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Kandi Sridhar, Pascaline Hamon, Jordane Ossemond, Said Bouhallab, Thomas Croguennec, et al.. Plant and animal protein mixed systems as wall material for microencapsulation of Mānuka essential Oil: Characterization and in vitro release kinetics. Food Research International, 2024, 187, pp.114419. 10.1016/j.foodres.2024.114419 . hal-04573021

**HAL Id: hal-04573021**

**<https://hal.inrae.fr/hal-04573021v1>**

Submitted on 13 May 2024

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# Plant and animal protein mixed systems as wall material for microencapsulation of Mānuka essential Oil: Characterization and *in vitro* release kinetics

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## ARTICLE INFO

### Keywords:

Plant proteins  
Animal proteins  
Protein mixed systems  
Wall material  
Mānuka essential oil  
Microencapsulation

## ABSTRACT

Combination of plant and animal protein diet is becoming a valuable source of nutrition in the modern diet due to the synergistic functional properties inherent in these protein complexes. Moreover, the synergy between animal and plant proteins can contribute to the high stability and improved solubility of the encapsulated bioactive ingredients (e.g., essential oils). Therefore, the study was designed to evaluate the plant (pea protein (PP) and lupine protein (LP)) and animal protein (whey protein, WP) mixed systems as a wall material for microencapsulation of mānuka essential oil, as an example of bioactive compound. Moreover, physicochemical properties and *in vitro* release profile of encapsulated mānuka essential oil were studied. Mānuka essential oil microcapsules exhibited low moisture content (5.3–7.1 %) and low water activity (0.33–0.37) with a solubility of 53.7–68.1 %. Change in wall material ratio significantly affected the color of microcapsules, while microcapsules prepared with 1:1 protein/oil ratio demonstrated a high encapsulation efficiency (90.4 % and 89.4 %) for protein mixed systems (PP + WP and LP + WP), respectively. Microcapsules further showed low values for lipid oxidation with a high oxidative stability and antioxidant activity (62.1–87.0 %). The zero order and Korsmeyer–Peppas models clearly explained the release mechanism of encapsulated oil, which was dependent on the type and concentration of the protein mixed used. The findings demonstrated that the protein mixed systems successfully encapsulated the mānuka essential oil with controlled release and high oxidative stability, indicating the suitability of the protein mixed systems as a carrier in encapsulation and application potential in development of encapsulated functional foods.

## 1. Introduction

Plant proteins are a valuable source of nutrition and are becoming increasingly popular in the modern diet (Prakash et al., 2023). Plant protein ingredients are often lower in saturated fat and cholesterol compounds, and can provide additional health benefits in the form of fiber, vitamins, and minerals. Among the plant protein sources, legumes, such as beans, lentils, and peas are the potential sustainable protein sources (Hadidi et al., 2023), which can be used in a variety of food products and can be a great way to reduce reliance on animal products while still maintaining a balanced diet. On the other hand, plant-based proteins may lack certain essential amino acids. Additionally, plant proteins exhibit lower solubility and emulsification efficiency that may

require additional processing or formulation adjustments (Ma et al., 2022). Therefore, a hybrid model containing animal and plant-based protein blends can offer a balanced and complete source of nutrition (Hinderink et al., 2021). By combining animal-based proteins (whey proteins) with plant-based proteins (pea and lupine), it is possible to develop a diet rich in all the essential amino acids along with bioactive compounds (Alves & Tavares, 2019; Grasberger et al., 2021). This can provide a balanced diet for people who follow a diet transition. Moreover, the protein mixed systems can enhance functional properties of proteins (e.g., stability, emulsification, foaming, binding, and adhesion) (Grasberger et al., 2021), thereby developing more stable, effective, and functional wall materials that can be used to protect and deliver a wide range of bioactive ingredients, such as essential oils.

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<https://doi.org/10.1016/j.foodres.2024.114419>

Received 4 March 2024; Received in revised form 19 April 2024; Accepted 22 April 2024

Available online 24 April 2024

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Mānuka (*Leptospermum scoparium*) essential oil is a natural product that has gained significant attention in recent years owing to its promising potential health advantages. It is extracted from the leaves and branches of the mānuka tree, a native to New Zealand (Mathew et al., 2020). Mānuka essential oil is known for its potent antimicrobial, anti-inflammatory, and antioxidant properties, which make it attractive for various applications in agri-food-pharma industries (Sharma et al., 2023). However, the use of mānuka essential oil is limited due to its sensitivity to environmental factors, such as light, heat, potential reactivity with other ingredients, and oxidation, which can lead to its degradation and loss of functionality. These limitations have led researchers to investigate different approaches to improve the stability and bioavailability of mānuka essential oils. Among the different approaches, encapsulation is one of the promising techniques that can address these challenges and improve the bioavailability and functionality of essential oils in food applications, particularly drug delivery studies (Sundar & Parikh, 2023). The choice of wall material plays a crucial role in determining the efficiency, stability, and bioavailability of the encapsulated essential oil. Previous studies have investigated various wall materials for the encapsulation of essential oils, including proteins, lipids, carbohydrates, and their combinations (Plati & Paraskevopoulou, 2023; Xiao & Ahn, 2023).

More recently, there has been growing interest in using mixed protein systems as potential wall materials in encapsulation of essential oils due to their sustainability, safety, film-forming properties, low-cost, and potential health benefits (Gimenez et al., 2023; Ma et al., 2024). For instance, mixed protein systems may exhibit different characteristics, such as surface charge, hydrophobicity, and their capacity to form a stable encapsulating matrix (Grasberger et al., 2024). These distinctive properties have the potential to influence the encapsulation process, subsequently affecting the overall encapsulation efficiency. A study by Guo et al. (2022) encapsulated the carvacrol, a major component of thyme and oregano oils, using a mixed system between a protein and a polysaccharide (i.e., whey protein isolate and high methoxyl pectin). Another study by Hosseinnia et al. (2017) optimized the *Ziziphora clinopodioides* essential oil encapsulation using whey protein isolate and pectin. Both studies showed good stability, protection against oxidation, and sustained release of the encapsulated oils, confirming the potential of plant and animal-based mixed protein systems for oil encapsulation. Currently, there are no studies exploring the potential of protein mixed systems as wall materials in the encapsulation of mānuka essential oil. Thus, working experience in microencapsulation and plant protein utilization prompted us to develop a stable and efficient encapsulation system to improve the bioavailability and functionality of mānuka essential oil in food applications and provide a safe alternative to synthetic encapsulation materials.

Therefore, the study was designed to evaluate the potential of plant and animal protein mixed systems as wall material in encapsulation of mānuka essential oil. Also, the study evaluated the physicochemical properties and *in vitro* release profile of encapsulated mānuka essential oil. The findings of this study provide valuable insights on the suitability of hybrid protein systems as wall materials in the encapsulation process and contribute to the development of encapsulated functional food and nutraceutical products.

