

Spray-dried microencapsulation of ginger extract using pea protein, chitosan, and pectin matrices

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ABSTRACT

Ginger (*Zingiber officinale* Roscoe), being an excellent functional ingredient, is recognized for its beneficial properties, including antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic activities. However, ginger bioactive compounds are susceptible to degradation and modification under processing and storage conditions. Simultaneously, their intense flavor has also limited their broader use in food formulations. Thus, microencapsulation could improve stability and mask the undesirable flavor of ginger's bioactive compounds. Therefore, the objective of the current study was to encapsulate the ginger extract in different wall materials to core ratios of pea protein isolates (PPI) and its combination with chitosan (CH) and pectin (PC). The resultant microcapsules were evaluated for their encapsulation efficiency, phenolic contents, antioxidant activity, storage stability, and in vitro digestion. The encapsulation efficiencies ranged from 68.3% to 86.9%, with PPI-PC (8:2 wall-to-core ratio) exhibiting the highest entrapment and producing microcapsules with the most uniform particle size distribution. To comprehensively evaluate the encapsulation performance, a composite scoring approach was employed, integrating multiple physicochemical attributes into a single metric. The analysis confirmed PPI-PC (8:2) as the optimal formulation, demonstrating superior encapsulation efficiency, stability, and antioxidant retention. These findings affirm the potential of microencapsulation in enhancing the stability and controlled release of ginger bioactives.

1. Introduction

Currently, there is a significant focus on the research of plant bioactives and phytochemicals, driven by their bioactivity and health augmenting traits (Reyes-Fermín et al., 2020). Ginger (*Zingiber officinale* Roscoe), a member of the Zingiberaceae family, is used for its antioxidant (El-Ghorab et al., 2010) and antimicrobial properties (Beristain-Bauza et al., 2019; Giriraju & Yunus, 2013). It has also gained recognition as an excellent source of bioactive and functional compound, making it important in the therapeutic treatment of various diseases and disorders (Mahomoodally et al., 2021). Beyond its traditional use, ginger is now widely associated with numerous health advantages, propelling its widespread incorporation into many commercial natural products within the expanding nutraceuticals and functional foods market. The medicinal properties attributed to ginger encompass a spectrum of activities, including anticarcinogenic (Karna et al., 2012), anti-inflammatory (Minghetti et al., 2007), and antidiabetic effects (Afshari et al., 2007). Various studies have also been

performed for the identification and quantification of both volatile and nonvolatile phytochemicals in ginger (Cha et al., 2020; El-Ghorab et al., 2010; Lu et al., 2022; Tohma et al., 2017), with gingerols and shogaols from the nonvolatile group comprising the major fraction of bioactive compounds (Shaukat et al., 2024). However, bioactive compounds such as gingerols, paradols, shogaols, and zingerones largely contributed to the therapeutic profile of ginger; among these, gingerol is recognized as the most important (Kaushal et al., 2017). The distinctive flavor of ginger is attributed to the association of its bioactive compounds (gingerol and shogaol) with zingerone (Singh et al., 2017).

Despite the potent therapeutic benefits of ginger-derived polyphenols, these compounds face significant stability challenges that limit their efficacy in nutraceutical applications. Factors such as exposure to light, heat, and oxygen can lead to rapid degradation and diminished biological activity of these sensitive molecules (Vieira da Silva et al., 2016). Additionally, the robust and pungent flavor of ginger hinders direct incorporation into foods, requiring masking strategies to maintain sensory acceptability (Shaukat et al., 2023a). Given these challenges,

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encapsulation represents a promising approach to enhance stability, control the release, and ultimately preserve the bioactivity of ginger polyphenols during storage and upon ingestion (Massounga Bora et al., 2018; Peanparkdee & Iwamoto, 2022). Of the available encapsulation technologies, microencapsulation using spray drying is a simple, effective and scalable technique (Akbarbaglu et al., 2021; Sato & Shimizu, 2025), and was employed in this study to encapsulate ginger extract (Busch et al., 2017).

For the encapsulation of bioactive compounds, the selection of a feasible coating or encapsulating material is also important to ensure the retention and stability of intended compounds. Pea protein, one of the most widely employed plant proteins, earned attention due to its good nutritional profile, health-promoting features, good digestibility, and lower allergic level (Zhang et al., 2022). However, its poor techno-functional properties create a barrier to its industrial applications (Tamnak et al., 2016). Considering the aforementioned challenges, the coating materials are used exclusively after a suitable treatment or in association with other encapsulating agents to achieve an ideal composition (Fernandes et al., 2012). The proteins, combined with other polymers, have been also employed in encapsulating bioactive compounds (Akbarbaglu et al., 2021). For instance, hydrolyzed pea protein complexed with maltodextrin has also emerged as a potential combination of biopolymers for spray-drying ingredients due to its emulsifying properties providing excellent volatile retention in the spray-dried product (Kurek & Pratap-Singh, 2020).

Chitosan, a cationic polysaccharide derived from the deacetylation of chitin, plays a crucial role in providing mechanical strength (Morin-Crini et al., 2019). Chitosan's advantageous properties, including low toxicity, biodegradability, and antimicrobial activity, make it ideal for encapsulating bioactive compounds like those found in ginger (Amalraj et al., 2020). Alongside other biopolymers, pectin also forms complexes that have demonstrated promise in microencapsulation studies. Pectin, derived from plant cell walls, is a structural polysaccharide with a large molecular weight that may be converted into a hydrogel and a flexible network of polymer chains (Freitas et al., 2021). Citrus fruits and apples are the most typical sources of commercial pectin, known for its emulsion stabilizing, gelling, and binding properties (Rehman et al., 2019). The conjugation of pectin with other food ingredients has also been studied to improve the functional properties of pectin (Eça et al., 2015). Considering the previous discussion, the binary polymer system, through integration of pea protein with other suitable copolymers, could evolve as a promising wall material for the encapsulation of plant bioactives.

A variety of bioactive compounds are microencapsulated in different wall materials (Jansen-Alves et al., 2019; Ricci et al., 2022; Tavares & Zapata Noreña, 2019; Vonghirundecha et al., 2022), but there is presently limited information regarding the microencapsulation of ginger extract through using drying technique. The potential combinations of pea protein, pectin, and chitosan as wall materials for plant extract encapsulation also remains largely unexplored. The novel combination of these wall materials, including pea protein, chitosan and pectin, in the form of binary biopolymer system are expected to innovate and substantially improve the encapsulation process. Hence, the aim of the present study was to produce microencapsulated ginger extract by spray drying using pea protein and its combinations with chitosan and pectin and PPI (pea protein isolate) and characterization of resultant microcapsules for their encapsulation efficiency and stability of phenolic contents, and antioxidant activity under storage conditions.

2. Material and methods

2.1. Preparation of ginger extract

The fresh ginger rhizomes were brought from the local supermarket at Al Ain (UAE) and transferred to the laboratory section of the Department of Food Science at the United Arab Emirates University

(UAE). Ginger rhizomes were properly washed under running tap water to remove the impurities, manually peeled, and then diced into thin slices measuring 2–3 mm in thickness. Hot air drying was carried out at 55 °C until moisture content was equilibrated. After drying, ginger slices were pulverized into powder and uniform size ginger powder was collected by passing through 50 mesh sieves. Then, the hydroethanolic extraction was performed by mixing 4 g of ginger powder in 100 mL of 70% ethanol, providing 1:25 sample-to-solvent ratio. Continuous stirring was provided to the sample mixture at 300 rpm for 12 h. After that, the sample mixture was centrifuged at 10,000 rpm for 10 min, the ginger powder was settled in the bottom, the supernatant was decanted, and the clear ginger extract was obtained by passing through a syringe filter. To acquire aqueous extract suitable for further food application, the ethanol was evaporated from ethanolic ginger extract through rotary evaporation performed at 60 °C. The bioactive value of the final ginger extract was further assessed by performing the total phenolic contents and antioxidant activity assays.

