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Antimicrobial activity of complexes formed from pea (*Pisum sativum* L.) proteins and red beet (*Beta vulgaris* L.) extract – effect of foam fractionation

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ABSTRACT

A novel protein system based on foam-fractionated pea (*Pisum sativum* L.) protein was developed to enhance the antimicrobial potential of red beet (*Beta vulgaris* L.) extract. The strategy involved the complexation of red beet extract (RBE) with foam-fractionated pea protein flour (PPF) to form a foam-fractionated PPF-RBE complex; complexation with non-foamed PPF was also studied for comparison. The non-foamed PPF-RBE exhibited a zeta-potential (ξ-potential) decrease from +19.467 ± 0.723 mV to +16.333 ± 0.493 mV, while the foam-fractionated PPF-RBE complexes demonstrated a similar reduction, transitioning from +21.633 ± 0.451 mV to +16.233 ± 0.321 mV. This suggests that the formation of the complexes involves a charge neutralizing effect. Both the non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes showed increased particle size as a result of interactions between the PPF and RBE, as demonstrated by dynamic light scattering (DLS) and transmission electron microscopy (TEM). Antimicrobial activity was exhibited by the RBE control and both PPF-RBE complexes (foamed and non-foamed). Specifically, foam-fractionated PPF-RBE was more effective in inhibiting and killing gram-negative bacteria. These foam-fractionated complexes lowered the minimum bactericidal concentration (MBC) of *Escherichia coli* (*E. coli*) by twofold in comparison to the RBE control. This investigation offers valuable insights into the potential of red beet extract as an antimicrobial agent and the enhanced functionality of foam-fractionated pea protein as a natural preservation ingredient for the food industry.

1. Introduction

Contamination of meat, fresh cut fruits, and other food by foodborne pathogens is a matter of great concern due to their potential to cause serious health problems (Zhao, Bai et al., 2021). Foodborne pathogens, including *Listeria monocytogenes, Escherichia coli, Staphylococcus aureus*, and *Salmonella typhimurium*, are primary contributors to food contamination, resulting in significant morbidity and mortality, and economic losses worldwide (Abebe, Gugsa & Ahmed, 2020; Bai et al., 2023; Bintsis, 2017). Despite the use of chemical preservatives and antibiotics to counteract the growth of microorganisms, there are negative health effects associated with their use including the emergence of antibiotic resistance (Capita & Alonso-Calleja, 2013). As a result, there has been a shift towards employing antimicrobial agents extracted from plants (Gyawali & Ibrahim, 2014). Prior research has investigated the presence of phenolic compounds in plant extracts, which have been associated with antibacterial properties (Choo, Wong & Nathan, 2020; Lima et al., 2019; Spórna-Kucab et al., 2023). These secondary metabolites are synthesized by plants as a means of protection against environmental factors (Elshafie, Camele & Mohamed, 2023). Consequently, plant extracts possess antioxidant and antibacterial properties, rendering them suitable for use as natural preservatives in the food industry. Red beets (*Beta vulgaris* L.) are a significant source of phenolic compounds (Kujala et al., 2002; Kusznierewicz et al., 2021; Sawicki, Bączek & Wiczkowski, 2016) and studies have shown that extracts from red beet can inhibit the growth of both gram-positive (e.g. *Staphylococcus aureus*) and gram-negative bacteria (e.g. *E. coli*) (Čanadanović-Brunet et al., 2011; Vulić et al., 2013).

The interaction between proteins and phenolic compounds can result in protein-phenolic complexes that have improved attributes (Zhang,

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Wang et al., 2022). For instance, studies have found that polyphenolic compounds had increased thermal stability after interacting with soy protein isolate fibrils (Zhao, Ma & Jing, 2020), as well as complex plant proteins including rice protein-pea protein Hu, Chen et al. (2023), and rice protein-soy protein isolate (Hu, Dai et al., 2023). Moreover, in a study by Chen, Kong et al. (2021), the interaction of soy protein isolate with thymol was found to increase antibacterial activity. Recent investigations conducted in our laboratory indicate that interactions between pea (*Pisum sativum* L.) proteins and red beet extract (RBE) at pH 3 improves the thermal stability of betanin Kumar et al. (2025a) and its long-term refrigeration stability (Kumar et al., 2025b). These interactions between pea protein and RBE were found to change the secondary and tertiary structures of the protein (Kumar et al., 2025b,c). This might result in a flexible protein structure that can bridge betanin to the surrounding proteins, leading to the formation of a network structure that could thereby enhances the polyphenolic stability (Hu, Dai et al., 2023).

These protein-phenolic interactions can also lead to improved functional properties, including solubility, emulsification, and foaming. For example, wheat germ protein-polyphenol interactions (primarily with epigallocatechin-3-gallate and chlorogenic acid) (Liu et al., 2024) and quinoa protein-tannic acid interactions (Qu et al., 2025) resulted in improvements in emulsification and foaming properties, with associated changes in the protein structure (Liu et al., 2024; Qu et al., 2025). We have also demonstrated that foam fractionation can be applied to pea proteins to significantly enhance their functional properties (Kumar & Brooks, 2025). Foam fractionation is an adsorptive bubble separation technique that facilitates the adsorption of proteins to the bubble surface by using their amphipathic properties (Wouters et al., 2017). With the introduction of air into the column, the proteins adsorb at the gas-liquid interface, with the hydrophobic regions of the proteins orienting towards the bubble surface and the hydrophilic regions aligning towards the aqueous phase. As the bubble rises through the column, the protein-rich foam (foamate) is collected at the top, and the bubble subsequently collapses to evaluate the enrichment and recovery percentage (Burghoff, 2012). In contrast to complexes formed with non-foamed pea protein and RBE, Kumar et al. (2025c) also showed that complexes formed with foam-fractionated pea protein and RBE exhibited greater antioxidant activity, likely due to structural changes in the foamed protein that exposed more hydroxyl groups (Kumar et al., 2025c). Perhaps these protein-phenolic interactions may enhance the properties of various food formulations, as naturally derived compounds rich in phenolic compounds and amino acids have been utilized as functional ingredients, such as mangosteen pulp in crackers (Saelee et al., 2024) and arabinoxylans extracted from cereals in bakery products and cookies (Hernández-Pinto et al., 2024).

In the present study, a novel protein system based on foam-fractionated pea protein and Red Beet Extract (RBE) was developed. It was hypothesized that foam fractionation would enhance the antimicrobial activity of the resultant complex. Therefore, the aim of this study was to investigate the antimicrobial activity of complexes formed with RBE and pea protein flour (PPF) (i.e. non-foamed and foam-fractionated PPF). Firstly, the phenolic compounds in RBE were profiled using Liquid Chromatography with Diode Array Detection and High-Resolution Mass Spectrometry (LC-DAD-HRMS). Then foam fractionation of PPF was conducted and the PPF-RBE complexes were prepared (Fig. 1). To investigate the characteristics of the PPF-RBE complexes that may affect antimicrobial activity, dynamic light scattering (DLS) was employed. This analysis was conducted to determine the zeta potential (ξ-potential) for evaluating the surface charges of the complexes which could contribute to the disruption of cell membranes, as well as the particle size which could affect the degree of contact with microbial cells. In addition, transmission electron microscopy (TEM) was used to observe any differences in the structural morphology of the complexes. Finally, the antimicrobial activities of the RBE control and both PPF-RBE complexes against gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) and gram-negative bacteria (*Salmonella enteritidis* and *E. coli*) were assessed. This study is the first time that the antimicrobial activity of PPF-RBE complexes has been investigated and to the best of our knowledge, the only study that examines the effect of foam fractionation on antimicrobial activity.