## 2. Materials and methods

### 2.1. Sample collection

Mānuka (*Leptospermum scoparium*) essential oil was purchased from Herbes & Traditions (Comines, France), while pea (46 % protein) and lupine (46 % protein) protein concentrates were obtained from Elementa, Saint-Nolff, France and Inveja, Pays de la Loire, France. Whey

protein concentrate (80 % protein) was obtained from Meggle GmbH, Wasserburg, Germany. The sample materials were kept in a digital humidity controller at a temperature of 18 °C and a humidity of 50 %.

### 2.2. Protein mixed systems

Individual sample dispersions (pea and lupine) were mixed with whey protein concentrate (1:1, v/v) at the same protein concentration (46 % protein). Samples were then thoroughly mixed with a magnetic stirrer at room temperature, lyophilized, and stored in air-tight Corning® Gosselin™ polypropylene food grade bottles at – 20 °C. Samples were coded as PP + WP (pea protein + whey protein) and LP + WP (lupine protein + whey protein).

### 2.3. Preparation of emulsion and microcapsules

Oil-in-water emulsion was prepared according to McCarthy et al. (2016). The protein samples (PP + WP and LP + WP) used as wall materials were dispersed in distilled water to form solutions of 10, 20, and 30 % (w/v) and the pH of the solutions were adjusted to  $7.08 \pm 0.02$  using NaOH and HCl. Then the samples were thoroughly mixed under a high shear at  $5000 \times g$  for 5 min using an ULTRA-TURRAX (IKA®, Staufen, Germany). The emulsion was prepared by adding 10 % mānuka essential oil to the wall material solution (protein/oil material ratio was maintained at 1:1, 2:1, and 3:1). The composition of solution was coded as PP + WP1, PP + WP2, and PP + WP3 as well as LP + WP1, LP + WP2, and LP + WP3 for the protein/oil material ratio of 1:1, 2:1, and 3:1, respectively. The mixtures were emulsified using an ULTRA-TURRAX at  $10,000 \times g$  for 5 min. The emulsion was transferred into disposable foil pans and frozen at – 20 °C for 5 h. Subsequently, the frozen samples were transferred into a freeze dryer chamber to initiate the freeze-drying (SERAIL, Le Coudray-Saint-Germer, France) at a condenser temperature of – 50 °C and chamber pressure of 0.05 mbar for 48 h. The freeze-dried samples were collected and gently ground to obtain a uniform fine flowing powder (Supplementary Figure S1). The prepared microcapsules were stored in air-tight Corning® Gosselin™ polypropylene food grade bottles and stored at 4 °C.

### 2.4. Characteristics of microcapsules

#### 2.4.1. Microcapsule morphology

A confocal scanning laser microscopy (Zeiss LSM-880, Carl Zeiss, Oberkochen, Germany) was performed to understand the different components distribution within the microcapsules. Briefly, reconstituted emulsions were prepared in deionized water based on total solids, followed by vortexing for 2 min. Then, sample (200 µL) was mixed with Nile red (0.10 %; 18 µL; staining oil) and Fast Green (1 %; 6 µL; staining protein), and incubated for 10 min at 20 °C. Nile red was excited at 488 nm and Fast Green was excited at 633 nm. Samples were placed on a cover slip and the images were taken with Zeiss Plan-apochromat 63X/1.40 oil objective.

#### 2.4.2. Moisture content and water activity ( $a_w$ )

The moisture content of the microcapsules was determined according to Le Priol et al. (2019). Briefly, lyophilized sample (1 g) was placed in pre-weighed Petri dishes and oven-dried (Memmert GmbH + Co. KG, Schwabach, Germany) at 120 °C until it reaches the constant weight loss. The moisture content was calculated by weighting the sample difference before and after drying according to the equation (1). For water activity, sample (5 g) was placed in a plastic cup and then placed in a water activity measurement chamber (LabSwift-aw, Novasina AG, Lachen, Switzerland) at  $20 \pm 2$  °C. After reaching the equilibrium, the water activity of sample was recorded.

$$\text{Moisture content (\%)} = \left[ \left( \frac{\text{Initial sample weight (g)} - \text{Sample weight after drying (g)}}{\text{Initial sample weight (g)}} \right) \times 100 \right] \quad (1)$$

#### 2.4.3. Solubility in water

The water solubility of the microcapsules was determined according to Francisco et al. (2020) with minor modifications. In brief, sample (0.50 g) was thoroughly mixed with deionized water (50 mL) and vortexed for 30 min. The mixture was then centrifuged (Biofuge Pico, Gemini BV., Apeldoorn, Netherlands) at  $3000 \times g$  for 10 min. Then, an aliquot of the supernatant (50 g) was transferred to pre-weighed Petri dishes and oven-dried at  $105^\circ\text{C}$  until it reaches the constant weight loss. The solubility (%) was calculated based on the Petri dishes weight difference before and after oven-drying.

#### 2.4.4. Encapsulation efficiency

Encapsulation efficiency (EE) of the microcapsules was determined according to Charles et al. (2021). In brief, lyophilized sample (2.50 g) was mixed with hexane (15 mL) and vortexed for 2 min. Then, the mixture was centrifuged for 30 min at  $12000 \times g$ . The clear organic phase was filtered and evaporated in the fume hood until the residue dried to constant weight loss. The amount of free surface oil was determined gravimetrically using blotting paper. The EE was calculated according to the following equation (2) and expressed as %:

$$\text{EE (\%)} = \left[ \left( \frac{\text{Oil content in sample (g)} - \text{Free surface oil of sample (g)}}{\text{Total oil (g)}} \right) \times 100 \right] \quad (2)$$

#### 2.4.5. Swelling rate

Swelling rate of microcapsules was performed according to Moorian et al. (2015) with minor modifications. Briefly, samples (50 mg) were placed in a saline phosphate buffer (0.10 M; 20 mL; pH 7.40) at room temperature and allowed to swell over 6 h. The swollen microcapsules were periodically removed and blotted with absorbent paper. The weight of the microcapsules was recorded and the swelling rate was determined according to equation (3).

$$\text{Swelling rate} = \left[ \left( \frac{\text{Swollen weight (g)}}{\text{Initial weight (g)}} \right) \right] \quad (3)$$

#### 2.4.6. Acid value

The acid value of microcapsules was performed according to Charles et al. (2021). Briefly, the sample (1 g) was mixed with hot ethanol (100 mL) containing 1 mL of phenolphthalein indicator (1 %, w/v) and vortexed for 3 min. The reaction mixture was titrated with 0.10 N sodium hydroxide (NaOH) to the first permanent pink color. The acid value was determined according to the following equation (4) and expressed as mg KOH/g of fatty acid.

$$\text{Acid value (mg KOH/g)} = \left[ \left( \frac{\text{Volume of titrant (mL)} \times M_{\text{NaOH}} \times 56.10}{\text{Weight of sample (g)}} \right) \right] \quad (4)$$