2.2. Total phenolic contents

The total phenolic contents of ginger extract were measured through Folin-Ciocalteu assay manifested by Shaikat et al. (2023) with small amendments. In brief, the aqueous ginger extract (50 µL) was incorporated into 2.5 mL of deionized water, and a quantity equal to 250 µL of Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) was also mixed with the sample solution. After providing 3 min of reaction time, 500 µL of 20% sodium carbonate solution was mixed with the sample solution. The total volume of the sample mixture was raised to 5 mL by pouring deionized water. The corresponding blank sample was also prepared by switching ginger extract with deionized water. These sample mixtures were incubated at room temperature for 60 min under dark conditions. Finally, the absorbance of the samples was measured at a wavelength of 725 nm against a conforming blank in a spectrophotometer (Multiskan SkyHigh Plate Reader, Thermo Scientific™, USA). The results of the total phenolic contents of ginger were expressed in mg of gallic acid equivalent in 100 mL of ginger extract (mg GAE/100 mL) against the standard curve of gallic acid.

2.3. Antioxidant activities

2.3.1. DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picryl-hydrazyl) assay was performed to evaluate the antioxidant activity of ginger extract according to the method described by Brand-Williams, Cuvelier and Berset (1995). In brief, 50 µL of ginger extract and its dilutions were integrated with 3 mL of methanolic solution of DPPH (0.1 mM) (Sigma-Aldrich, Steinheim, Germany). The concordant blank was also prepared by substituting the methanol with extract. After 1 hour of incubation in the dark, the absorbance of the samples was recorded spectrophotometrically at the wavelength of 515 nm. The antioxidant activity, in terms of DPPH radical scavenging activity, was described as mg of Trolox equivalent per liter of the ginger extract, attended against the Trolox standard curve.

2.3.2. ABTS radical scavenging activity

ABTS (2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity was measured following the method of Re et al. (1999), with minor adjustment. The ABTS reagent (7 mM) (Sigma-Aldrich, Steinheim, Germany) and potassium persulphate (2.5 mM) were dissolved in distilled water and left for 16 h in dark conditions to facilitate the reaction. The acquired ABTS solution was diluted with the required amount of ethanol to establish the absorbance of ABTS solution between 0.7–0.8 at 734 nm. Later on, the ginger extract (100 µL) was combined with 900 µL of ABTS reagent solution, homogenized, and incubated for 30 min in dark environment. The corresponding ABTS blank sample was also fabricated by swapping the extract with ethanol.

The antioxidant activity, in terms of ABTS radical scavenging activity, was described in micromoles of Trolox equivalent per milliliter of the ginger extract, attended against the standard curve of Trolox.

2.4. Production of ginger extract-loaded microcapsules via spray drying

The feed material for spray drying was prepared through a combination of different ingredients, as presented in Table 1. The solutions of all three types of polymers were prepared by dissolving protein and pectin in distilled water while chitosan in an acidified water (1% acetic acid) through continuous stirring and then these suspensions were finally centrifuged to collect particle-free polymer solutions. For the preparation of extract and polymer blends, all of the ingredients, as per ratios described in Table 1, were homogenized by high shear homogenizer (ULTRA-TURRAX T25 D, IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 7000 rpm for 2 min.

The encapsulation of ginger extract was carried out through a mini spray dryer (Büchi B-290, Flawil, Switzerland). The spray drying was performed by administrating feed solutions at the processing parameters of inlet temperature, outlet temperature, feed flow rate, air pressure, and aspiration of 160 °C, 70 ± 5 °C, 7.5 mL/min, 30 mbar and 70%, respectively. The resultant spray-dried microcapsule samples were collected from the spray dryer and stored in sterilized airtight glass vials at 4 °C until further analysis.

2.5. Characterization of spray dried microcapsules

2.5.1. Scanning electron microscopy (SEM)

The spray-dried microcapsules were sputter coated with gold, and their morphology was evaluated by Environmental scanning electron microscope (ESEM Quanta 250 FEG, FEI Company, Hillsboro, OR, USA) at an acceleration voltage of 10 kV and magnification of 8300 ×.

2.5.2. Physicochemical characterization

The moisture content of the microcapsules was determined as a known quantity of the samples (250 mg) placed in a hot air oven at 105 °C for 5 h. Subsequently, the loss in weight was used to calculate the moisture contents (%) of these microcapsules.

$$\text{Moisture Content (\%)} = \left(\frac{\text{Weight Loss in Oven Drying}}{\text{Sample Weight}} \right) \times 100 \quad (1)$$

The solubility of microcapsules was measured through dissolving 250 mg of microcapsule samples in 10 mL of water through orbital shaking at 75 rpm for 10 min and then centrifuging the resultant solution at 3000 rpm for 10 min. The supernatant was decanted and dried in a hot air oven at 105 °C for 12 h. The water solubility (%) was described as the weight of the dried supernatant over the initial sample weight.

$$\text{Solubility (\%)} = \left(\frac{\text{Weight of dried supernatant}}{\text{Sample Weight}} \right) \times 100 \quad (2)$$

The hygroscopicity of microcapsules were analyzed by placing glass petri plates containing 250 mg of each sample in a desiccator loaded with saturated sodium chloride solution (relative humidity: ~ 76%) for 1 week at ambient temperature. After one week, the weight of the

samples was measured, and the hygroscopicity (%) of microcapsules was presented as the weight of adsorbed moisture per 100 g of the spray-dried microcapsules. The particle size distribution of ginger extract-based microcapsules was analyzed using a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Panalytical Ltd., Malvern, UK). For the standard operating procedure, the particles were treated as opaque using the Fraunhofer approximation, with a density of 0.5 g/cm³. The obscuration levels were maintained between 0.1% and 15%. The sample dispersion was achieved with an air pressure of 4 barg and a feed rate set to 25%. Each measurement cycle included three repetitions of 10 s each, and the results were averaged to determine the particle size distribution. The results are presented for Sauter mean diameter (D [3,2]) and span values to evaluate the effects of the different formulations on particle size distribution.

2.5.3. Encapsulation efficiency

The encapsulation efficiency of ginger extract microcapsules was determined by following the method of Jansen-Alves et al. (2019) with some modifications. The spray-dried microcapsule samples (25 mg) were incorporated in 1 mL of 70% ethanol and manually mixed to wash the surface phenolic contents. The sample mixture was centrifuged at 3000 rpm for 5 min, and 100 µL of the supernatant was collected and proceeded for the measurement of surface phenolic contents. The remaining sample mixture was further vortexed and subjected to orbital shaking at 200 rpm for 1 hour. After shaking, the samples were sonicated in a sonication water bath for 15 min, and then, the samples were left overnight for maximum dissolution of microcapsules. After overnight dissolution, the samples were again sonicated for a further 15 min and vortexed for 20 s. These extensive treatments were executed to ensure the optimal dissolution of microcapsules and release of phenolic compounds. Finally, the sample mixtures were centrifuged at 6000 rpm for 5 min, and the supernatant was collected to represent the total phenols in microcapsules. Both supernatants were further analyzed for their total phenolic contents through Folin-Ciocalteu assay following the protocol described above in Section 2.2. The encapsulation efficiency (EE) was calculated by using the following formula:

$$\text{EE (\%)} = \left(\frac{\text{Total Phenolics in Microcapsule} - \text{Surface Phenolics}}{\text{Total Phenolics in Microcapsule}} \right) \times 100 \quad (3)$$

2.5.4. Fourier transform infrared spectroscopy (FTIR) analysis

The structural evaluation of spray dried microcapsules was conducted through FTIR spectroscopy equipped with a universal attenuated total reflectance (UATR) unit (Perkin-Elmer Inc., Norwalk, CT, USA). The samples were positioned over Diamond/ZnSe crystal plate, and the measurements were documented against a spectral range of 4000 cm⁻¹ to 400 cm⁻¹ at the ambient temperature.