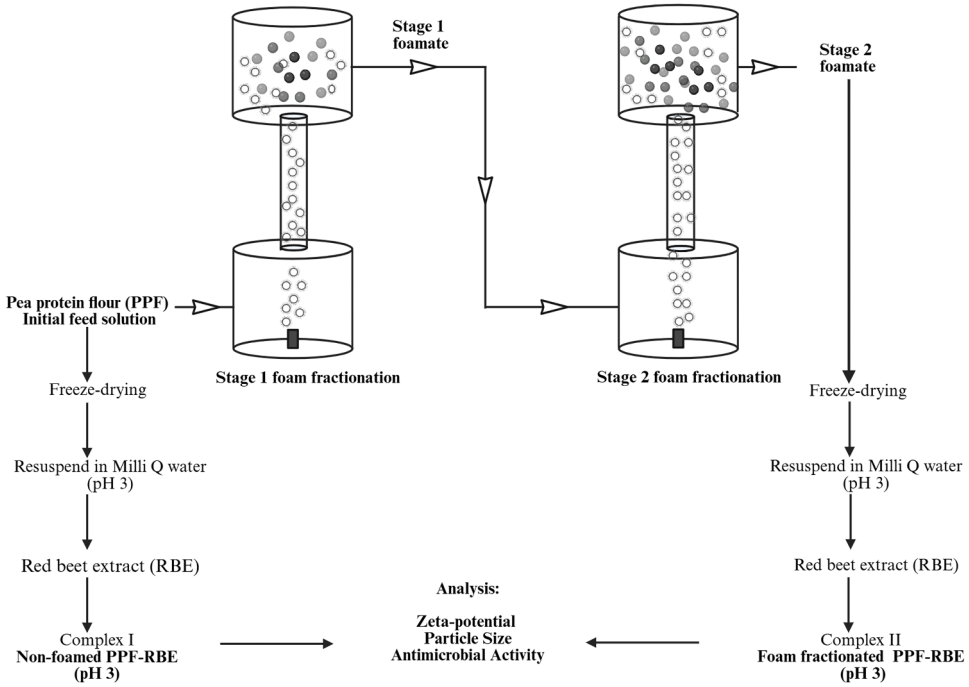


Fig. 1. Schematic representation of foam fractionation and the experimental procedure for preparation of non-foamed and foam fractionated PPF-RBE complexes.

2. Materials and methods

2.1. Materials

Pea protein flour, containing 19.84 ± 0.04 % protein (dry basis, w/w), was obtained from Parrheim Foods (Saskatoon, SK, Canada) and red beets (var. Detroit Red) were acquired from a local farm in Nova Scotia. Sodium acetate buffer (pH 3.0) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). A Pierce™ BCA Protein Assay Kit (Catalogue number: 23,225), NaOH and HCl, along with other analytical grade chemicals, were obtained from Thermo Fisher Scientific Inc. (Ottawa, ON, Canada).

2.2. Preparation of non-foamed and foam-fractionated pea protein flour (PPF) solutions

About 90 g pea protein flour (PPF) was dispersed in 1350 mL of Milli Q water (1:15 v/v), stirred for 60 min, and the pH was adjusted to 9.0. The insoluble material was then removed by centrifugation at 1500 × *g* for 30 min at 20 °C using a Sorvall T1 centrifuge with a T41 swing-out rotor, catalogue number 11,210,435 (Thermo Scientific, USA). The supernatant was filtered with Whatman paper 1, adjusted to pH 7.0, and kept at 4 °C overnight to ensure complete protein hydration. The soluble protein concentration of the pea protein flour (PPF) standard solution was 11.20 ± 0.09 mg/mL (BCA assay). Non-foamed PPF solution was obtained by diluting this solution with Milli Q water to 1 mg/mL, followed by pH adjustment to 4.5, then to 12, and finally to 7, so that it would undergo similar pH treatment as the foam-fractionated sample.

Foam-fractionated PPF was obtained from two-stage foam-fractionation according to the methodology outlined by Kumar and Brooks (2025). Firstly, the PPF standard solution was diluted with Milli Q water to a soluble protein concentration of 1 mg/mL and the pH adjusted to 4.5, then stage 1 foam-fractionation was performed with an initial liquid load volume of 4000 mL, and an air flow rate of 800 mL/min. The resultant foam (foamate) was collected and collapsed to form a solution which was then adjusted to pH 12 to solubilize any precipitates that may have formed and then pH 7 for measurement of soluble protein which was determined as 2.21 ± 0.14 mg/mL using the BCA assay. This foamate solution was then used as feed solution for the second stage of foam-fractionation after adjustment to pH 4.5 with a liquid loading volume of 1500 mL and an air flow rate of 300 mL/min. The stage 2 foamate was collected and the resulting solution (foamed or foam-fractionated PPF) was adjusted to pH 12 and then 7, with a final soluble protein concentration of 5.78 ± 0.52 mg/mL. Finally, both the non-foamed PPF and foam-fractionated PPF solutions were freeze-dried (FreeZone 2.5 Plus freeze dryer, Labconco, Kansas City, MO, USA) to form powders and stored in airtight bags at −20 °C until further analysis.

Soluble protein solutions were obtained for complexation experiments using 1 g of each freeze-dried PPF sample (non-foamed and foamed) dissolved in 100 mL of Milli Q water at 20 °C for 30 min. The samples were then adjusted to pH 3 for later complexation (according to Kumar et al., 2025b) and stirred for another 30 min, followed by centrifugation at 17,000 × *g* for 20 min at 4 °C (Avanti® JE, Beckman Coulter, JA-25.50 Fixed-Angle Rotor, Fullerton, CA, USA) to remove insoluble precipitates. The soluble protein solutions (supernatant) were subsequently used to form complexes with RBE for the investigation of DLS, TEM, and antibacterial properties.

2.3. Red beet extract (RBE) preparation

Fresh red beets (var. Detroit Red) were chopped along with their skins, removing the ends, followed by the separation of juice from the pomace by cold pressing using Breville Juice Fountain™ Plus Centrifugal Juicer (Breville, Australia). Subsequently, the red beet pomace was freeze-dried and stored at −20 °C. To prepare red beet extract (RBE), freeze-dried beet pomace was sieved and ground into a powder. Then, the powder was dissolved in Milli Q water at a weight-to-volume ratio of 1:15 (w/v) and subjected to ultrasonic treatment to aid extraction at 40 kHz and 100 W for 1 min at 20 °C (Branson 2510R-DTH sonicator, Branson Ultrasonics Corporation, Danbury, CT, USA). The sample was centrifuged at 8000 × *g* for 20 min at 4 °C (Avanti® JE, Beckman Coulter) and the supernatant was collected. The resultant pellet was rinsed with a known volume of Milli Q water, centrifuged and the supernatants were combined and then filtered using a Whatman type 1 filter. To remove the mucilage from samples, 80 % ethanol was mixed with the combined supernatant at a ratio of 2:1 (v/v) and the mixture was kept overnight at −20 °C. The next day, the solution was centrifuged at 3004 × *g* for 20 min at 4 °C using a Sorvall T1 centrifuge (Thermo Scientific, USA), and the resulting supernatant was filtered through a 0.45 μm filter. The ethanol was removed using rotary evaporation, after which the samples were freeze dried, and kept at −20 °C until further analysis.

2.4. Total phenolics content of red beet extract (RBE)

The total phenolic content was evaluated using the Folin-Ciocalteu method (Singleton & Rossi, 1965), wherein 125 μL of the sample was combined with 625 μL of 2 N Folin & Ciocalteu's phenol reagent (F9252, Sigma-Aldrich). After 5 min, a saturated solution containing 625 μL of sodium carbonate (75 g/L) was added. After an hour of incubation at room temperature, the resulting blue samples were analyzed at 765 nm using Genesys 10S UV–Vis spectrophotometer. A control was prepared using the same procedure as the samples, with the only difference being the use of water instead of the sample. Quantitative evaluation was conducted utilizing a standard calibration curve of gallic acid in water at four different concentrations between 0.01−0.10 mg/mL such that the total phenolic content of freeze-dried RBE powder was determined as 55.11 ± 0.77 μmoL GAE/g dry weight.