#### 2.5. Total phenolic and flavonoid contents, and antioxidant activity

To quantify the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity, sample (1 mg) was suspended in distilled water (1 mL) and centrifuged at 900 rpm for 10 min. The supernatant was used to determine the TPC, TFC, and antioxidant activity. TPC content was determined using the Folin–Ciocalteu's reagent

according to Nantitanon et al. (2010). Briefly, supernatant (20  $\mu\text{L}$ ) was thoroughly mixed with Folin–Ciocalteu reagent (45  $\mu\text{L}$ ) and sodium carbonate solution (135  $\mu\text{L}$ ; 7.50 %). The resulting mixture was allowed to stand for 2 h at room temperature, after which the absorbance was measured spectrophotometrically at 750 nm. The results were expressed as milligrams of equivalent gallic acid per 100 g (mg GAE/100 g). For TFC, supernatant (500  $\mu\text{L}$ ) was mixed with  $\text{NaNO}_2$  (5 %, 0.15 mL), and the mixture was allowed to stand for 5 min. Then,  $\text{AlCl}_3$  solution (10 %; 0.30 mL) was added and thoroughly mixed. After 6 min incubation, NaOH (1 mL; 1 M) was added, and distilled water was used to adjust the volume to 5 mL. The absorbance was then measured at 510 nm against a blank (Chang et al., 2013). The results were expressed as milligrams of quercetin equivalent per 100 g (mg QE/100 g).

Free radical scavenging assay was carried out based on Musa et al. (2011) with some modifications. Briefly, the supernatant (1 mL) was mixed with 500  $\mu\text{L}$  methanolic DPPH solution (1 mM). The mixture was kept at room temperature for 30 min and measured at 517 nm using a UV/VIS spectrophotometer (Thermo Multiskan Go, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). A control was prepared following the afore-mentioned procedure without the sample. The DPPH scavenging ability was calculated according to equation (5) and expressed as percentage.

$$\% \text{ inhibition} = \left[ \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \right] \quad (5)$$

where,  $A_{\text{control}}$  = absorbance of control and  $A_{\text{sample}}$  = absorbance of the sample

#### 2.6. Oxidative stability

The oxidative stability of microcapsules was evaluated according to Di Giorgio et al. (2022). Briefly, microcapsules (0.22 g) were mixed with TBARS reagent (2.50 mL), sonicated for 10 min, and heated ( $95^\circ\text{C}$ ) in a boiling water bath (Grant Instruments™ JB Nova, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) for 10 min to a pink color. After cooling, samples were centrifuged at  $3600 \times g$  for 20 min, and the absorbance of sample at 532 nm was measured using a UV/Vis spectrophotometer. A control was prepared as described above with mānuka essential oil. The TBARS were calculated using 1,1,3,3-tetraethoxypropane standard and expressed as mg malonaldehyde (mg MDA/L) per L of oil.

#### 2.7. Color analysis

Color analysis of microcapsules was performed according to Castel et al. (2018) in a lab-scale hand-held colorimeter (Chroma meter, Konica Minolta, Inc, Tokyo, Japan) after a calibration with a white tile (y, 87.50; x, 0.31; y, 0.32). The color parameters, such as  $L^*$ ,  $a^*$ , and  $b^*$  were recorded, and the total color difference ( $\Delta E$ ) was calculated as shown in equation (6).

$$\Delta E = \left[ \left[ (L^* - L^*_1)^2 + (a^* - a^*_1)^2 + (b^* - b^*_1)^2 \right]^{1/2} \right] \quad (6)$$

where,  $L^* - L^*_1$ ,  $a^* - a^*_1$ , and  $b^* - b^*_1$  are difference in lightness, red intensity, and yellow intensity, respectively.

## 2.8. *In vitro* release and kinetic modelling

The release profile of mānuka essential oil microcapsules was assessed *in vitro* using simulated gastrointestinal fluids (Kairam et al., 2020). Simulated gastric fluid (SGF) was prepared by dissolving 2 g sodium chloride (NaCl) and 7 mL of 36 % hydrochloric acid (HCl) in 900 mL of distilled water. Subsequently, 5 g of pepsin was added to the solution, and the pH was adjusted to 1.20 using 0.10 M HCl. The final volume of the SGF solution was adjusted to 1000 mL with distilled water. For the preparation of simulated intestinal fluid (SIF), 1.25 g of pancreatin and 6.80 g of potassium dihydrogen phosphate were dissolved in 800 mL of distilled water. To this solution, 77 mL of 0.20 M NaCl was added, and the mixture was stirred overnight at 4 °C using a magnetic stirrer. The pH of the solution was then adjusted to 6.80 using 1 M NaOH, and the final volume of the SIF solution was adjusted to 1000 mL with distilled water.

To evaluate the *in vitro* release of mānuka essential oil from microcapsules, microcapsules (5 g) were placed in SGF (50 mL), followed by the addition of distilled water (50 mL). The resulting mixture was then incubated at a temperature of 37 °C (IKA® KS4000 i control) for a duration of 0.50 h, allowing the separation of the supernatant from the microcapsules through filtration. Subsequently, SIF (50 mL) was added to the swollen microcapsules, and the mixture was gently stirred for 6.50 h. The released oil was subsequently extracted from the system using hexane in a separating funnel. This extraction process was repeated twice using hexane, and the quantity of released oil was determined gravimetrically. The cumulative % oil released in both SGF and SIF was determined by comparing the amount of released oil to the total oil. All measurements were conducted in triplicate.

The *in vitro* release profile of microcapsules was modelled using the zero order (i.e., %cumulative oil release vs time), first order (i.e., log % cumulative oil release vs time), Higuchi's model (i.e., %cumulative oil

release vs  $\sqrt{\text{time}}$ ), and Korsmeyer-Peppas model (i.e., log %cumulative oil release vs log time) according to Nasri et al. (2020) as shown in equations (7)–(10).

$$\text{Zero order model : } \left[ \left( \frac{Q_t}{Q_\infty} \right) = (Kt) \right] \quad (7)$$

$$\text{First order model : } \left[ \left( \frac{Q_t}{Q_\infty} \right) = (1 - e^{-Kt}) \right] \quad (8)$$