2.5.5. Thermal analysis

The thermal characterization of microcapsules was performed through differential scanning calorimetry (DSC) (DSC Q100, TA Instruments, USA). The samples (5 mg) were loaded in hermetically sealed

Table 1

Feed formulations of spray-dried ginger extract microcapsules based on total solid content.

Microparticle type	Polymer (wall material) composition in fraction			Polymer:Extract (Wall:Core) ratio
	Pea protein isolates (PPI)	Chitosan (CH)	Pectin (PC)	
PPI-1	1.0	0.0	0.0	9:1
PPI-2	1.0	0.0	0.0	8:2
PPI-CH-1	0.9	0.1	0.0	9:1
PPI-CH-2	0.9	0.1	0.0	8:2
PPI-PC-1	0.9	0.0	0.1	9:1
PPI-PC-2	0.9	0.0	0.1	8:2

aluminum pans and placed in a heating chamber against an empty pan serving as a reference. The samples were heated between a temperature range of 20 to 250 °C at a scanning rate of 10 °C per min and a nitrogen flow rate of 40 mL/min.

2.5.6. Storage stability

The spray-dried microcapsule samples were stored in sterile glass vials at 23 °C and 45 °C. Subsequently, microcapsules were analyzed to assess the effect of storage on the color, total phenolic contents, and antioxidant activity. The pertinent and crucial controls, including background correction and matrix subtraction, were also carried out for more accuracy.

2.5.7. Color analysis

The color analysis was performed of freshly spray-dried microcapsules and the microcapsule sample was obtained at the end of storage through a Hunter Lab digital colorimeter (Hunter Color Lab Elex EZ, USA). The color values for L^* (Lightness), a^* (Redness) and b^* (Yellowness) were documented and further utilized to calculate the browning index (BI) of microcapsule through the following equation:

$$BI = \frac{100 (x - 0.31)}{0.17} \quad (4)$$

where,

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (5)$$

2.5.8. Total phenolic contents

The spray-dried samples, against the storage intervals of 0, 7, 14, 21, and 28 days, were fully dissolved following the protocol described in Section 2.5.3 and the supernatant was evaluated for their total phenolic contents through Folin-Ciocalteu method following the procedure described above in Section 2.2.

2.5.9. Antioxidant activity

The antioxidant activity of fresh microcapsules and samples acquired after the termination of storage period was measured to determine the antioxidant stability of microcapsules during storage.

2.5.9.1. DPPH radical scavenging activity. The antioxidant activity, concerning DPPH radical scavenging activity, of stored microcapsules was also evaluated following the method of Busch et al. (2017), with some adjustments. The supernatant from fully dissolved samples (50 µL) was combined with 1 mL of methanolic solution of DPPH (0.1 mM). The sample mixture was mixed well and kept under dark conditions for 30 min. After incubation, the absorbance of samples was recorded at the wavelength of 517 nm, and DPPH radical scavenging activity (%) was determined using the following formula:

$$\text{DPPH Radical Scavenging Activity (\%)} = \left(\frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}} \right) \times 100 \quad (6)$$

2.6. In vitro digestion

In vitro digestion of microcapsules was also performed to analyze the release of bioactive compounds under simulated digestion conditions following the methods of Lee and Chang (2020) and Vonghirundecha et al. (2022), with some amendments. To evaluate the in vitro gastric digestion, the spray-dried ginger extract sample (1 g) was incorporated into 20 mL of simulated gastric fluid, prepared by dissolving pepsin (0.06 g/g of sample) and sodium chloride (0.18 g/g of sample). The pH of the resultant mixture was adjusted at 1.5 by dropwise addition of 5 M HCl. The sample mixture was incubated at 37 °C and rotary shaking of 75 rpm for 2 h to provide simulated gastric conditions. After 2 h, the

sample mixtures were transferred to an ice bath for 10 min to stop the reaction, and 1 mL out of 20 mL of sample mixture was removed, centrifuged at 15,000 rpm for 5 min, and the supernatant was collected as the gastric digestion sample. To proceed with the simulated intestinal digestion, the pH of the remaining sample mixture was adjusted at 7.5 using 1 N NaOH solution. The mixture of pancreatin, potassium phosphate and bile salts (5 mM NaTC) in the ratio of 0.1:2:1 (0.6 g/g of sample) was also added to the sample mixture. The acquired sample mixture was subjected to simulated intestinal conditions at 37 °C and rotary shaking of 110 rpm for 02 h. After two hours, the mixture was again subjected to the ice bath for 10 min to stop the reaction. The sample mixture was centrifuged at 15,000 rpm for 10 min, and the supernatant was collected as the sample against total (gastrointestinal) in vitro digestion. The supernatants from both simulated digestion processes were analyzed for their total phenolic contents through Folin-Ciocalteu assay following the protocol mentioned in Section 2.2.

2.7. Quantitative ranking of encapsulation formulations

A composite scoring approach was employed to evaluate the effectiveness of different encapsulation formulations by integrating multiple physicochemical properties into a single metric. The parameters considered were categorized based on whether lower or higher values were desirable. Particle size, span, moisture content, BI, hygroscopicity, and gastric release were minimized, while solubility, EE, TPC, and antioxidant activities (DPPH and ABTS) were maximized. To ensure comparability, Min-Max Normalization was applied, scaling all parameters between 0 and 1 using the formulas:

$$X_{\text{normalized}} = \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad (7)$$

for parameters that should be maximized, and

$$X_{\text{normalized}} = 1 - \frac{X - X_{\min}}{X_{\max} - X_{\min}} \quad (8)$$

for those that should be minimized. Particle size and EE were given double weight to emphasize their significance in determining particle stability and encapsulation efficiency. The composite score for each formulation was calculated as a weighted sum:

$$\text{Composite Score} = \sum \omega_i X_{\text{normalized},i} \quad (9)$$

where ω_i represents the weight of each parameter. Formulations were ranked based on their composite scores, with higher values indicating superior performance.

2.8. Statistical analysis

Data collected from all the experiments, performed in triplicates, was presented as their mean values $\pm$ standard deviations. The acquired data was analyzed by performing one-way analysis of variance (ANOVA) and Fisher's test to establish the significant difference (p-value < 0.05) among samples through statistical package software Minitab™ version 20 (Minitab, LLC; PA, USA).

3. Results and discussion

3.1. Bioactive characterization of ginger extract

The bioactive profile of the ginger extract was assessed by quantifying its total phenolic contents and antioxidant activities using the DPPH and ABTS assays. The aqueous ginger extract was recognized with an appreciable amount of total phenolic contents around 216.7 ± 15.2 mg GAE/100 mL, which is almost two-fold the total phenolic content obtained in our previous study where glycerol was employed as a green

solvent for the extraction of bioactive compounds from ginger (Shaikat et al., 2023b). The ginger extract also exhibited an adequate antioxidant value of 4.09 ± 0.33 mg Trolox Eq./L and 61.1 ± 14.7 μ M Trolox Eq./mL against DPPH and ABTS radical scavenging activities, respectively. Generally, the rotary vaporized aqueous ginger extract was reported with a comparatively better bioactive profile, also supported by previous research records (Tanweer et al., 2020). The hydroethanolic solution in different concentrations was reported as an excellent solvent with commendable extraction efficiency against various types of food matrices (Cha et al., 2020). At the meantime, the food application of ethanolic extracts is not preferred due to certain safety and regulatory issues (Kowalska et al., 2021). Thus, rotary evaporation was performed

to attain the ethanol-free aqueous ginger extract, suitable to incorporate into blends to spray dry.