2.5. Analysis of phenolic compounds in red beet extract (RBE)

2.5.1. Sample preparation for LC-DAD-HRMS analysis

The phenolic compound composition in the freeze-dried RBE was determined using LC-DAD-HRMS analysis. RBE was dissolved in Milli Q water at a concentration of 10 mg/mL. The samples were adjusted to pH 3 and stored overnight at 4 °C. These samples were centrifuged the next day to remove any precipitates. The supernatant was then frozen at −20 °C and freeze-dried. Subsequently, 100 mg of the freeze-dried RBE was dissolved in 3 mL of Milli Q water. This was loaded onto a 3 mL (500 mg) C18 SPE cartridge (activated and preconditioned) and the column was then washed with approximately 50 mL water to elute most of the betanin, which is the main pigment present in red beet, characterized by a red purple color (Borjan et al., 2022; Kujala et al., 2002). This was done to obtain an enriched fraction of additional phenolic compounds. Then, the phenolics rich fraction retained on the column was eluted with methanol for LC-DAD-HRMS analysis.

2.5.2. LC-DAD-HRMS analysis

LC-DAD-HRMS was performed on an UltiMate 3000 UHPLC pump coupled to a Q Exactive™ Orbitrap High Resolution Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA), equipped with HESI-II probe for electrospray ionization. UV signal was acquired simultaneously using an UltiMate 3000 Diode Array Detector, with LC eluent delivered through DAD before reaching the MS. Data were acquired in both positive and negative polarities separately at 70,000 instrument resolution, with mass range from *m/z* 90–1000, in data-dependent acquisition (DDA) mode, where the most abundant ions were subjected to MSMS at 17,500 resolution. Optimized MS parameters included a sheath gas flow rate of 50, auxiliary gas flow rate of 10, spray voltage of 3 kV, capillary temperature of 350 °C, heater temperature of 250 °C and normalized collision energy (NCE) performed at 30 %. Separation was performed on an Acquity UPLC HSS-T3 column (100 × 2.1 mm, 1.7 μm, Waters,

Milford, MA), using a mobile phase of de-ionized water (A) and acetonitrile (B), both with 0.1 % formic acid, at 0.4 mL/min, with gradient starting from 5 % B, held for 5 min, increased linearly to 60 % B in 15 min, then to 100 % in 1 min and held for 2 min before returning to initial condition. Data were processed using Xcalibur v 4.4 (Thermo Fisher Scientific) and identification of phenolic compounds was based on accurate masses and MS/MS fragmentation patterns that matched literature data.

2.6. Preparation of non-foamed PPF-RBE and foam-fractionated PPF-RBE complex

A stock solution of Red Beet Extract (RBE) (10 mg/mL) was prepared by dissolving 1 g of freeze-dried RBE in 100 mL of Milli Q water. The RBE stock solution was diluted to generate different concentrations of RBE (2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, and 10 mg/mL). To prepare PPF-RBE complexes (both foamed and non-foamed), 7.5 mL of each soluble Pea Protein Flour (PPF) protein solution (pH 3.0) was mixed with 7.5 mL of RBE solution (at the selected RBE concentration) in a 1:1 ratio (v/v). The final concentration of RBE in each complex system thus varied from 1 to 5 mg/mL. Similarly, the PPF-Water control (foamed and non-foamed) was prepared in a 1:1 (v/v) ratio by mixing 7.5 mL of each soluble PPF solution (pH 3.0) with 7.5 mL of Milli Q water. The RBE-Water controls were prepared by mixing varying concentrations of the RBE stock solution with Milli Q water in a 1:1 (v/v) ratio, resulting in final RBE concentrations ranging from 1 to 5 mg/mL. The complex and control samples were shaken (200 rpm) overnight to facilitate complex formation at room temperature (Series 25 Incubator Shaker, New Brunswick Scientific, NJ, USA) and subsequently centrifuged the following day at 3004 × *g*, for 20 min at room temperature (Sorvall T1 centrifuge, Thermo Scientific, USA) to remove any precipitates from the soluble complex solutions.

2.7. Dynamic light scattering (DLS)

ξ-potential and particle size distribution of non-foamed PPF, non-foamed PPF-RBE complexes, foam-fractionated PPF, and foam-fractionated PPF-RBE complexes were evaluated using a Zetasizer (Nano-ZS, Malvern Instruments Ltd., Worcestershire, UK). The dispersant was deionized water, with a refractive index of 1.330 and a viscosity of 0.8872. To measure the particle size, the samples were diluted tenfold and a light scattering angle of 173° was used. Particle size measurements were acquired by permitting the samples to reach equilibrium for 120 s and calibrating the measurement settings.

2.8. Transmission electron microscopy (TEM)

To assess the variations in microstructure, the freeze-dried proteins (non-foamed and foamed) and their complexes were examined using a transmission electron microscope (JEM-1230, JEOL). Freeze-dried powders were dissolved in Milli Q water (5 mg/mL) and filtered to remove large particulates through 0.22 μm filters. Afterward, a drop of the sample was placed on the carbon coated grid. A gentle stream of water was applied to remove any excess samples from the grid. Subsequently, the grid was coated with a small amount of 2 % uranyl acetate stain, which was promptly rinsed off the grid, and a second droplet of 2 % uranyl acetate was applied to the grid for 30 s, followed by the removal of any residual stain using filter paper. The grid was left to air-dry overnight before imaging with TEM. Finally, the microstructures were analyzed at an acceleration voltage of 80 kV and images were acquired with a digital camera (Hamamatsu-HR) at a magnification of 60,000x.

2.9. Antimicrobial activity

2.9.1. Preparation of test compounds

The antimicrobial activity of the non-foamed and foam fractionated protein systems in complex with Red Beet Extract (RBE) was investigated. Freeze-dried powders of both the non-foamed and foam fractionated Pea Protein Flour (PPF) samples, were reconstituted in sodium acetate buffer (pH 3.0) at a final protein concentration of 30 mg/mL. Stock solutions of RBE powder (2 g/mL) were prepared in sodium acetate buffer (pH 3.0) and then diluted to final concentrations of 1000 mg/mL, 500 mg/mL, 250 mg/mL, 125 mg/mL, and 62.5 mg/mL. The non-foamed and foamed PPF solutions were mixed with the RBE solution at a 1:1 (v/v) ratio. As a result, the non-foamed and foam-fractionated PPF concentrations in all the complex samples was 15 mg/mL, and RBE concentrations were 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL. Similarly, the RBE control was prepared by mixing Milli-Q water and RBE solution in a 1:1 (v/v) ratio. This resulted in final RBE concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, and 31.25 mg/mL, to match the RBE concentrations in the complexes.

2.9.2. Preparation of microtitre plates

96 well microtiter plates were prepared such that each well had a final volume of 100 μL. Test compounds, (RBE, non-foamed PPF-RBE, and foam fractionated PPF-RBE), were diluted in Mueller-Hinton broth to achieve their final test concentrations of 400, 200, 100, 50 and 25 mg/mL. The final PPF concentration in the non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes remained constant at 12.5 mg/mL.

2.9.3. Determination of antimicrobial activity

Antimicrobial susceptibility testing was performed using broth microdilution panels, following the methodology and quality control recommendations outlined in the most current CLSI M07 document at the time of testing (CLSI, 2024). Briefly, each bacterial isolate was sub-cultured from frozen stock on 5 % sheep blood agar (SBA) and incubated overnight at 35 °C in ambient air. Bacterial suspensions were prepared in 2 mL sterile distilled water equal to a 0.5 MacFarland density standard (1.5×10^8 cfu/mL). The final inoculum was prepared by adding the 2 mL suspension to 38 mL of sterile distilled water resulting in a bacterial concentration of 5×10^5 cfu/mL. A 95-pin inoculator, delivering 10 μL was used to add the inoculum to each well. The plates were incubated at 35 °C in ambient air for 20–24 h prior to examination. All experiments were done in duplicate. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of test compound that inhibited bacterial growth (i.e. lack of visible growth). The minimum bactericidal concentration (MBC) was also determined by sub-culturing those wells lacking visible bacterial growth onto SBA and incubating at 35 °C for 20–24 h. The MBC was defined as the lowest concentration of test compound that completely inhibited bacterial growth.