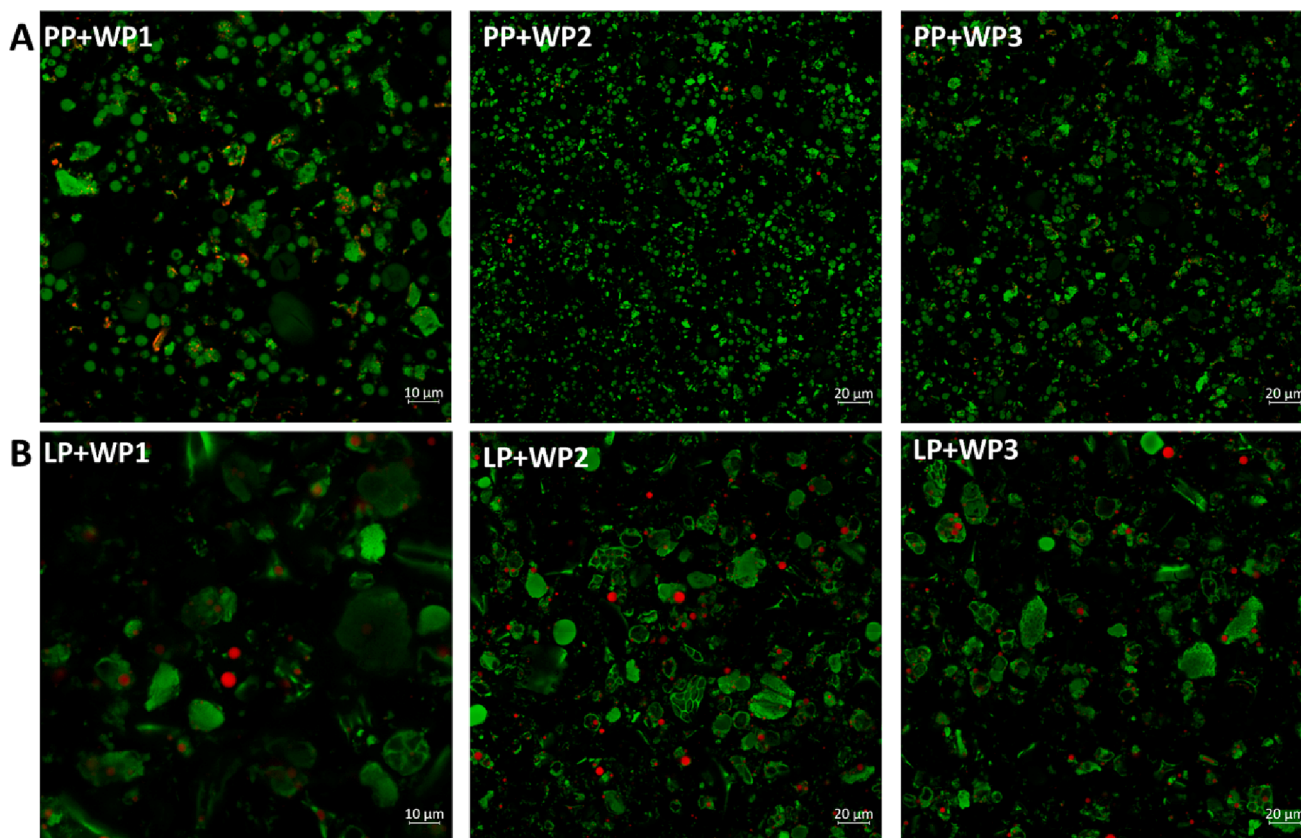
$$\text{Higuchi model : } \left[ \left( \frac{Q_t}{Q_\infty} \right) = (Kt^{\frac{1}{2}}) \right] \quad (9)$$

$$\text{Korsmeyer – Peppas model : } \left[ \left( \frac{Q_t}{Q_\infty} \right) = (Kt^n) \right] \quad (10)$$

Where,  $Q_t$ , the percentage of oil released at time  $t$ ;  $Q_\infty$ , total % oil;  $Q_t/Q_\infty$ , the fractional release of oil at time  $t$ ;  $k$ , release rate constant;  $n$ , diffusional release exponent (0.50, Fickian diffusion;  $0.50 < n < 1$ , anomalous (non-Fickian) diffusion; 1, case-II transport;  $>1$ , super case-II transport), which indicates the oil release mechanism;  $t$ , time.

## 2.9. Statistical analysis

The experiments were conducted with a minimum of three independent determinations and the results were presented as a mean  $\pm$  standard deviation. Statistical analysis was performed using multivariate analysis of variance (MANOVA) and Duncan's multiple range tests at a significance level of  $p < 0.05$  by the commercial IBM®SPSS® Statistics version 22.0 (IBM Ltd., Armonk, NY, USA) for Microsoft® Windows® 10. The kinetics model fitting was performed using the Microsoft® Excel® Add-in software (DDSolver).



**Fig. 1.** Confocal micrographs of reconstituted mānuka essential oil emulsion. (A) PP+WP and (B) LP+WP. Scale bar: 10 or 20  $\mu\text{m}$ . For sample codes, refer to section 2.3.

### 3. Results and discussion

#### 3.1. Morphology

The internal structure of reconstituted emulsion system revealed the regular distribution of active ingredient (labelled in red) within a continuous green-labeled protein matrix (Fig. 1). These images further demonstrated the matrix type encapsulation model without distinct core or shell. Both PP + WP and LP + WP formulations exhibited a similar trend in particle size variation (10  $\mu\text{m}$  to 20  $\mu\text{m}$ ). The smallest particle size was observed in both PP + WP1 and LP + WP1 formulations (10  $\mu\text{m}$ ), while the larger particle sizes were observed in PP + WP2, PP + WP3, LP + WP2, and LP + WP3 formulations (20  $\mu\text{m}$ ). The variation in particle size could be related to protein-to-oil material ratios, chemical properties of wall materials, and emulsification conditions (Linke et al., 2020; Zhao et al., 2019). The consistency in particle size within each group of formulations (PP + WP and LP + WP) suggested that the protein source (pea protein or lupine protein) has a significant impact on particle size, while whey protein acts as a consistent component contributing to the overall encapsulation process. Furthermore, the loss of sphericity was observed due to the consequences of the freeze-drying process and the nature of the wall materials (Zhuo et al., 2004). Additionally, agglomeration, caking, and the formation of a dry crust on the surface of the droplets during the drying process can also impact the sphericity of the microcapsules (Kou et al., 2023). An alteration in the protein material ratio within the PP + WP and LP + WP samples resulted in a shift in particle size. Notably, this particle size change remained consistent in both samples when the protein-to-oil material ratio was adjusted to 1:2 and 1:3. A study by Wang et al. (2020) showed the similar findings in their study, where quercetin was embedded within a continuous matrix of binary complex (i.e., soy protein isolate + polysaccharide). In another investigation by Jiang et al. (2014), the distribution of oil particles in native and alkali-treated pea proteins exhibited significant heterogeneity under different storage conditions, while Li et al. (2012) concluded the uniform distribution of oil droplets in a protein medium stabilized by sodium caseinate.