3.2. Characterization of ginger extract microcapsules

3.2.1. Morphology and size distribution

The morphology of the microcapsules produced from different wall materials was observed from the SEM images shown in Fig. 1. The SEM micrographs revealed that most of the particles possessed a generally spherical structure, with noticeable dents and concavities on the surface. Among the formulations, PPI-PC-2 displayed the most uniform and smooth structure, aligning with its narrow size distribution, while PPI-

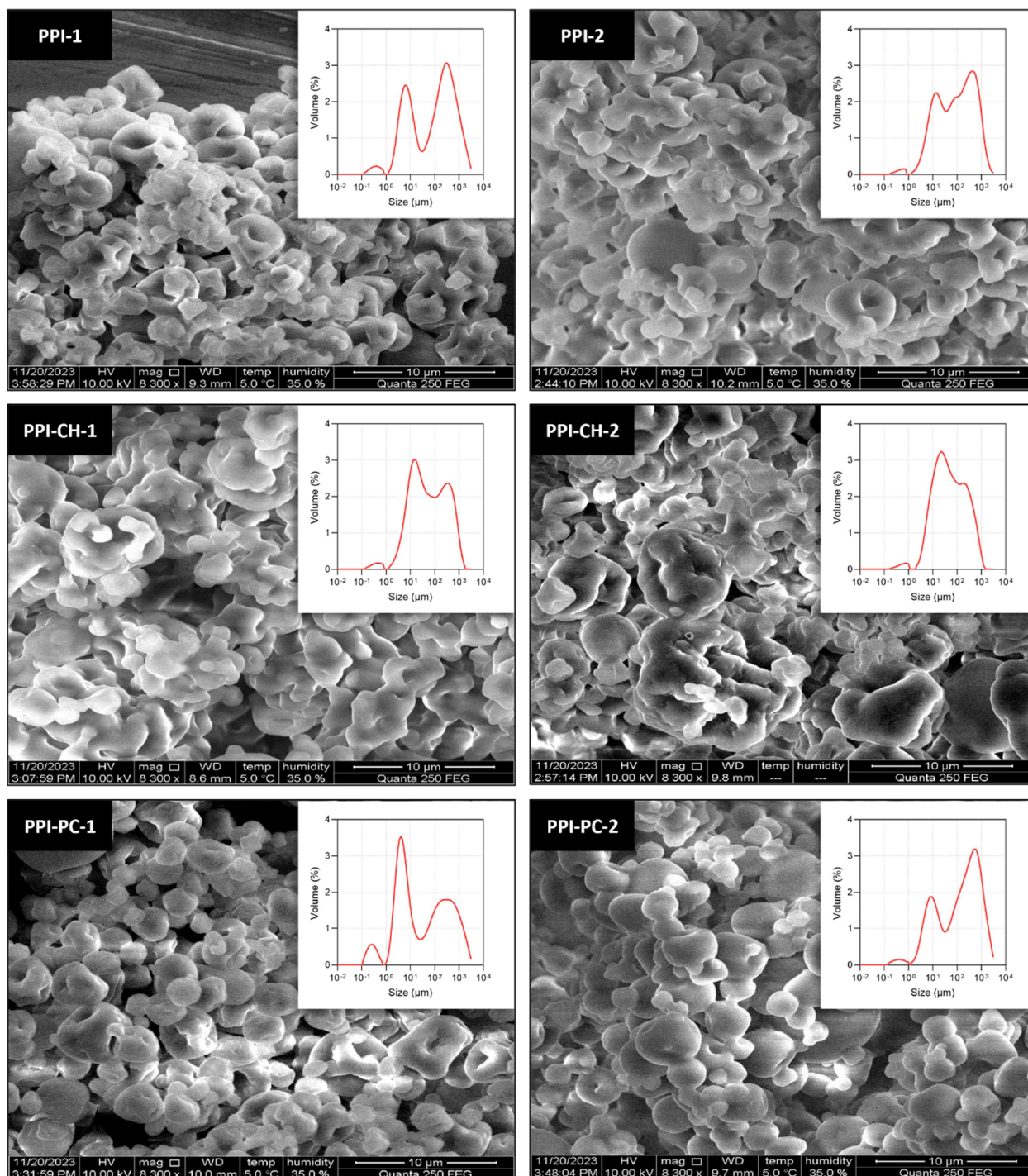


Fig. 1. Scanning electron micrographs (8300 $\times$) of spray-dried ginger extract microcapsules. The detailed composition of each formulation is provided in Table 1.

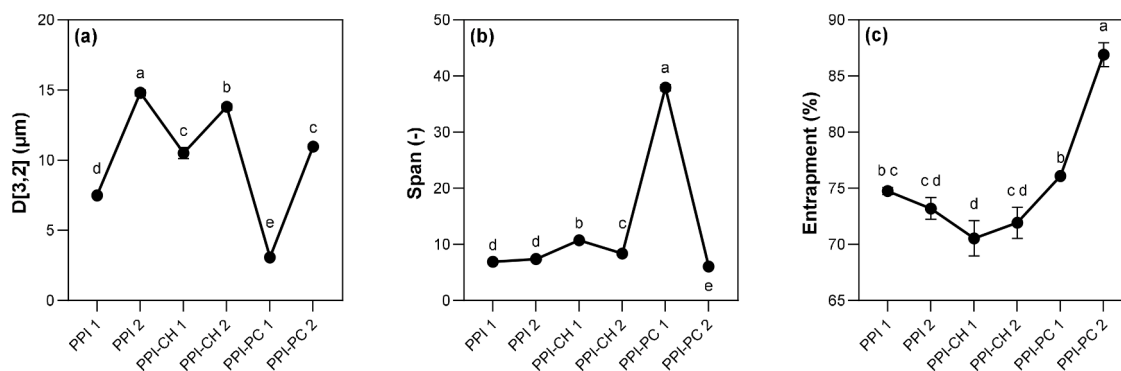


Fig. 2. Particle size D[3,2] (a), span (b), and entrapment efficiency (c) of ginger extract-loaded microcapsules.

CH-2 exhibited the most irregular and bulky particles. A significant level of agglomeration was observed in PPI-CH-1, followed by PPI-CH-2, suggesting that the inclusion of chitosan contributed to particle clustering.

The observed shrinkage and surface dents likely resulted from relatively low drying temperatures, although the type of polymer used also played a crucial role in shaping particle morphology. Osamede Airouyuwa and Kaewmanee (2019) previously reported that PPI-based microparticles, compared to those made with SPI, showed greater levels of agglomeration, shrinkage, and surface imperfections, with these deformities being exacerbated by chitosan addition. In contrast, the inclusion of pectin improved the morphological properties of the microcapsules, resulting in a smoother and more uniform structure. In another study, combining pectin with whey protein produced microcapsules with enhanced morphological properties compared to whey protein alone (Choi & Chang, 2018). Moreover, morphology appears closely correlated with encapsulation efficiency, as microcapsules with minimal structural impairments and smoother surfaces tend to exhibit superior encapsulation efficiencies (Ahn et al., 2008).

The particle size (D[3,2]) and size distribution uniformity (span values) provide valuable insights into the encapsulation quality and performance of the microcapsules. Among the six microcapsule types, PPI-2 exhibited the highest particle size value, indicating a larger average particle size, while PPI-PC-1 showed a significantly smaller particle size, aligning with its focus on fine encapsulation within the micro-scale range (Fig. 2a). In terms of uniformity, PPI-PC-2 had the lowest span values, suggesting a narrow size distribution and consistent particle formation, which is desirable for stable and uniform encapsulation (Fig. 2b). Conversely, PPI-CH-1 displayed the highest span, indicative of a broader particle size range and more heterogeneous encapsulation, which could lead to variable release profiles.