2.9.4. Bacterial isolates

Four gram-positive organisms (*Listeria monocytogenes* (clinical isolate, CI-23–1), *L. monocytogenes* (ATCC-19,113), MSSA (Methicillin-susceptible *Staphylococcus aureus*, ATCC-29,213), MRSA (Methicillin-resistant *S. aureus*, ATCC-6538)) and four gram-negative organisms (*S. enteritidis* (CI-22–4), *S. enteritidis* (ATCC-25,928), *E. coli* (CI-23–9) and *E. coli* (ATCC-25,922) were tested. All isolates were maintained in a glycerol-skim milk solution at −70 °C. Prior to testing, each isolate was revived and sub-cultured on SBA, incubated overnight at 35 °C in ambient air, and sub-cultured a second time prior to use.

2.10. Statistical analysis

Each experiment was conducted three times, unless otherwise indi-

cated. The results were presented as the mean ± standard deviation (SD). Analysis was conducted using a one-way ANOVA multiple comparisons test with a significance level of $p < 0.05$ in GraphPad Prism 10, Version 10.3.0 (507), to assess the differences between the samples. All graphs were prepared using OriginPro, Version 2024 (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. LC-DAD-HRMS profiling of phenolic compounds in red beet extract (RBE)

From the phenolic enriched fraction obtained from RBE using solid phase extraction, 24 phenolic compounds were tentatively identified based on the observed accurate masses and MS/MS fragment results

Table 1
HPLC retention times (min), Vis spectra (λ_{max} values) and mass spectrometric (MS spectral data) and MS/MS fragments (positive mode, + or negative mode, -) of tentatively identified phenolic compounds from Red Beet Extract (RBE) by LC-DAD/HRMS.

Compound	Name	Molecular formula	Retention time (min)	λ_{max} (nm)	Observed accurate mass (m/z)			Major MS/MS fragments	Reference
					$[M+H]^+$	$[M+Na]^+$	$[M-H]^-$		
1	Betanin	$C_{24}H_{26}N_2O_{13}$	4.69	535	551.15063		549.13586		Fernando et al., 2022, Sawicki, Bączek & Wiczkowski, 2016
2	17-Decarboxy-betanin	$C_{23}H_{26}N_2O_{11}$	7.51	506	507.16113		505.14627		Sawicki, Bączek & Wiczkowski, 2016
3	Isobetanin	$C_{24}H_{26}N_2O_{13}$	8.29	532	551.15082		549.13599		Kusznierewicz et al., 2021
4	17-Decarboxy-isobetanin	$C_{23}H_{26}N_2O_{11}$	8.91	523	507.16089		505.16019	345 (+)	Kusznierewicz et al., 2021, Sawicki, Bączek & Wiczkowski, 2016
5	2-Decarboxy-betanin	$C_{23}H_{26}N_2O_{11}$	8.96	504	507.16098		505.14636		Sawicki, Bączek & Wiczkowski, 2016
6	2-Decarboxy-isobetanin	$C_{23}H_{26}N_2O_{11}$	8.98	502	507.16110		505.14636		Sawicki, Bączek & Wiczkowski, 2016
7	Neobetanin	$C_{24}H_{24}N_2O_{13}$	9.37	457	549.13507		547.12018	387 (+)	Kusznierewicz et al., 2021, Sawicki, Bączek & Wiczkowski, 2016
8	Feruloyl glucose	$C_{16}H_{20}O_9$	9.40	457		379.09982	355.10336	260, 193, 178, 149, 134 (-)	Kujala et al., 2002, Kusznierewicz et al., 2021
9	17-Decarboxy-neobetanin	$C_{23}H_{24}N_2O_{11}$	9.48	445	505.14532		503.13052	343, 297 (+)	Fernando et al., 2022, Kusznierewicz et al., 2021, Sawicki, Bączek & Wiczkowski, 2016
10	Tyramine-betaxanthin (miraxanthin III)	$C_{17}H_{18}N_2O_5$	10.19	457	331.12888				Kusznierewicz et al., 2021, Sawicki, Bączek & Wiczkowski, 2016
11	Betanidin	$C_{18}H_{16}N_2O_8$	10.25	531	389.09811				Kusznierewicz et al., 2021, Sawicki, Bączek & Wiczkowski, 2016, Sawicki & Wiczkowski, 2018
12	3-Methoxytyramine-betaxanthin	$C_{18}H_{20}N_2O_6$	10.44	466	361.13931		359.12460	317, 269, 211, 151, 119 (+)	Kusznierewicz et al., 2021, Sawicki, Bączek & Wiczkowski, 2016
13	Leucine-betaxanthin (vulgaxanthin IV)	$C_{15}H_{20}N_2O_6$	10.49	463	325.13937			191 (+)	Sawicki, Bączek & Wiczkowski, 2016
14	Vulgaxanthin IV isomer	$C_{15}H_{20}N_2O_6$	10.69	450	325.13943		323.12457		Sawicki, Bączek & Wiczkowski, 2016
15	β-d-fructofuranosyl-α-d-(6-O-(E)-feruloylglucopyranoside	$C_{22}H_{30}O_{14}$	10.83	323		541.15240	517.15552	193, 175 (-)	Bokern et al., 1991
16	2,17-Bidecarboxy-neobetanin	$C_{22}H_{24}N_2O_9$	11.27	450	461.15558		459.14066		Slatnar et al., 2015
17	Tryptophan-betaxanthin	$C_{20}H_{19}N_3O_6$	11.58	433	398.13464				Sawicki, Bączek & Wiczkowski, 2016
18	5,5′,6,6′-Tetrahydroxy-3,3′-biindolyl	$C_{16}H_{12}N_2O_4$	11.61	405	297.08713		295.07214		Kujala et al., 2002
19	6′-O-Feruloyl-betanin	$C_{34}H_{34}N_2O_{16}$	11.70	531	727.19775		725.18408	551, 389 (+)	Slatnar et al., 2015
20	6′-O-Feruloyl-isobetanin	$C_{34}H_{34}N_2O_{16}$	11.78	530	727.19751		725.18451	551, 389 (+)	Slatnar et al., 2015
21	Decarboxy-betanin/isobetanin isomer	$C_{23}H_{26}N_2O_{11}$	12.17	450	507.16116		505.14618	370, 313, 236, 194, 138 (+)	Sawicki, Bączek & Wiczkowski, 2016
22	Ferulic acid	$C_{10}H_6O_4$	12.26	323	195.06528			177, 149, 134, 117, 89 (+)	Kusznierewicz et al., 2021
23	N-trans-Feruloylhomovanillylamine	$C_{19}H_{21}NO_5$	14.67	222	344.14908		342.13428	177, 145 (+)	Kujala et al., 2002
24	Betavulgarin	$C_{17}H_{12}O_6$	17.64	222	313.07019			198, 180 (+)	Kujala et al., 2002

(Table 1 and Fig. 2, S1) and in comparison to published data. The phenolic compounds include 18 betalains (betacyanin and betaxanthin derivatives), 4 ferulic acid and its conjugates (ferulic acid, feruloyl glucose, 6′-O-ferulylsucrose, N-trans-feruloylhomovanillylamine), an isoflavone (betavulgarin), and a hydroxyindole (5,5′,6,6′-Tetrahydroxy-3,3′-biindolyl). In the enriched phenolic extract, 6′-O-Ferulylsucrose (arillatose B, **15**) and betavulgarin (**24**) were identified as some of the major phenolic components present.

3.2. Dynamic light scattering (DLS)

The DLS results for ξ-potential are presented in Fig. 3. In the absence of Red Beet Extract (RBE), the absolute ξ-potential values of the non-foamed PPF (+22.667 ± 0.252 mV) and foam-fractionated Pea Protein Flour (PPF) (+24.500 ± 1.039 mV) exhibited minimal variations at pH 3. This suggests that both the non-foamed and foam-fractionated PPF controls are reasonably stable. Foam-fractionation may have enhanced inter-particle electrostatic repulsion interactions due to the protein unfolding when adhering to the bubble, as shown by the larger ξ-potential for the foam-fractionated PPF. The positive ξ-potential value can be attributed to the ionized NH_3^+ groups in both the non-foamed and foam-fractionated PPF controls under acidic conditions, consistent with other research on pea proteins (Doan & Ghosh, 2019). However, the RBE control samples from 1–5 mg/mL showed no significant difference in ξ-potential value, ranging from −3.540 ± 0.526 mV to −4.310 ± 0.337 mV ($P > 0.05$). This negative charge is consistent with previous studies showing that betanin in an RBE aqueous solution from pH 2–3 showed mono-anionic charges, which can be explained by the deprotonation of the C2−COOH and C15−COOH groups (da Silva et al., 2019).