#### 3.2. Moisture content, water activity, and solubility

Moisture content, water activity, and solubility of mānuka essential oil microcapsules are shown in Table 1A. The moisture content of the samples ranged from 5.3 to 7.1 %, in which the LP + WP samples significantly ( $p < 0.05$ ) showed higher moisture content compared to PP + WP samples. The water activity values were relatively consistent across all the samples, ranging from 0.33 (PP + WP1) to 0.37 (LP + WP1). Solubility was higher in the LP + WP compositions, aligning with the moisture content trend observed in the samples. A study by Le Priol et al. (2019) highlighted the lack of standardized specifications for moisture content in dry food formulations utilizing plant-animal proteins as wall materials. In their study, sunflower oil microcapsules with moisture content ranging from 1.60 to 8.20 % were prepared using

**Table 1A**  
Moisture content, water activity, and solubility of mānuka essential oil microcapsules<sup>1</sup>

Samples	Moisture (%)	Water activity	Solubility (%)
PP + WP1	5.80 $\pm$ 0.21 <sup>b</sup>	0.33 $\pm$ 0.01 <sup>a</sup>	53.66 $\pm$ 1.02 <sup>a</sup>
PP + WP2	5.27 $\pm$ 0.21 <sup>a</sup>	0.34 $\pm$ 0.01 <sup>a</sup>	57.73 $\pm$ 1.60 <sup>b</sup>
PP + WP3	6.00 $\pm$ 0.10 <sup>c</sup>	0.35 $\pm$ 0.01 <sup>a</sup>	53.86 $\pm$ 1 <sup>a</sup>
LP + WP1	7.14 $\pm$ 0.22 <sup>e</sup>	0.37 $\pm$ 0.01 <sup>a</sup>	65.44 $\pm$ 5.80 <sup>d</sup>
LP + WP2	6.70 $\pm$ 0.17 <sup>d</sup>	0.36 $\pm$ 0.01 <sup>a</sup>	68.10 $\pm$ 1.96 <sup>e</sup>
LP + WP3	7.13 $\pm$ 0.23 <sup>e</sup>	0.36 $\pm$ 0.01 <sup>a</sup>	61.49 $\pm$ 3.03 <sup>c</sup>

<sup>1</sup>Data were expressed as mean  $\pm$  standard deviation. The mean values sharing different lower-case superscripts (a-e) within the column represented statistically significant ( $p < 0.05$ ) differences based on MANOVA and Duncan's multiple range tests. For sample codes, refer to section 2.3.

commercially available plant protein extracts as wall materials. The study concluded that the observed differences in moisture content could be attributed to proteins affinity for water and the diffusivity of water through the wall material. Similar moisture content of 6.13–8.04 % was also reported by Zhao et al. (2022) for encapsulated flavonoids using the combination of different proteins and maltodextrin wall materials. A study investigated the encapsulation of cumin (*Cuminum cyminum* L.) seed essential oil in soybean protein using freeze-drying (Najjaa et al., 2020). The results showed that the freeze-drying process resulted in stable and spherical particles with high encapsulation efficiency and low moisture content. Generally, the moisture content can be influenced by the ratio of wall material (protein) to core material (oil). In our study, both PP or LP combined with WP at 1:2 showed a significantly lower moisture content. The higher moisture content in LP + WP compositions could be attributed to the properties of the different wall materials (i.e., nature of proteins) used and their ability to retain moisture (Zhao et al., 2022). The lower moisture content is one of the major parameters that maintain the quality of the encapsulated oil, contributing significantly to the preservation of its quality and extending its shelf-life (Selim et al., 2021). However, determination of moisture content is insufficient to assess the stability of dried food formulations as different perishability levels can be observed even among foods with similar moisture content (Nielsen, 2010). The findings further revealed that all microcapsules had low water activity, which could be attributed to the effective water evaporation during freeze-drying (Kerr, 2013). Moreover, the low water activity ( $\leq 0.37$ ) of samples suggested that the samples were less prone to microbial spoilage and have a lower risk of microbial growth (Allen, 2018). The differences in solubility could be ascribed to encapsulating materials that can form a solid matrix around the oil droplets, thereby increasing their dispersibility in water. A study by Zhao et al. (2022) studied the solubility *Cornus officinalis* flavonoids microcapsules using protein/maltodextrin combinations as wall material. The study findings indicated that the use of a protein/maltodextrin system as the wall material resulted in an enhancement of the solubility of *C. officinalis* flavonoids. In our study, the combination of PP or LP with WP can affect the solubility of the encapsulated oils. Overall, these findings revealed that combining plant proteins (LP or PP) with animal proteins (WP) holds promise as a viable option for encapsulating mānuka essential oil. This combination demonstrated the ability to achieve reduced moisture content and low water activity of mānuka essential oil for various applications.

#### 3.3. Encapsulation efficiency, swelling rate, and acid value

Encapsulation efficiency, swelling rate, and acid value of mānuka essential oil microcapsules are presented in Table 1B. The encapsulation efficiency varied based on different protein/oil material ratio and the combination of proteins used in the encapsulation process. The highest encapsulation efficiencies were achieved when the protein/oil material ratio was 1:1. Both PP + WP1 and LP + WP1 (i.e., protein/oil ratio of

**Table 1B**  
Encapsulation efficiency, swelling rate, and acid value of mānuka essential oil microcapsules<sup>1</sup>

Samples	Encapsulation efficiency (%)	Swelling rate	Acid value (mg KOH/g)
PP + WP1	90.39 $\pm$ 3.37 <sup>e</sup>	2.35 $\pm$ 0.04 <sup>c</sup>	9.06 $\pm$ 0.70 <sup>c</sup>
PP + WP2	78.62 $\pm$ 1.68 <sup>d</sup>	1.59 $\pm$ 0.02 <sup>a</sup>	8.79 $\pm$ 0.32 <sup>b</sup>
PP + WP3	66.95 $\pm$ 2.08 <sup>a</sup>	1.63 $\pm$ 0.01 <sup>a</sup>	8.41 $\pm$ 0.56 <sup>a</sup>
LP + WP1	89.42 $\pm$ 2.24 <sup>e</sup>	2.11 $\pm$ 0.01 <sup>b</sup>	10.28 $\pm$ 0.85 <sup>e</sup>
LP + WP2	74.20 $\pm$ 0.53 <sup>c</sup>	2.20 $\pm$ 0.01 <sup>b</sup>	8.45 $\pm$ 0.56 <sup>a</sup>
LP + WP3	70.73 $\pm$ 0.30 <sup>b</sup>	2.19 $\pm$ 0.01 <sup>b</sup>	9.35 $\pm$ 0.32 <sup>d</sup>

<sup>1</sup>Data were expressed as mean  $\pm$  standard deviation. The mean values sharing different lower-case superscripts (a-e) within the column represented statistically significant ( $p < 0.05$ ) differences based on MANOVA and Duncan's multiple range tests. For sample codes, refer to section 2.3.

1:1) demonstrated the highest encapsulation efficiencies (90.4 and 89.4 %), respectively. This indicated that an equal proportion of protein to oil ratio led to an improved encapsulation efficiency. As the protein ratio increases to 2:1 and 3:1, the encapsulation efficiency of samples decreased for both protein mixtures. These results agreed with the findings of [Le Priol et al. \(2019\)](#), in which authors reported the encapsulation efficiency of 69–91 % for the encapsulated soybean oil using different plant proteins. Similarly, studies by [Selim et al. \(2021\)](#) and [Avramenko et al. \(2016\)](#) observed the differences in encapsulation efficiency of fish and flaxseed oil, respectively, with change in wall material (maltodextrin, gum Arabic, and whey protein; unhydrolyzed or hydrolyzed lentil protein isolate). The differences in encapsulation efficiency between the PP + WP and LP + WP samples could be attributed to the unique properties and interactions of the PP or LP with WP. PP and LP may exhibit different characteristics in terms of molecular weight, structure, particle size, surface charge, hydrophobicity, and ability to form a stable encapsulating matrix. These properties can influence the encapsulation process and affinity for encapsulated oil.

