÖ; conceptualization, resources, writing—review and editing.

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Data Availability The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Competing Interests The authors declare no competing interests.

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