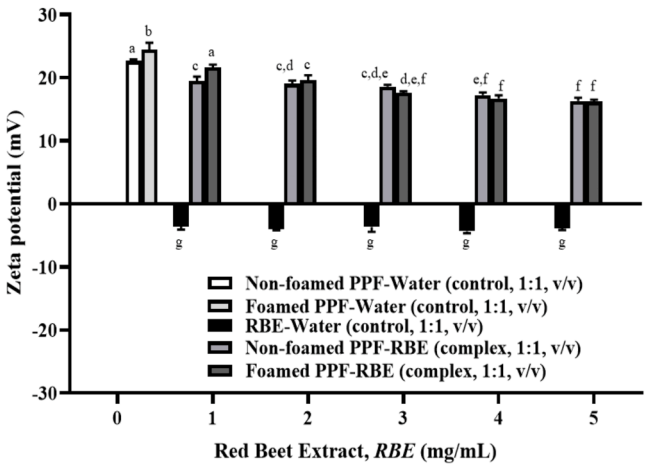


Fig. 3. Zeta (ξ)-potential analysis of non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes. (0): Foam-fractionated PPF- Water, (1): Foam-fractionated PPF- 1 mg/mL RBE, (2): Foam-fractionated PPF- 2 mg/mL RBE, (3): Foam-fractionated PPF- 3 mg/mL RBE, (4): Foam-fractionated PPF- 4 mg/mL RBE, and (5): Foam-fractionated PPF- 5 mg/mL RBE. Distinct letters indicate that the values exhibit significant variation ($P < 0.05$). Where RBE = Red Beet Extract and PPF = Pea Protein Flour.

The absolute ξ-potential value of the pure RBE changed after interactions with the non-foamed PPF and foam-fractionated PPF (Fig. 3).

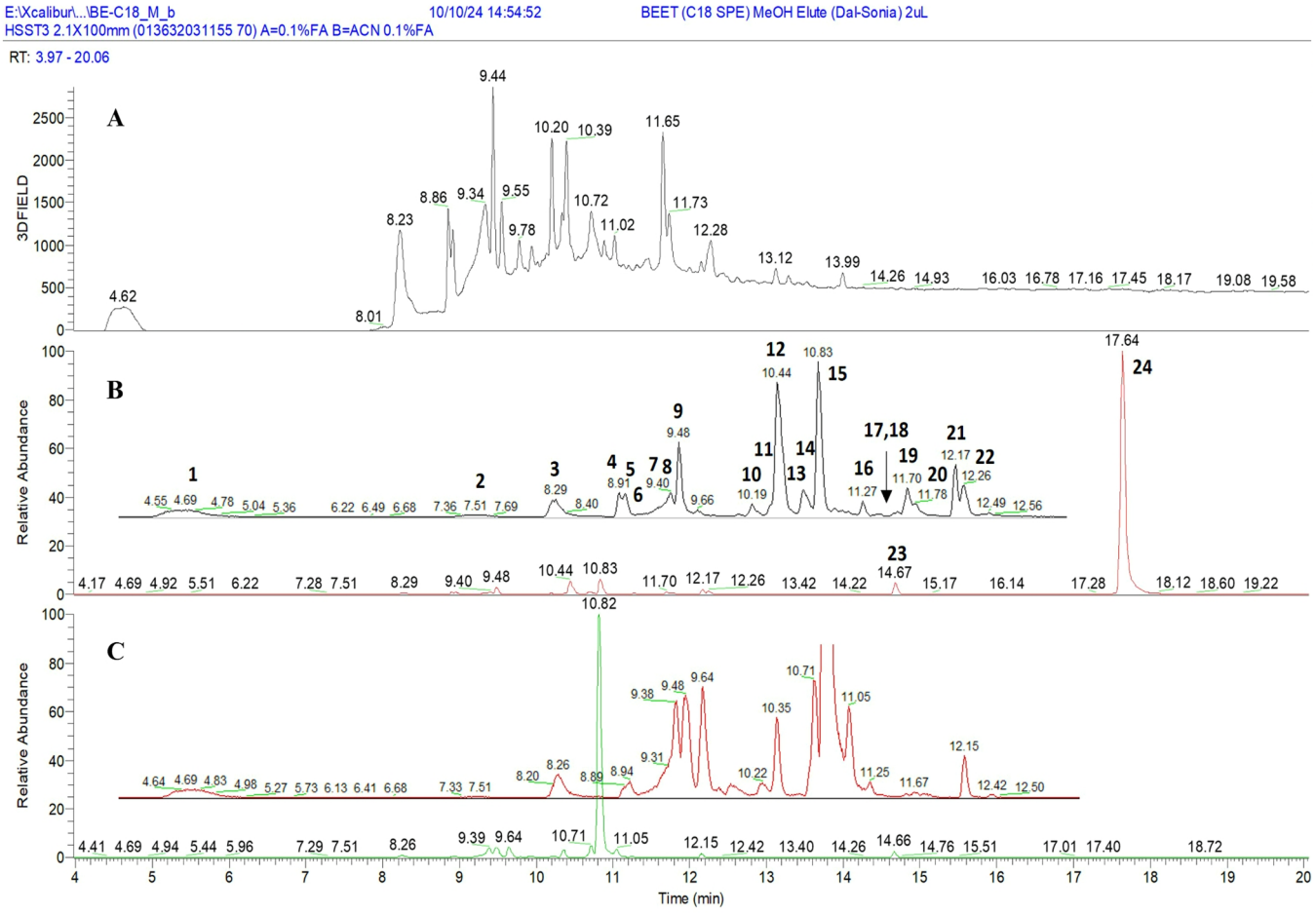


Fig. 2. LC-DAD/HRMS chromatograms of Red Beet Extract. (A) DAD extracted chromatogram at 400–600 nm; (B) HRMS extracted ion chromatogram of identified compounds, in positive mode (with zoomed-in insert); (C) HRMS extracted ion chromatogram of identified compounds, in negative mode (with zoomed-in insert).

As the concentration of RBE increased, the non-foamed PPF-RBE complex exhibited a decrease in ξ-potential value from +19.467 ± 0.723 mV to +16.333 ± 0.493 mV, whereas the foam-fractionated PPF-RBE complex exhibited a ξ-potential decrease within the range from +21.633 ± 0.451 mV to +16.233 ± 0.321 mV. The results indicate that the decline in ξ-potential of both the non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes were similar, without any significant difference ($P > 0.05$). These findings demonstrate that the charged groups on the surface of both the complexes were different than on the surface of non-foamed PPF and foam-fractionated PPF controls. One possible explanation for this phenomenon is the charge neutralization effect, a mechanism by which the negative charges on the RBE could potentially counterbalance the positive charges on both non-foamed and foam-fractionated PPF when they bind through electrostatic interaction (Fig. 4). This implies that the anionic compounds in the RBE have the potential to interact with distinct cations present on non-foamed PPF and foam-fractionated PPF. Similar findings have been reported for the interaction between soybean protein isolate and naringenin (Wang et al., 2024). Furthermore, the decrease in ξ-potential observed in both the complex systems could have been due to a reduction in electrostatic repulsion (Chen, Li et al., 2024). Comparable outcomes attributed to the charge shielding effect were reported for the interaction between anthocyanins and whey protein isolate/bovine serum albumin proteins were reported by Zang et al. (2021), and for the interaction between soybean meal hydrolysate and polyphenol complexes (Zhang, Xu et al., 2023). The reduction in electrostatic repulsion could have also resulted in the formation of soluble aggregates in both complex systems, causing the particle size to increase after complexation.