Samples with a 1:1 protein/oil material ratio (PP + WP1 and LP + WP1) have relatively higher swelling rate values compared to those with higher ratio (2:1 and 3:1) ([Table 1B](#)). As the wall material ratio progressed to 2:1 and 3:1, the swelling rate of samples decreased. This indicated that an equal proportion of protein/oil material ratio leads to increased swelling of the encapsulating material, while the higher proportion of the protein (wall material) in relation to the oil (core material) may limit the ability of the matrix to absorb water effectively, resulting in reduced swelling. However, the ratio of 1:1 promoted the formation of an encapsulating matrix with a high affinity for water absorption, which could be ascribed to the balanced interaction between the proteins and the surrounding medium (i.e., water) ([Loo et al., 2021](#)). These results were slightly higher than the findings of [Singh et al. \(2018\)](#), who reported a swelling rate of 0.911 to 0.959 for  $\alpha$ -tocopherol microcapsules using pectin and sodium alginate.

On the other hand, the acid values varied among the different samples ([Table 1B](#)). Generally, the acid value is a measure of the amount of free fatty acids present in a substance. It is a crucial parameter in assessing the quality and freshness of fats and oils, particularly in food and industrial applications. As the ratio increased, the acid values generally decreased for both PP + WP and LP + WP combinations. The variation in their acid values could be attributed to the inherent characteristics of these proteins. For example, our study reported the lower acid value for PP + WP than LP + WP combinations, indicating the effectiveness of PP + WP as wall materials in ensuring the stability of encapsulated mānuka essential oils due to their ability to form thicker interfacial protein films at the droplet interface.

Overall, these findings highlighted the importance of the protein/oil

material ratio and the combination of specific proteins in determining the encapsulation efficiency, swelling rate, and acid value of mānuka essential oil. The 1:1 ratio of protein/oil demonstrated higher encapsulation efficiencies and improved swelling rate compared to higher wall material ratio. Moreover, choice of protein combination and the ratio of protein/oil material can significantly influence the oxidative stability of the system, which should be optimized for the development of efficient encapsulation systems for mānuka essential oil.

#### 3.4. Total phenol contents and antioxidant activity

Total phenolic and flavonoid contents, and antioxidant activity of mānuka essential oil microcapsules are represented in [Table 1C](#). Total phenolic content (TPC) of all samples varied from 3.2 to 9.1 mg GAE/g. For the PP + WP and LP + WP combinations, there was an increase in the TPC compared to PP, WP, and LP alone. The combination of PP + WP1 displayed a TPC of 8.5 mg GAE/g, which decreased by 1.07-fold when the protein/oil ratio shifted to 3:1. A similar trend was observed for the LP + WP combinations, with the decreased TPC as the protein/oil ratio increased. This indicates that a higher proportion of protein in the emulsion may lead to a slight reduction in the TPC. Similar trend was followed for total flavonoid content (TFC) of mānuka essential oil microcapsules. For instance, the TFC for LP + WP3 combination at a 3:1 ratio was significantly ( $p < 0.05$ ) lower (0.80 mg QE/g) than the other combinations. Similarly, PP + WP2 and PP + WP3 combinations exhibited a significantly decreased TFC compared to PP + WP1, suggesting that an increased proportion of the protein material may slightly decrease the TFC.

On the other hand, the antioxidant activity of mānuka essential oil microcapsules varied from 54.7 to 87.0 % ([Table 1C](#)). Among the samples, PP + WP1 exhibited the highest antioxidant activity at 87.0 %, followed by PP + WP2 at 80.8 %. PP + WP1 had a slightly lower antioxidant activity at 74.2 %. In the LP + WP-based combinations, LP + WP1 showed an antioxidant activity of 77.7 %, while LP + WP2 and LP + WP3 had values of 70.5 % and 62.1 %, respectively. As expected, the observed antioxidant activity pattern was consistent with the TPC and TFC of the mānuka essential oil microcapsules, indicating a potential proportionality between the antioxidant activity and the quantity of oil encapsulated in the microcapsules. A study by [Ez-zoubi et al. \(2023\)](#) revealed a direct and positive relationship between the TPC of the plant extract and its antioxidant activity. Moreover, these findings were in line with the observed pattern in encapsulation efficiency, indicating that the oil material present in the microcapsules played a significant role in demonstrating the ability to scavenge DPPH radicals ([Moser et al., 2017](#); [Zhao et al., 2022](#)). Therefore, these results indicated that the choice of protein combination and protein/oil material ratio significantly affected the antioxidant activity of the mānuka essential oil microcapsules. Protein/oil ratio at 1:1 exhibited greater antioxidant potential compared to another ratio. Overall, these findings highlighted the potential of using pea protein/lupine-based combinations with whey protein at specific protein/oil ratio to develop microcapsules with improved antioxidant properties.

#### 3.5. Oxidative stability

The TBARS values of the mānuka essential oil microcapsules over a storage of 90 days are illustrated in [Fig. 2](#). At the beginning of the storage period (0 days), the control sample (free mānuka essential oil) had a significantly higher TBARS value of 2.77 mg MDA/L oil compared to PP + WP and LP + WP combination microcapsules. As the storage time increased, there was a progressive increase in TBARS values of all samples, indicating an increase in lipid peroxidation. However, the TBARS values of PP + WP and LP + WP combinations exhibited a slower rate of lipid peroxidation over the storage compared to control, which could be related to the encapsulation process that prevented the susceptibility of the samples to oxidative degradation. By the end of the

**Table 1C**

Total phenolic and flavonoid contents, and antioxidant activity of mānuka essential oil microcapsules<sup>1</sup>

Samples	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH activity (%)
PP (control)	7.60 ± 4.77 <sup>f</sup>	1.61 ± 0.10 <sup>e</sup>	59.03 ± 6.10 <sup>b</sup>
WP (control)	3.24 ± 1.63 <sup>a</sup>	0.90 ± 0.12 <sup>c</sup>	60.58 ± 8.44 <sup>b</sup>
LP (control)	4.46 ± 0.94 <sup>b</sup>	0.86 ± 0.03 <sup>b</sup>	54.68 ± 7.52 <sup>a</sup>
Mānuka essential oil	6.29 ± 0.79 <sup>d</sup>	2.06 ± 0.10 <sup>f</sup>	82.92 ± 3.76 <sup>g</sup>
PP + WP1	8.45 ± 1.01 <sup>h</sup>	2.65 ± 1.07 <sup>h</sup>	86.96 ± 3.35 <sup>h</sup>
PP + WP2	8.45 ± 0.78 <sup>h</sup>	2.35 ± 0.64 <sup>g</sup>	80.75 ± 1.07 <sup>g</sup>
PP + WP3	7.84 ± 1.61 <sup>g</sup>	2.35 ± 0.64 <sup>g</sup>	74.23 ± 1.07 <sup>e</sup>
LP + WP1	7.08 ± 0.95 <sup>e</sup>	1.66 ± 0.02 <sup>e</sup>	77.65 ± 1.61 <sup>f</sup>
LP + WP2	9.10 ± 0.18 <sup>h</sup>	1.53 ± 0.10 <sup>d</sup>	70.51 ± 2.68 <sup>d</sup>
LP + WP3	6.04 ± 0.40 <sup>c</sup>	0.80 ± 0.02 <sup>a</sup>	62.13 ± 2.84 <sup>c</sup>

<sup>1</sup>Data were expressed as mean ± standard deviation. The mean values sharing different lower-case superscripts (a-h) within the column represented statistically significant ( $p < 0.05$ ) differences based on MANOVA and Duncan's multiple range tests. TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl, GAE, gallic acid equivalent; QE, quercetin equivalent. For sample codes, refer to [section 2.3](#).