The impact of the wall-to-core ratio was also evident in the size distribution, with the 8:2 formulations (e.g., PPI-2 and PPI-CH-2) generally showing larger particles and slightly higher Span values than their 9:1 counterparts, likely due to increased wall material concentration influencing particle growth. Overall, the particle size and Span data reveal that formulations combining PPI with pectin achieved favorable encapsulation properties. While PPI-PC-2 demonstrated the narrowest spread, indicating highly uniform encapsulation, PPI-PC-1 exhibited the smallest particle sizes across all formulations. This smaller size contributed to a broader distribution with a higher proportion of particles in the nano range, which may enhance bioavailability and controlled release potential.

3.2.2. Encapsulation efficiency

The encapsulation efficiency (EE%) across all treatments ranged from 68.3% to 86.9% (Fig. 2c), highlighting the influence of wall material type on encapsulation performance. The wall materials differ in their stabilization and film-forming capacities, which significantly affect encapsulation efficacy. The formulation PPI-PC-2, which combines PPI

with a higher ratio of pectin, demonstrated the highest encapsulation efficiency, reaching up to 86.9%. This superior performance may be attributed to pectin's ability to enhance the solid-like properties of the film, as reflected in an increase in interfacial dilatational modulus (Gharsallaoui et al., 2007), which improves the stability and encapsulation of bioactive compounds within the matrix. Consistent with the findings of current study, the research conducted by Choi and Chang (2018) have also disclosed that the complexation of whey protein concentrates with pectin polysaccharides significantly improved the encapsulation efficiency of gallic acid. Similarly, Hu et al. (2024) reported an encapsulation efficiency of ~79.7% for camellia seed oil using a pea peptide-maltodextrin system, further supporting the role of pea-derived proteins in enhancing microencapsulation performance.

In contrast, formulations with PPI-CH showed the lowest encapsulation efficiencies, with values around 68.3%. The reduced efficiency in these combinations could be linked to the high viscosity of chitosan, which may hinder effective encapsulation. Additionally, the combination of PPI and chitosan as carriers might promote particle adherence and agglomeration, leading to less effective encapsulation (Moser et al., 2017). A similar trend was also found in another study where whey protein isolates and chitosan complexes were employed for the encapsulation of garlic extract. The highest retention efficiency recorded in this study was 61.40% which is even significantly lower than chitosan-based complexes of current the study (Tavares & Zapata Noreña, 2019).

3.2.3. Physicochemical characterization

The physicochemical characteristics of the food ingredients in powdered form are crucially important for their subsequent utilization in food and commercial applications (Chen & Özkan, 2007). In color evaluation, all the microencapsulated samples were found with almost similar L^* values ranging from 88.2 ± 0.04 to 89.7 ± 0.20 while the a^* and b^* values were significantly different (Table 2). Overall, considering the range of L^* values and positive values for both the remaining parameters (a^* and b^*), it could be deduced that the color of all samples was falling between light yellow to light orange. The moisture content of spray-dried microcapsules refers to the remaining water in the wall material during spray drying process. The moisture content of ginger extract microcapsules ranged from 6.1 ± 0.12 to $7.2 \pm 0.12\%$, where PPI-CH-1 was documented with highest moisture content of $7.2 \pm 0.12\%$ (Table 2).

The microcapsules produced with pure pea protein were recorded with the highest water solubility, but all the samples witnessed a satisfactory value of water solubility ranging from 91.1 ± 2.2 to $96.1 \pm 0.6\%$. The solubility of microcapsules was decreased when PPI was combined with chitosan and pectin because the drying process did not have any considerable impact on the solubility of the spray dried product (Manickavasagan et al., 2015). The solubility of the ginger extract microcapsules agrees with the best treatment of microencapsulated citron extract in a combination of whey protein,

Table 2
Physicochemical characteristics of spray-dried microcapsules.

Treatments	Color parameters			Moisture content (%)	Solubility (%)	Hygroscopicity (%)
	L*	a*	b*			
PPI-1	89.7 ± 0.20 ^a	1.43 ± 0.18 ^{ab}	12.1 ± 0.46 ^c	6.4 ± 0.30 ^c	96.1 ± 0.56 ^a	16.6 ± 0.58 ^a
PPI-2	89.6 ± 0.03 ^a	0.73 ± 0.03 ^c	14.5 ± 0.10 ^b	6.1 ± 0.16 ^b	94.7 ± 1.39 ^{ab}	16.9 ± 0.58 ^a
PPI-CH-1	89.6 ± 0.04 ^a	1.49 ± 0.04 ^a	14.9 ± 0.09 ^b	7.2 ± 0.23 ^a	92.8 ± 1.94 ^{ab}	13.7 ± 0.10 ^b
PPI-CH-2	88.2 ± 0.04 ^a	1.23 ± 0.05 ^b	18.3 ± 0.18 ^a	6.6 ± 0.15 ^b	91.1 ± 2.20 ^b	12.1 ± 0.97 ^{bc}
PPI-PC-1	89.4 ± 0.11 ^a	1.21 ± 0.13 ^b	12.4 ± 0.06 ^c	6.3 ± 0.20 ^c	91.9 ± 2.51 ^{ab}	11.7 ± 1.19 ^c
PPI-PC-2	88.9 ± 0.34 ^a	0.93 ± 0.11 ^c	14.7 ± 0.29 ^b	6.1 ± 0.12 ^c	92.4 ± 1.39 ^{ab}	12.3 ± 0.26 ^{bc}

Note: Data is presented as values ± SD while values with different letters differ significantly ($p < 0.05$) by Fisher's LSD test.

maltodextrin and gum arabic, using the same concentrations of each polymer (Mahdi et al., 2020).

The hygroscopicity of microcapsules is an important parameter to perceive their keeping quality and food operations. The pea protein alone as a wall material exhibited higher hygroscopicity than its combination with chitosan and pectin. The addition of chitosan and pectin influenced the hygroscopicity of the microcapsules, possibly due to their synergistic interactions and alignments arranged in a manner that hinders moisture absorption from the environment.

3.2.4. Fourier transform infrared spectroscopy

The chemical interactions and configurations developed because of the integration of polymer substances and the bioactive ingredients were analyzed through FTIR spectroscopy. The FTIR spectra of ginger extract encapsulated in PPI and its combinations with chitosan and pectin are shown in Fig. 3. The broad band area, which is usually extended between the range of 3000 to 3700 cm^{-1} is, corresponds to the stretching of the hydroxyl group (Lee & Chang, 2020), which was also detected in the ginger extract microcapsules with a little deviation from each other. The peak for PPI-1 sample was spotted at 3061 cm^{-1} against the hydroxyl

group stretching, but it was witnessed at 3279 ± 1 against the rest of the spray-dried samples.

The formation of new linkages and interactions between protein and polysaccharides, as the chemical changes and transitions caused by the Maillard reaction, are analyzed against the characteristic bands of amide I, amide II, and amide III (Jiang et al., 2022), which was identified against PPI-1 sample at 1645, 1543 and 1400 cm^{-1} , respectively. The bands shift against these amide groups was noticed against all of the samples prepared with the combinations of PPI with chitosan and pectin. The most prominent transitions were discovered in PPI-CH-2 where the corresponding peaks were witnessed at 1634, 1539, and 1398 cm^{-1} , respectively. The characteristics absorption band for the carbohydrates was generally located between the spectral wavelength of 1000–1100 cm^{-1} (Choi & Chang, 2018), representing the C—O, C—C stretching, and C—H bending spectral areas. These carbohydrate-specific bands were observed in the samples supplemented with chitosan and pectin, and it was also noticed that in the samples fabricated with higher ginger extract concentration, these characteristic peaks were slightly more stretched, most likely due to the better interaction of polysaccharides with phenolic contents.