DLS experiments were also conducted to investigate the particle size distribution of the complex systems. As shown in Fig. 5, the particle size distribution of non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes was examined using varying RBE concentrations (1–5 mg/mL). It was found that both the pure non-foamed PPF and foam-fractionated PPF complexes exhibited the bimodal size distribution. Other studies have also shown the bimodal distribution of pea protein isolate (Abioye, Obeme-Nmom & Udenigwe, 2024) and the mixed pea globulins (Chihi et al., 2016). The average particle size (z-average) of the non-foamed PPF alone was 105.52 ± 6.47 nm (Table S1) and the z-average of the foam-fractionated PPF alone was 95.93 ± 0.36 nm (Table S2).

After their interaction with RBE, the DLS analysis demonstrated a peak shift towards the larger particle size in both the non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes (Fig. 5A and B). Particularly, the non-foamed PPF-RBE complexes showed a shift in the primary peaks of the particle size distribution toward larger particle sizes, and a slight increase in peak intensity with increasing RBE concentrations was apparent after the RBE concentration reached 3 mg/mL (Fig. 5A). Furthermore, the z-average of the non-foamed PPF-RBE complexes displayed an increase in size from 114.13 ± 2.54 nm to 121.16 ± 0.61 nm. These non-foamed PPF-RBE complexes did not exhibit any significant difference in the z-average after the RBE concentration reached 3 mg/mL (Table S1). Meanwhile, the foam-fractionated PPF-RBE complexes also exhibited a significant shift toward larger particle sizes, which suggest a substantial increase in particle size as the RBE concentration increased from 1–5 mg/mL, along with a corresponding increase in the peak intensities of this shift (Fig. 5B). In addition, the z-average of the foam-fractionated PPF-RBE complexes significantly increased in size from 98.78 ± 1.18 nm to 110.24 ± 0.86 nm (Table S2). Thus, it can be concluded that the complex formation increases the particle size in both the non-foamed PPF-RBE and foam-fractionated PPF-RBE complexes. These results are consistent with the particle size increase observed in the interaction between pea protein isolate-catechin (Zhang, Zhang et al., 2022) and rice-soy complex plant protein-betanin (Hu, Dai et al., 2023).

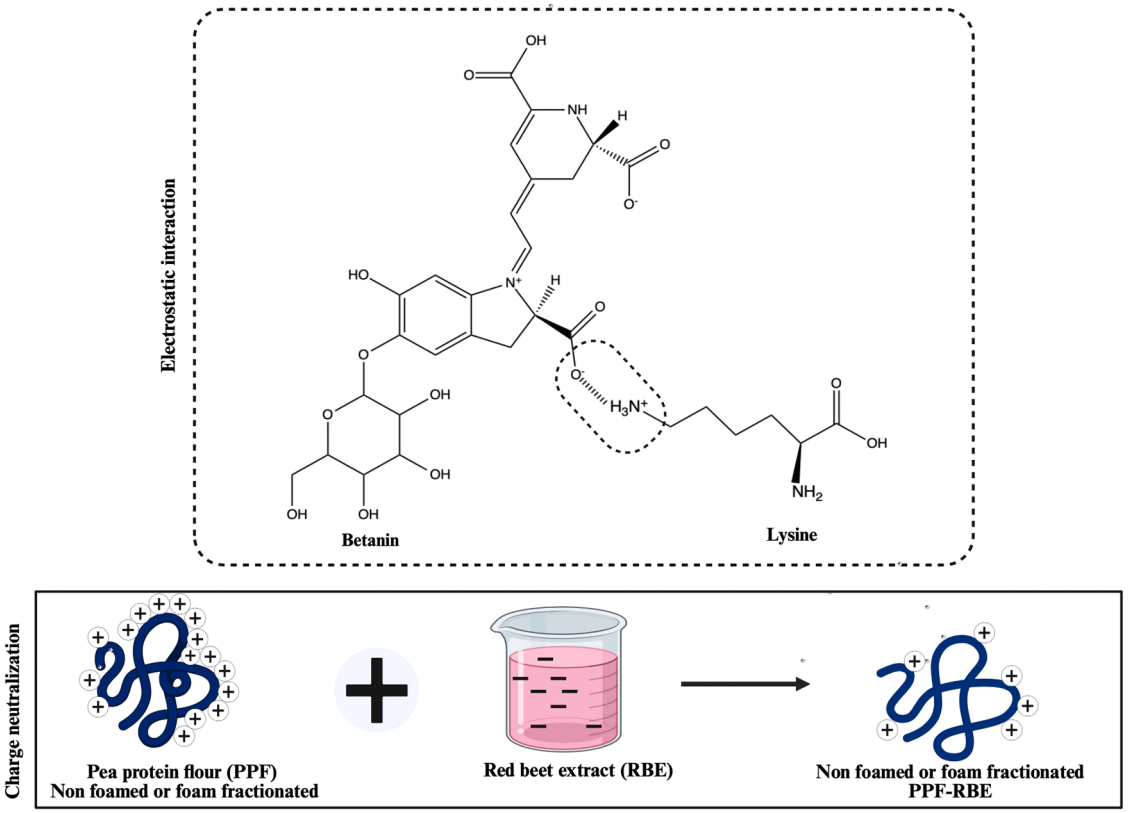


Fig. 4. Schematic showing possible mechanism for the electrostatic interactions between amino acid residues (e.g., lysine in PPF) and phenolic compounds (e.g., betanin from RBE).

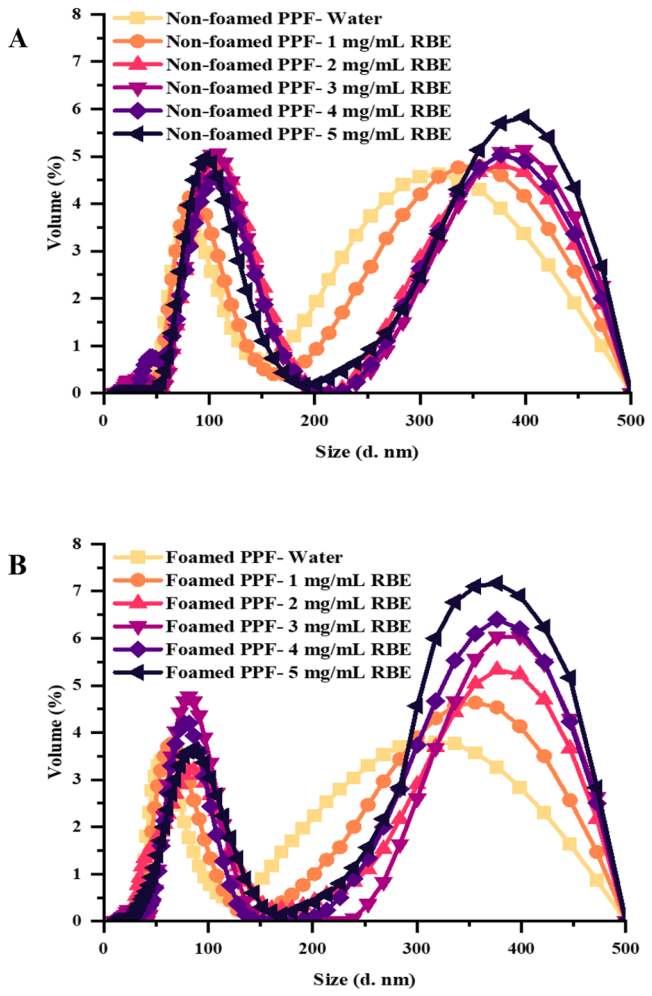


Fig. 5. Particle size distribution analysis. (A) non-foamed PPF-RBE and (B) foam-fractionated PPF-RBE complexes. Where RBE = Red Beet Extract and PPF = Pea Protein Flour.