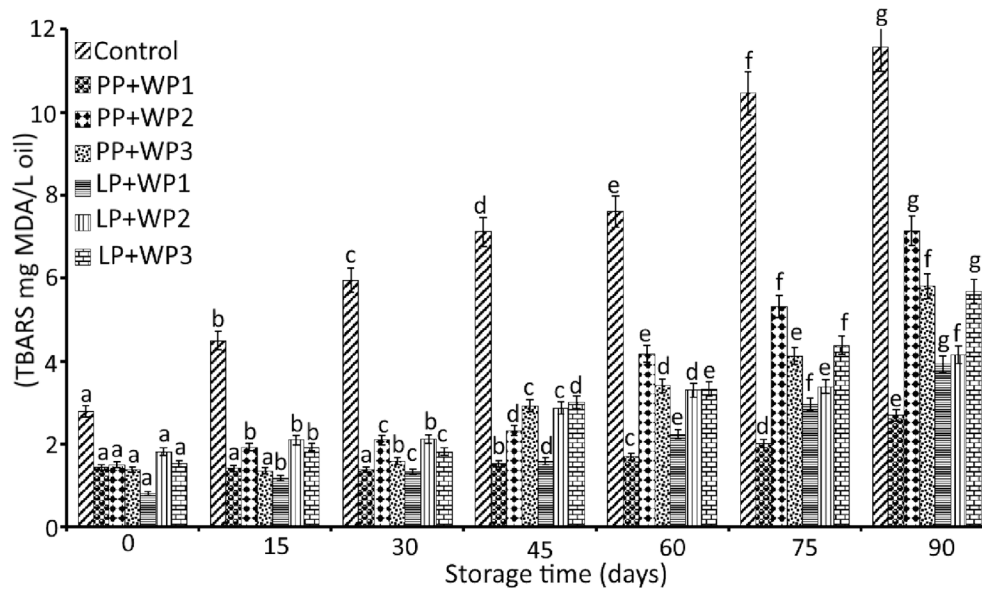


Fig. 2. TBARS values of mānuka essential oil microcapsules incubated at 25 °C during 90 days storage. Error bar represents the standard deviation from the mean of three independent replications ( $n = 3$ ). Different lowercase letters (from a to g) among the storage time (0–90 days) represent significant differences ( $p < 0.01$ ) based on MANOVA and Duncan's multiple range tests. TBARS, thiobarbituric acid reactive substances. For sample codes, refer to section 2.3.

storage period (90 days), the control sample exhibited the highest TBARS value of 11.56 mg MDA/L oil. This indicated a significant level of lipid peroxidation and oxidative damage, suggesting that the samples experienced substantial oxidative deterioration over the course of 90 days.

On day 0, PP + WP1 samples showed insignificant ( $p > 0.05$ ) differences in TBARS values of mānuka essential oil microcapsules. As the storage time increased to 75 days, PP + WP1 samples showed an increased rate of lipid peroxidation, which was 1.40-fold higher oxidation than the initial stages of storage. For LP + WP samples, the rate of lipid peroxidation appeared to be relatively slow (0.80 mg MDA/L oil) during the initial stage of storage, as indicated by the gradual increase in TBARS values up to the day 30. However, after 30 days, there was a gradual acceleration in the rate of lipid peroxidation, as evidenced by the larger increments in TBARS values between subsequent storage days. Comparatively, PP + WP1 and LP + WP1 samples exhibited lower TBARS values (0.80–3.92 mg MDA/L oil), suggesting a potential protective effect against lipid oxidation. By the end of the storage period (90 days), the control sample exhibited the highest TBARS value (11.96 mg MDA/L oil), while PP + WP1 and LP + WP1 consistently displayed the lowest TBARS values (<3.92 mg MDA/L oil), which indicated its superior antioxidant capacity in preventing lipid peroxidation. Interestingly, as the protein/oil material ratio increased, there was an indefinite trend in TBARS values across most PP or LP combinations with WP. A study by Avramenko et al. (2016) investigated the oxidative stability of flaxseed oil microcapsules using lentil proteins as wall material. In this study, the TBARS values of flaxseed oil microcapsules varied from 0.72 to 1 mg MDA/L oil over 35 days of storage, which was in line with our findings after day 30. Another study by Wang et al. (2018) reported the increased TBARS values for free oil compared to encapsulated peony seed oil using a combination of whey protein isolate, corn syrup, and soy lecithin. This study concluded the high oxidative stability of encapsulated oil due to antioxidative properties of oil and their ability to bind metals through the involvement of carboxyl groups found in amino acids. Furthermore, these findings align with the observed pattern in encapsulation efficiency, total phenolics, and antioxidant activity, suggesting that the oil contained within the microcapsules played a crucial role in showcasing its capacity to effectively scavenge free radicals during storage (Moser et al., 2017; Zhao et al., 2022). In accordance with the present results, an earlier study

Table 2  
Color of mānuka essential oil microcapsules<sup>1</sup>

Samples	Color parameters			
	L*	a*	b*	ΔE
PP + WP (Control)	58.12 ± 3.53 <sup>f</sup>	0.94 ± 0.13 <sup>d</sup>	13.09 ± 1.14 <sup>b</sup>	–
LP + WP (Control)	57.41 ± 0.60 <sup>e</sup>	1.03 ± 0.13 <sup>d</sup>	18.76 ± 0.08 <sup>e</sup>	–
PP + WP1	49.60 ± 1.85 <sup>a</sup>	0.49 ± 0.07 <sup>c</sup>	8.15 ± 0.91 <sup>a</sup>	9.87 ± 2.51 <sup>e</sup>
PP + WP2	48.54 ± 1.70 <sup>a</sup>	0.50 ± 0.01 <sup>b</sup>	8.01 ± 0.22 <sup>a</sup>	10.85 ± 2.78 <sup>f</sup>
PP + WP3	51.94 ± 0.41 <sup>c</sup>	0.48 ± 0.05 <sup>b</sup>	7.89 ± 0.19 <sup>a</sup>	8.13 ± 3.26 <sup>d</sup>
LP + WP1	57.40 ± 0.89 <sup>c</sup>	−0.23 ± 0.11 <sup>a</sup>	15.63 ± 0.13 <sup>c</sup>	3.38 ± 0.05 <sup>b</sup>
LP + WP2	55.51 ± 0.42 <sup>d</sup>	−0.24 ± 0.03 <sup>a</sup>	15.56 ± 0.23 <sup>c</sup>	3.98 ± 0.62 <sup>c</sup>
LP + WP3	57.03 ± 0.22 <sup>e</sup>	−0.35 ± 0.01 <sup>a</sup>	16.01 ± 0.27 <sup>d</sup>	3.17 ± 0.20 <sup>a</sup>