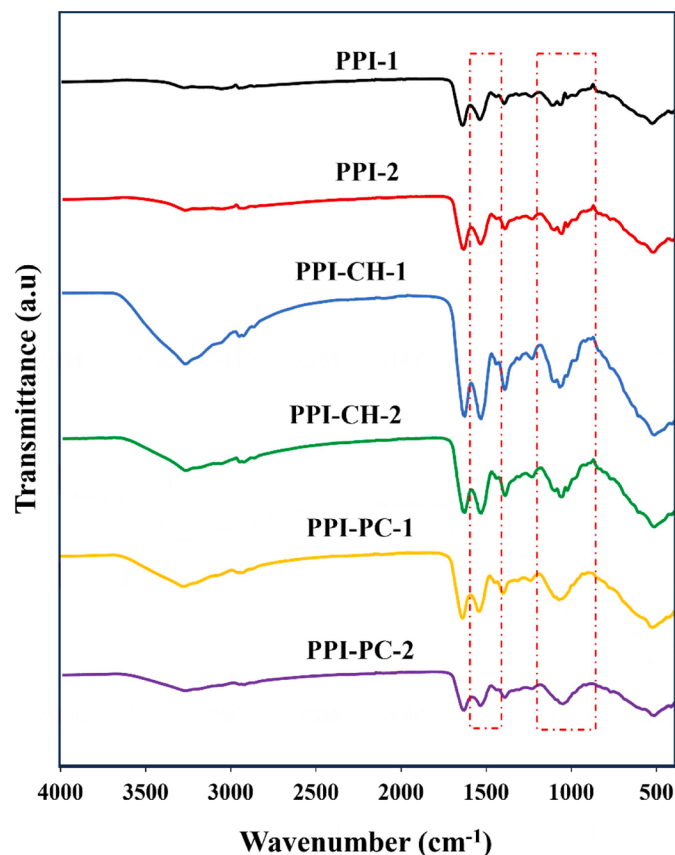


Fig. 3. FTIR spectra of spray-dried microcapsules of ginger extract.

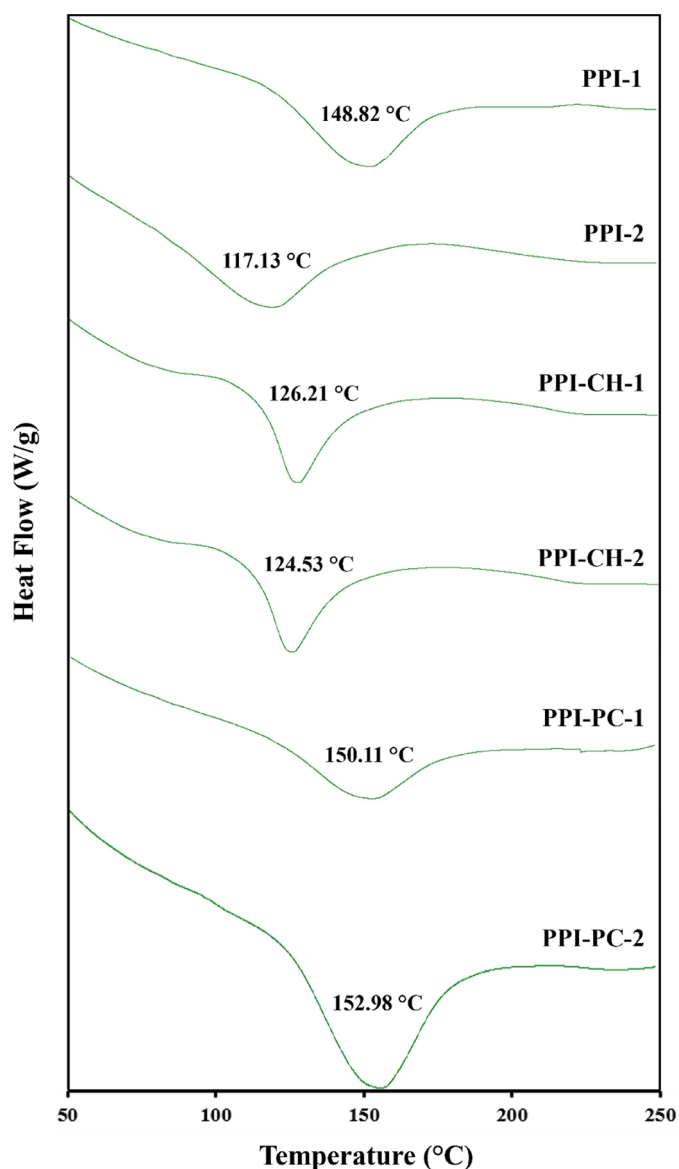


Fig. 4. Differential scanning calorimetry thermograms of the spray-dried ginger extract microcapsules.

3.2.5. Thermal stability

The thermal stability of spray-dried ginger extracts in the form of DSC thermograms is presented in Fig. 4. The thermal stability of pea protein-based microcapsules having wall to core ratio of 9:1 was analyzed through its endothermic peak identified at 148.82 °C, which was regressed in the case of PPI-2 where the concentration of ginger extract was increased. The thermal stability of microcapsules was significantly ($p < 0.05$) decreased with the formulation of chitosan with pea protein as PPI-CH-1, and PPI-CH-2 was melted at 117.13 °C and 126.21 °C whereas the combination of pea protein with pectin slightly improved the thermal stability of microcapsules and even it was not dropped with the increment of ginger extract as it was evident in PPI-2. The intercross linking of pea protein with pectin improved its stability and prevented the denaturation of protein against high-temperature treatment (Esfanjani et al., 2015).

3.3. Storage stability

3.3.1. Browning index

The browning index is a critical indicator of the appearance, quality, and sensory characteristics of dried food products. The browning index renders an insightful illustration of food product color by considering the cumulative impact of all color parameters (Nazir et al., 2022). The color changes during storage in ginger extract-based microcapsules can also be seen visually in Fig. 5(a), while the browning index of these samples is presented in Fig. 5(b). The browning index of freshly prepared spray-dried samples of PPI-CH treatments was significantly higher than their corresponding freshly fabricated PPI and PPI-PC samples. This could be attributed to the onset of a higher degree of Maillard reaction in PPI-CH samples in contrast to PPI-PC, as was discussed earlier in FTIR analysis. When protein and chitosan are combined, the carbonyl groups from chitosan and the amino acids present in protein may react to produce Schiff bases. The further disintegration of these Schiff bases may lead to the formation of the brown pigment melanoidin (Ricci et al., 2022). A gradual increase in the browning index was observed for all the spray-dried samples during storage. However, for both PPI-CH treatments, the increment in the browning index was quite significant at 45 °C, which may limit their suitability for longer-term storage. During storage, color changes also occurred due to the oxidation process as high temperature provides a favorable environment for oxidation, but the abrupt increase in PPI-CH samples indicated that the chitosan is more prone to oxidation given that chitosan contains many carbonyl groups, which may serve as the reaction spots for oxygen (Cheng et al., 2017).

3.3.2. Total phenolic contents

The spray-dried samples were also evaluated for their total phenolic contents stability against the storage temperatures of 23 °C and 45 °C for 28 days, and the results are shown in Fig. 6. The loss of TPC was not

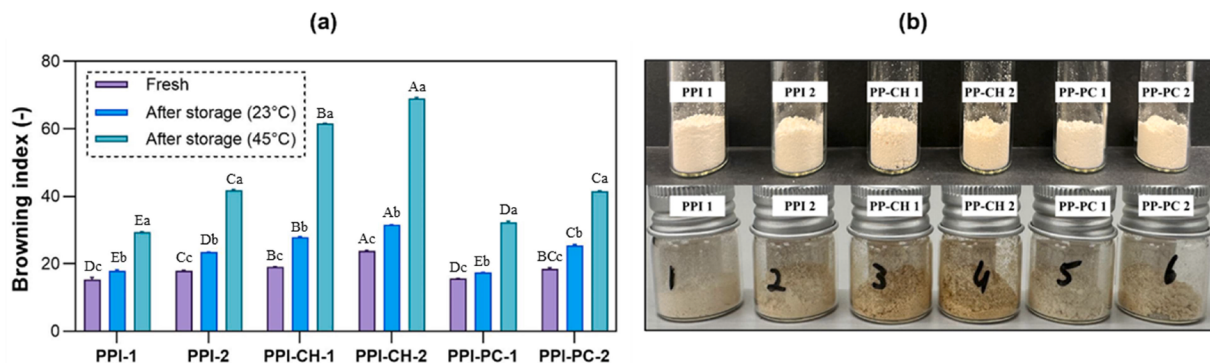


Fig. 5. (a) Browning index of ginger extract microcapsules during 28 days of storage at 23 °C and 45 °C. Bars represent mean values $\pm$ SD. Data bars with different letters differ significantly ($p < 0.05$) by Fisher's LSD test, with capital letters indicating differences among treatments at the same interval and small letters indicating differences among intervals within the same treatment. (b) Images of fresh (top) and stored (bottom) microcapsules at 45 °C.