3.3. Transmission electron microscopy

The morphological structures of the foam-fractionated Pea Protein Flour (PPF) and their complexes prepared with Red Beet Extract (RBE) at pH 3 were captured with transmission electron microscopy (TEM) (Fig. 6). As shown in Fig. 6A, foam-fractionated PPF alone demonstrated a network of polydisperse interconnected smaller spherical structures, suggesting the presence of dense bridging chains by various globulins. Spherical aggregates were also reported for pea protein isolate in the study by Abioye, Obeme-Nmom and Udenigwe (2024). Similar to the current work, a branching network was observed between spherical aggregates of pea protein isolate at a pH of 7.0 (Tran et al., 2024). It is possible that these networks in the foam-fractionated PPF control might have emerged as a result of the foam fractionation, where the unfolding of proteins as they attach to bubbles could have caused more significant bridging than in the non-foamed PPF control. Other industrial separation procedures, such as resuspension, precipitation, and spray drying, have been associated with the presence of interconnected network structures in pea protein isolate (Tran et al., 2024).

After the addition of RBE at lower concentration (Fig. 6B), the spherical structures demonstrate a tendency to aggregate and form chainlike linear structures rather than generating extensive network structures. This also agrees with the bimodal distribution in Fig. 5B Comparable observations were found for low concentration epigallocatechin gallate (ECGC) in a soybean protein-ECGC complex (Zhou et al., 2020). When the concentration of RBE increased, the foam-fractionated PPF-RBE exhibits an increase in the size of the spherical aggregates, which then begin to form networks (Fig. 6C). These networks and spherical aggregates further enlarge with a slight increase in RBE concentration (Fig. 6D). Similar findings were obtained for the interaction between pea protein isolate and curcumin at a medium concentration (Abioye, Obeme-Nmom & Udenigwe, 2024). Eventually, the development of distinct and longer individual chains with larger spherical clusters was observed with further increase in RBE concentration in the foam-fractionated PPF-RBE complex (Fig. 6E). At the highest concentration of RBE, the foam-fractionated PPF-RBE complex exhibited a network of more aggregated spherical structures and larger complex chains (Fig. 6F). These TEM observations align with the results from particle size analysis for foam-fractionated PPF-RBE complexes (Fig. 5B). Comparable trends with increasing polyphenol

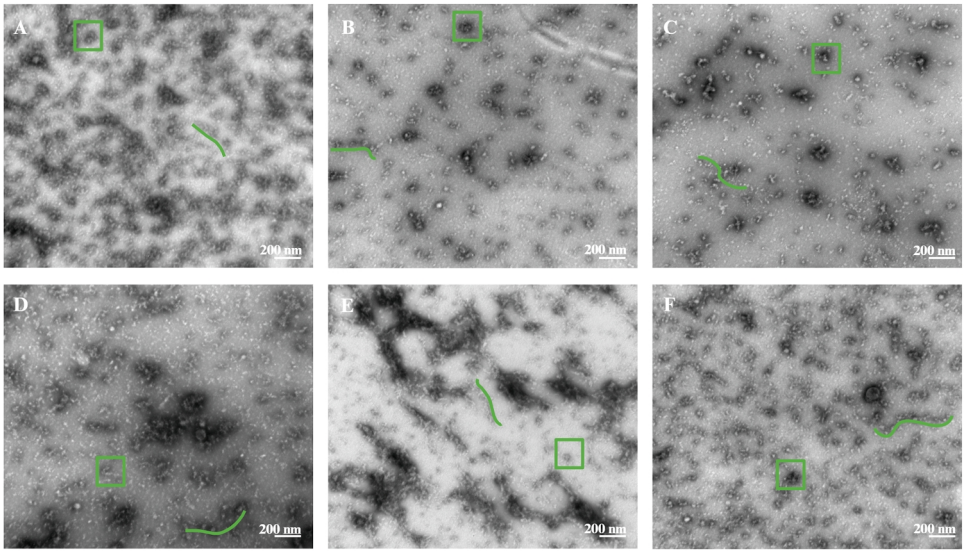


Fig. 6. Transmission electron micrograph (TEM) images of foam-fractionated PPF and foam-fractionated PPF-RBE complexes. (A): Foam-fractionated PPF- Water, (B): Foam-fractionated PPF- 1 mg/mL RBE, (C): Foam-fractionated PPF- 2 mg/mL RBE, (D): Foam-fractionated PPF- 3 mg/mL RBE, (E): Foam-fractionated PPF- 4 mg/mL RBE, and (F): Foam-fractionated PPF- 5 mg/mL RBE. Green markings are to included to identify spherical structures (boxes) and chains (wavy lines). Images were captured at 60,000x magnification. Where RBE = Red Beet Extract and PPF = Pea Protein Flour.

concentration in a soybean protein-ECGC complex were also observed in a previous study (Zhou et al., 2020). Previous studies have demonstrated that alterations in protein structure can occur when protein-phenolic complexes form such as with soybean protein and chlorogenic acid (Yun et al., 2024), and soy protein isolate and catechin (Dai et al., 2022). It is possible that RBE could potentially engage in the unfolding of non-foamed PPF and foam-fractionated PPF, thereby revealing the previously hidden hydrophobic pockets of the protein. This would enhance the binding and aggregation between the PPF-RBE complexes (both non-foamed and foamed), as discussed in a recent study on pea protein–curcumin complexes (Abioye, Obeme-Nmom & Udenigwe, 2024). In this manner, RBE could serve as a bridge for both the non-foamed and foam-fractionated PPF molecules through non-covalent interactions, resulting in an enhanced aggregation (Dai et al., 2022).

3.4. Antimicrobial activity

The antibacterial efficacy of the non-foamed PPF-RBE complex and foam-fractionated PPF-RBE complex were assessed in comparison to the pure Red Beet Extract (RBE) control. In agreement with previous studies on antimicrobial activity of pea protein isolate (Li et al., 2024), both the pure non-foamed Pea Protein Flour (PPF) and foam-fractionated PPF controls did not exhibit any inhibitory activity against gram-positive and gram-negative bacterial strains, even at their highest soluble concentration of 30 mg/mL (data not provided). The MICs and MBCs for the RBE controls, non-foamed PPF-RBE complexes, and foam-fractionated PPF-RBE complexes are shown in Table 2.

The MICs of the RBE control were ≤ 25, ≤ 50, ≤ 25, and ≤ 25 mg/mL against MSSA-29,213, MRSA-6538, *L. monocytogenes CI-23–1* and *L. monocytogenes* 19,113, respectively. In contrast, the MICs of all the gram negatives against the RBE control were ≤ 50 mg/mL. Although the MBC of RBE control was predictably higher for all gram-positive organisms, there was a notable difference in the MBCs between *Staphylococcal* and *Listeria* isolates. The MBC for both *Staphylococcus* isolates was 100 mg/mL, similar to the gram negatives, however, *Listeria* spp. consistently had a higher MBC of 200 mg/mL. Although the number of isolates tested does not allow us to do a statistical analysis, we consistently observed a slight increase in activity of the RBE control against our gram-positive isolates compared to the gram negatives. This observable increase in activity did not, however, translate into enhanced bactericidal activity once the MBC was calculated, particularly against *L. monocytogenes*.

For the non-foamed PPF-RBE complex, there appeared to be no notable difference in the MICs between the RBE control and the non-foamed PPF-RBE complex for most of the gram-negative bacteria, however the MIC against *E. coli* 25,922 exhibited a decrease of one to two dilutions. In contrast, all gram-negative bacteria exhibited a decrease in the MBC of non-foamed PPF-RBE complex by one dilution. When the non-foamed PPF-RBE complex was tested against the gram-positive isolates, no increase in activity was observed based on the MIC, however, the MBC appeared to decrease by one dilution.

For the foam-fractionated PPF-RBE complex, the MIC decreased one to two dilutions compared to RBE controls against the gram-negatives. Additionally, the MBC decreased one to two dilutions compared to the RBE controls. For the gram-positives, the foam-fractionated PPF-RBE complex did not show much difference in activity based on the MIC, however there appeared to be some observable increase in the bactericidal activity against both strains of *Staphylococcus*. No difference in bactericidal activity against *Listeria* was observed compared to the control.