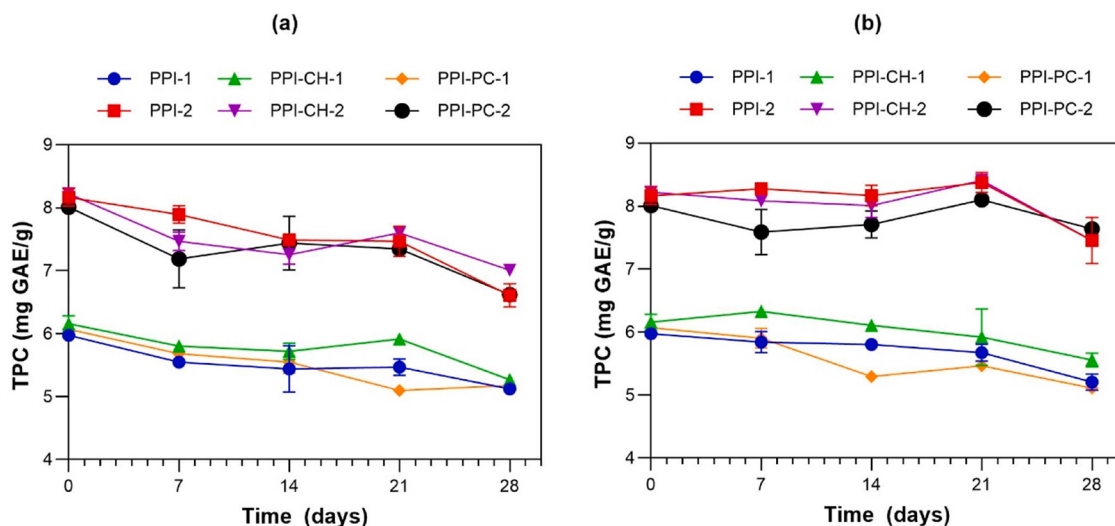


Fig. 6. Total phenolic contents of ginger extract-loaded microcapsules during storage at (a) 23 °C and (b) 45 °C.

significant ($p > 0.05$) during the storage duration till 21 days at both temperatures, and the combination of wall material offers significantly better protection for microencapsulated ginger extract. The total phenolic of microcapsules prepared using a PPI-2, PPI-CH-2, and PPI-PC-2 exhibited higher total phenolic of 8.31, 8.02, and 7.91 mg GAE g^{-1} , respectively, as compared to PPI-1, PPI-CH-1 and PPI-PC-2 which possessed 5.96, 6.28, and 5.97 mg GAE g^{-1} , respectively. This is because the combination of PPI-2, PPI-CH-2, and PPI-PC-2 was supplemented with a higher concentration of ginger extract during the formulation of microcapsules (Jayasundera et al., 2011). The total phenolic content experienced a significant decrease on the 28th day of storage at both 23 °C and 45 °C against all the treatments studied. This decline is due to the heat sensitivity of phenolic compounds, leading to their degradation at high temperatures, or might be due to oxidation (both enzymatic and non-enzymatic), hydrolysis, and polymerization (Cao et al., 2012). Overall, all the combinations exhibited a protective effect, indicating their ability to protect phenolic compounds from degradation at high-temperature conditions.

3.3.3. Antioxidant activity

The antioxidant activity of spray dried microcapsules for 28 days of storage at temperatures 23 and 45 °C was evaluated by DPPH assay, measured in % inhibition and is shown in Fig. 7. Regarding DPPH scavenging activity, no significant difference was detected during the storage period at both storage temperatures.

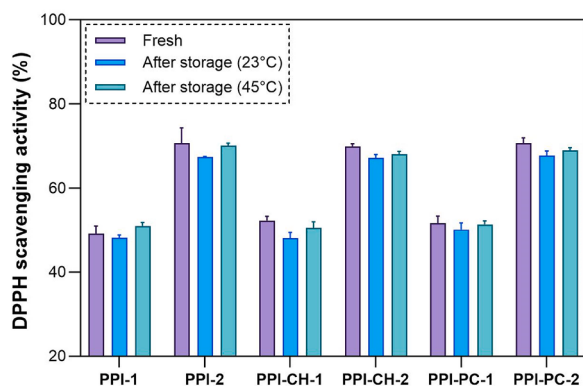


Fig. 7. Effect of storage at 23 °C and 45 °C on the DPPH radical scavenging activity of spray-dried ginger extract microcapsules. Bars represent mean values $\pm$ standard deviations.

These findings indicate the antioxidant stability of spray-dried ginger extract which could be attributed to the presence of a thicker protective layer around the core material, which also resists the leakage of antioxidants from their covering shells. This could be due to the combination of pea protein, pectin, and chitosan causing a thickening of the protective wall surrounding the active substances, subsequently limiting the release rate of active compounds. This aligns with the assertion made by Kanha et al. (2021), suggesting that a thicker wall has the potential to decrease the release of active compounds throughout the storage duration.

3.4. In-vitro digestion of ginger extract-loaded microcapsules

The phenolic release profile of microencapsulated ginger extract samples under a simulated digestion environment is shown in Fig. 8. Microcapsules derived from pea protein and the combination of pea protein with chitosan and pectin were used to evaluate the gastrointestinal phenolic release of ginger extract. After a thorough investigation, it was found that although the pea protein and chitosan combination (PPI-CH-1) showed the highest percentage of total gastrointestinal phenolic release of $78.63 \pm 3.25\%$ but it was not significantly different from other two types of the wall material having the same concentration of ginger extract. The greater gastric release of phenols was also recorded against the same treatment (PPI-CH-1) which was also

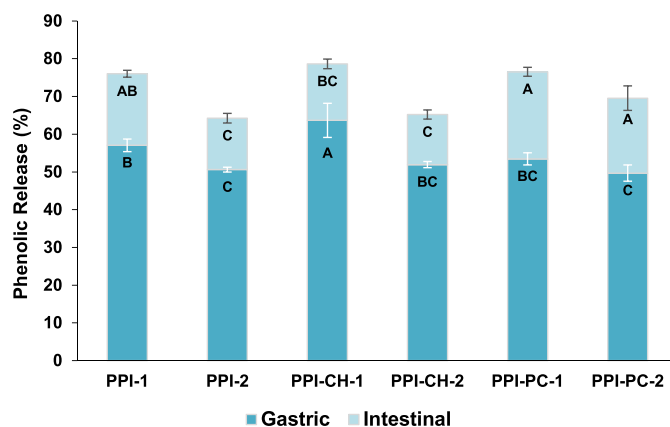


Fig. 8. Phenolic release from spray-dried ginger extract microcapsules during in vitro gastric and intestinal digestion. Bars represent mean values $\pm$ standard deviations, and different letters indicate significant differences ($p < 0.05$).