These results clearly demonstrate that RBE exhibits antimicrobial activity against both gram-positive and gram-negative bacteria. Reports in the literature associate phenolic compounds in RBE, such as those identified in the present work (Table 1), with antibacterial properties (Kumar & Brooks, 2018; Wijesinghe & Choo, 2022). Even though the specific mechanisms responsible for the antibacterial effects of RBE remain unclear, it has been recently suggested that the antibacterial activity of RBE may be associated with the capacity of phenolic compounds to penetrate the peptidoglycan layer of gram-positive bacteria, rendering them highly susceptible to inactivation. Moreover, gram-negative bacteria, such as *E. coli*, can be affected by small hydrophilic phenolic compounds (e.g. betacyanins), which can pass through porin channels of the outer membrane. As such, these compounds might alter membrane permeability and disrupt the pH gradient. As a result, ATP levels decrease with the loss of proton motive force ultimately leading to cell death (Yong et al., 2018).

The findings showed that the non-foamed PPF-RBE complex appeared to slightly increase the bactericidal activity in comparison to RBE control in gram-negatives while the foam-fractionated PPF-RBE complex increased both the inhibitory and bactericidal activity against the gram-negative organisms. For the gram-positive bacteria, the bactericidal activity was improved against the *Staphylococcus* for both the PPF-RBE complexes in comparison to RBE control. This phenomenon might be attributed to the electrostatic interaction between PPF proteins and RBE, which alters the charge of the RBE, as observed by ξ-potential analysis (Fig. 3). The enhanced antibacterial activity of the PPF-RBE complexes can be attributed to the a number of factors: (i) Formation of positively charged PPF-RBE complexes (Fig. 3), which promotes effective binding to the negatively charged surfaces of microorganisms, potentially leading to the binding and disruption of bacterial membranes (Shao et al., 2020); (ii) Structural changes in PPF-RBE complexes from α-helix to β-sheets due to complex interactions (Kumar et al., 2025c; Lian et al., 2025), as foam-fractionated PPF proteins exhibit a higher degree of β-sheet structure (Kumar et al., 2025c) and this structure contributes to the amphipathic arrangement of the protein molecules, promoting their entry into bacterial membranes, potentially enhancing membrane affinity, insertion, and disruption (Jin et al., 2005; Zhang, Lin et al., 2009); (iii) Structural alterations in the PPF-RBE complexes resulting in the exposure of additional buried hydrophobic amino acid residues (Kumar et al., 2025c) as well as exposure of ionizable charge groups in the PPF-RBE complex (Fig. 3), which would enable

Table 2
MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) for the RBE control and PPF-RBE complexes (foamed and non-foamed) are reported according to the test concentrations investigated. *L.monocytogenes* -CI-23–1: *Listeria monocytogenes* (clinical isolate, CI-23–1), *L.monocytogenes −19,113: Listeria monocytogenes* (ATCC-19,113), MSSA (Methicillin-susceptible *Staphylococcus aureus,* (ATCC-29,213), MRSA (Methicillin-resistant *S. aureus* (ATCC-6538) and four gram-negative organisms, *Salmonella enteritidis* (CI-22–4), *S. enteritidis* (ATCC-25,928), *Escherichia coli* (CI-23–9) and *E. coli* (ATCC-25,922) were tested. Where RBE = Red Beet Extract and PPF = Pea Protein Flour.

Organisms	RBE- Water (control, 1:1, v/v)		Non-foamed PPF-RBE (complex, 1:1, v/v)		Foam-fractionated PPF-RBE (complex, 1:1, v/v)	
	MIC mg/mL	**MBC mg/mL**	**MIC mg/mL**	**MBC mg/mL**	**MIC mg/mL**	**MBC mg/mL**
Gram-positive bacteria						
MSSA - 29,213	≤ 25	≤ 100	50	50	≤ 25	≤ 50
MRSA - 6538	≤ 50	≤ 100	50	50	≤ 25	≤ 50
L.monocytogenes -CI-23–1	≤ 25	≤ 200	50	100	≤ 25	≤ 200
L.monocytogenes −19,113	≤ 25	≤ 200	50	100	≤ 25	≤ 200
Gram-negative bacteria						
S. enteriditis- CI-22–4	≤ 50	≤ 100	50	50	≤ 25	≤ 50
S. enteriditis - 25,928	≤ 50	≤ 100	50	50	≤ 25	≤ 50
E.coli – CI-23–9	≤ 50	≤ 100	50	50	≤ 12	≤ 25
E. coli −25,922	≤ 50	≤ 100	25	50	≤ 25	≤ 25

their diffusion across the lipid bilayer, resulting in disruption of bacterial membrane (Delcour, 2009). Where foam fractionation has been reported to cause the unfolding of proteins resulting in greater structural flexibility and exposure of hydrophobic sites on the proteins (Ekici, Backleh-Sohrt & Parlar, 2005). Consequently, the foam-fractionated PPF-RBE complex, comprising flexible PPF proteins, may facilitate penetration across the bacterial cell membrane, particularly in gram-negative bacteria. This flexibility might allow the foam-fractionated PPF-RBE complexes to form strong bindings with various target sites and varying lipid compositions on the bacterial membrane leading to improved inhibitory and bactericidal effects. Furthermore, the particle size analysis demonstrated that the foam fractionated PPF-RBE complexes were somewhat smaller than the non-foamed PPF-RBE complexes. In comparison to the non-foamed PPF-RBE complexes, this may have contributed to the increased degree of contact between the foam fractionated PPF-RBE complexes and microbial cells, as demonstrated in the previous study by Walker and Altman (2005). Although further research is required, our study indicates that foam-fractionated PPF-RBE complexes may effectively serve as a potential antibacterial substituent in food systems.

4. Conclusions

This study identified many phenolic compounds present in RBE. The findings of this work demonstrate that foam-fractionated proteins, produced using foam fractionation, effectively interact with phenolic compounds in RBE, utilizing a unique, straightforward, cost-effective, and scalable method. It was found that the ξ-potential and particle size of the protein and antimicrobial activity of RBE were affected by the formation of PPF-RBE complexes. The interactions involved in establishing the PPF-RBE complexes (both non-foamed and foamed) led to a reduction in ξ-potential, indicating a charge neutralization effect resulting from complex formation. Furthermore, the particle size of the foam-fractionated PPF-RBE complexes was observed to significantly increase after the complex formation, although the non-foamed complexes were larger. In comparison to non-foamed PPF-RBE complex, the foam-fractionated complex demonstrated enhanced antibacterial activity attributable to the alterations in the charge and structure of PPF proteins from foam-fractionation.

These findings provide valuable insights into practical applications for the utilization of pea proteins and phenolic compounds in the food industry. This study would be valuable as the complexes formed at pH 3, which could simulate gastric conditions. In particular, the use of foam-fractionated PPF proteins-RBE complexes promises to enhance the antibacterial properties of various pea protein-based systems in the food industry. The improved antibacterial properties by the foam fractionated PPF-RBE complexes indicate their potential as bio-preservatives in food formulations (e.g., yogurt, protein-rich beverages), natural ingredient in cosmetic formulations (e.g., emulsions), and active packaging films. However, foam fractionation is a relatively new technology, and the utilization of functional ingredients that are derived from foam fractionation is limited. Further investigation is required to examine the incorporation of foam fractionated PPF-RBE complexes as functional ingredients and to perform toxicity and safety assessments of those formulated foods.

Optimizing the operational parameters for foam fractionation for industrial-scale applications and assessing the ability of the resultant foam-fractionated proteins to form complexes with RBE, as well as their potential to maintain the storage stability of RBE, are required for future research. This method would offer an innovative approach for the development of the bioeconomy utilizing pea proteins and natural colorants. Furthermore, it is essential to perform a further assessment of the antibacterial properties of residual betanin, and its degradation products produced during storage to enhance their utility in food and pharmaceutical applications.

Ethical declaration

No animal or human subjects were involved in this research.

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CRediT authorship contribution statement

Sonia Kumar: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Ross J. Davidson:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Roumiana Stefanova:** Methodology, Investigation, Data curation. **Joseph P. M. Hui:** Writing – review & editing, Methodology, Investigation, Data curation. **Junzeng Zhang:** Writing – review & editing, Methodology, Investigation. **Marianne Su-Ling Brooks:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

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.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.101347.

Data availability

Data will be made available on request.

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