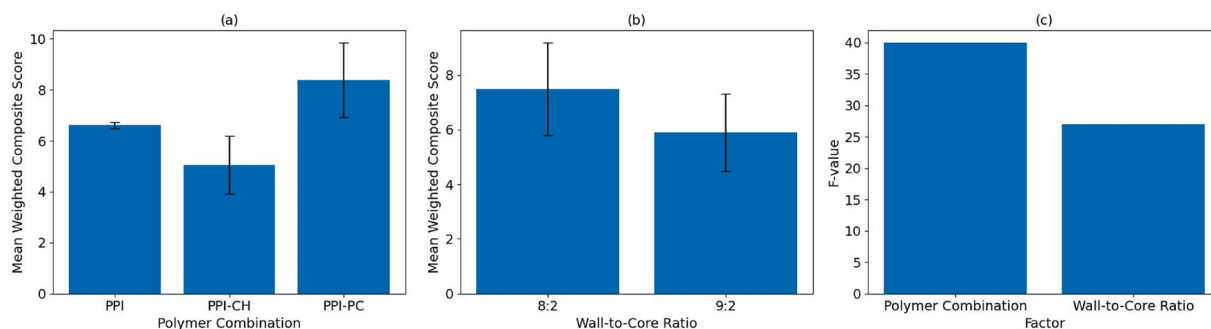


Fig. 9. Comparison of weighted composite scores for different encapsulation formulations: a) Mean composite scores for different polymer combinations, b) Mean composite scores for different wall-to-core ratios (8:2 and 9:1), c) ANOVA F-values, indicating the relative impact of polymer combination and wall-to-core ratio on the composite score. Error bars represent standard deviations.

significantly higher than all of the other samples. In the meantime, the pea protein-pectin complexes emerged as most promising combination of wall materials in terms of greatest phenolic release in simulated intestinal conditions and the highest phenolic release of $23.08 \pm 1.16\%$ under intestinal digestion was attended against PPI-PC-1. Furthermore, both treatments of microcapsules made from the combination of pea protein and pectin (PPI-PC) were comparatively resilient in the stomach and exhibited relatively a minimum phenolic release. These same microcapsules, however, demonstrated a marked increase in phenolic release in the intestinal environment, suggesting the possibility of targeted or controlled release profiles that are influenced by the particular composition of the encapsulation materials. Moreover, [Assadpour, Jafari and Maghsoudlou \(2017\)](#) have also reported a similar behavior of whey protein-pectin encapsulated folic acid under gastric conditions where these capsules exhibit resistance to the acidic environment containing pepsin against the release of core material. Mostly, the intestinal release of bioactive compounds has been considered desirable due to the large surface area, lower transit time and better absorption in the intestinal environment. Another interesting release trend was also observed among the treatments based on the concentration of the extract. The total phenolic release under simulated digestion conditions was significantly higher for the treatments having lower concentration of ginger extract (PPI-1, PPI-CH-1 and PPI-PC-1) as compared to the treatments incorporated with higher amount of ginger extract (PPI-2, PPI-CH-2 and PPI-PC-2). Additionally, the microcapsules with concavities, pores, and other structural defects were also more susceptible to extrinsic factors like moisture, pH, and enzymes in gastric conditions ([Choi & Chang, 2018](#)) which could be the primary factor behind the higher gastric release of phenolic contents in the microcapsules formulated from the blend of pea protein and chitosan. This finding highlights the critical function of careful encapsulation material selection in both preventing gastric degradation and coordinating customized release patterns in the gastrointestinal tract.

3.5. Quantitative ranking of encapsulation formulations

The composite scoring approach provided a systematic evaluation of encapsulation performance by integrating multiple physicochemical properties into a single metric. The results demonstrated notable variations among different polymer combinations and polymer fractions which are presented in [Fig. 9](#). Among the polymer combinations, formulations containing PPI-PC exhibited the highest composite scores ([Fig. 9a](#)), indicating superior encapsulation performance compared to PPI-CH and PPI alone. This suggests that the incorporation of pectin as a co-wall material enhanced the overall quality of the encapsulated ginger extract. Conversely, formulations based solely on PPI-CH exhibited the lowest composite scores, implying less effective encapsulation under the given processing conditions.

When comparing wall-to-core ratios, formulations with a 9:1 ratio

generally achieved higher composite scores than those with an 8:2 ratio ([Fig. 9b](#)), suggesting that a slightly higher polymer content favored improved encapsulation properties. This difference was statistically significant ($p < 0.001$), although the effect was less pronounced than that observed for polymer combination. Polymer combination also showed a highly significant effect ($p < 0.001$) ([Fig. 9a, c](#)), confirming that the selection of wall materials had a more substantial influence on encapsulation quality than polymer concentration.

Statistical analysis using two-way ANOVA further confirmed that polymer combination exerted a significantly greater effect on the composite score than polymer fraction, as indicated by the higher F-value ([Fig. 9c](#)). This finding highlights the crucial role of wall material selection in optimizing the encapsulation of ginger extract. The inclusion of polysaccharide contributed to enhanced entrapment efficiency, stability, and antioxidant retention, resulting in higher overall scores. In contrast, the polymer fraction had a smaller yet significant effect, implying that adjusting the wall-to-core ratio can fine-tune encapsulation properties but is not the primary determinant of performance.

4. Conclusion

In this study, the physicochemical, morphological, encapsulation, and antioxidant characteristics of spray-dried microcapsules coated with pea protein and its combinations with chitosan and pectin were investigated. The combination of pea protein with pectin (PPI-PC) emerged as the most promising wall material for the microencapsulation of ginger extract, outperforming the other two formulations. This conclusion was derived from various characterization techniques, including environmental scanning electron microscopy (ESEM), physicochemical analysis, thermal stability assessment, encapsulation efficiency, antioxidant activity, storage stability, and *in vitro* digestion. ESEM micrographs revealed a smooth and uniform morphology in PPI-PC microcapsules, suggesting improved structural integrity. The encapsulation efficiency analysis confirmed the highest entrapment of ginger bioactives in these microcapsules, further supporting their potential as an effective delivery system.

To provide a comprehensive assessment, a composite scoring approach was employed to integrate multiple physicochemical properties into a single metric. This systematic evaluation reinforced the superiority of PPI-PC microcapsules, which consistently achieved the highest composite scores. The analysis also revealed that polymer combination had a significantly greater impact on encapsulation performance than polymer fraction, highlighting the crucial role of material selection in optimizing microencapsulation. Furthermore, formulations with an 8:2 wall-to-core ratio generally exhibited higher composite scores, suggesting a more favorable balance between core and wall materials. PPI-PC microcapsules also demonstrated superior thermal stability, while all formulations exhibited notable storage stability, effectively retaining phenolic content and antioxidant activity over

time. Additionally, the in vitro digestion analysis confirmed that PPI-PC microcapsules ensured the safe delivery of bioactive compounds to the stomach, allowing for their subsequent absorption in the intestine.

The findings highlight the potential of plant-protein-polysaccharide matrices (particularly PPI-PC) for use in functional foods, nutraceuticals, and controlled-release formulations. Future work should include in vivo validation, sensory evaluation, and application trials in real food and nutraceutical systems to assess consumer acceptability and product performance. Furthermore, exploring ternary biopolymer combinations and optimizing release mechanisms may yield even more robust delivery systems. Such advancements will contribute to the broader utilization of plant-based encapsulation materials for stabilizing and delivering bioactive compounds.

Ethical statement – Studies in humans and animals

This study did not involve any experiments on humans or animals. All experimental procedures were conducted using plant-based materials and in vitro analytical methods.

CRediT authorship contribution statement

Muhammad Nouman Shaikat: Writing – original draft, Methodology, Investigation, Formal analysis, Writing – review & editing. **Ahmad Rabbani:** Investigation, Formal analysis, Writing – original draft. **Biagio Fallico:** Supervision, Conceptualization, Validation. **Akmal Nazir:** Writing – review & editing, Supervision, Resources, Conceptualization, Data curation, Methodology, Software.

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.

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Data availability

Data will be made available on request.

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