<sup>1</sup>Data were expressed as mean ± standard deviation. The mean values sharing different lower-case superscripts (a-f) within the column represented statistically significant ( $p < 0.05$ ) differences based on MANOVA and Duncan's multiple range tests. L\*, a\*, and b\* are differences in lightness, red intensity, and yellow intensity, respectively; ΔE, total color difference. For sample codes, refer to section 2.3.

demonstrated the lower TBARS values for encapsulated oil using whey protein compared to free oil over 50 days of storage (Selim et al., 2021). Overall, the findings suggested that the protein combinations and protein/oil material ratios influence the oxidative stability of the microcapsules. The presence of plant-animal protein combinations, particularly differences in pea or lupine proteins may contribute to better antioxidant activity and protection against lipid oxidation of mānuka essential oil microcapsules during storage.

### 3.6. Color analysis

The color parameters of mānuka essential oil microcapsules for different protein combinations are shown in Table 2. The L\* value for mānuka essential oil microcapsules prepared with PP with WP was



significantly lower than the  $L^*$  value of the control sample (PP + WP and LP + WP). The  $L^*$  values for PP + WP1, PP + WP2, and PP + WP3 were significantly lower (i.e., darker color) compared to the control group (PP + WP). However, LP + WP1 and LP + WP3 had  $L^*$  values similar to the control group (LP + WP), indicating comparable lightness levels. The darker color observed in PP + WP than in LP + WP combinations may be attributed to the characteristics of pea proteins, as they tend to have a slightly darker color compared to lupine proteins (Rivera et al., 2022). Moreover, lupine proteins might have a lesser impact on the overall color of the microcapsules. For  $a^*$  values, microcapsules prepared using PP + WP and LP + WP combinations significantly ( $p < 0.05$ ) exhibited a different red-green color compared to the control groups (PP + WP and LP + WP). For instance, the addition of oil led to a reduction in  $a^*$  values for PP + WP with no notable impact of the protein/oil ratio. Likewise, a similar trend was observed in LP + WP, where the decrease in  $a^*$  values was more pronounced. The LP + WP1, LP + WP2, and LP + WP3 samples exhibited a shift towards the green color (negative  $a^*$ ) with statistically insignificant ( $p > 0.05$ )  $a^*$  value. The PP + WP and LP + WP combination-based microcapsules exhibited a decrease in the yellow-blue color, as indicated by their lower  $b^*$  values compared to the control. Similarly, variations in  $L^*$  and  $a^*$  values of encapsulated oils were documented by Ogrodowska et al. (2017), Kurek and Pratap-Singh (2020), and Santos et al. (2020) using different plant and animal-based proteins. The effect of changing the protein/oil material ratio on the  $b^*$  values of microcapsules using PP + WP combinations was found to be statistically insignificant ( $p > 0.05$ ). However, among the LP + WP combinations, LP + WP3 showed a relatively lower significant difference ( $p < 0.05$ ) in  $b^*$  value compared to LP + WP2 and LP + WP1, indicating consistent shifts towards the green color across different protein/oil material ratio.

On the other hand,  $\Delta E$  significantly ( $p < 0.03$ ) varied among the samples (Table 2). The PP + WP combinations exhibited variations in  $\Delta E$ , which was  $\geq 8$ -times higher than the control sample. Similarly, the LP + WP combinations also demonstrated variations in  $\Delta E$ , which were found to be  $\geq 3$ -fold higher than the control sample. This implies that the impact on PP + WP systems is noticeable to anyone, whereas for LP + WP systems, only trained experts may observe the difference. However, changing in protein/oil material ratio in both LP or PP with WP samples followed an indefinite trend. These findings also accord with earlier observations that showed the change in  $\Delta E$  for the hempseed (*Cannabis sativa*) oil (Kurek & Pratap-Singh, 2020) and *C. officinalis* flavonoids (Zhao et al., 2022) using plant-based (hemp, pea, and rice) protein-maltodextrin and protein (whey isolate protein, soy isolate protein, and gelatin)-maltodextrin combinations as wall material, respectively. Collectively, the findings highlighted the importance of protein combinations and protein/oil material ratio on the color characteristics of mānuka essential oil microcapsules.

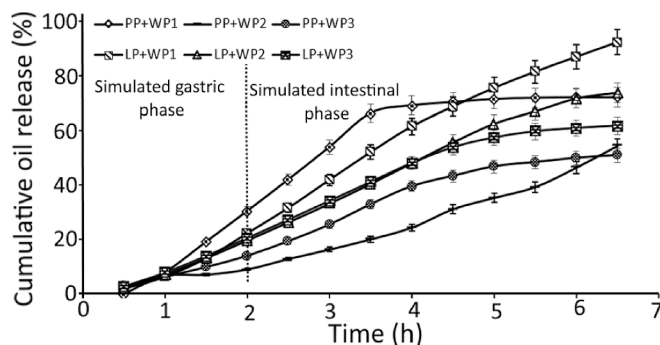


Fig. 3. *In vitro* release profiles of microencapsulated mānuka essential oil. Error bar represents the standard deviation from the mean of three independent replications ( $n = 3$ ). For sample codes, refer to section 2.3.

### 3.7. *In vitro* release and kinetic modelling

*In vitro* release behavior of mānuka essential oil microcapsules is shown in Fig. 3. Following a 2-h digestion in SGF, PP + WP and LP + WP combination mānuka essential oil microcapsules released approximately 9.1 to 30.6 % of the oil, respectively. Upon entering the intestinal digestion phase under SIF, there was a notable increase in oil release from the microcapsules, which was 1.35–5.0.-fold higher cumulative release compared to the *in vitro* release under SGF conditions. This could be the consequences of the enzymes (i.e., pancreatin) present in SIF that digested the microcapsules protein material or increase the permeability of protein material (Wang et al., 2018). Both combinations (PP + WP and LP + WP) showed a steady cumulative increase over the time at 37 °C. The LP + WP1 showed the highest cumulative release of 92.3 %, followed by LP + WP2 (73.9 %) and PP + WP1 (72.0 %), while PP + WP3 exhibited the lowest cumulative release of 50.9 % after 6.50 h in gastro-intestinal conditions. This indicated the dependence of cumulative release behavior on the protein and oil material combinations. PP + WP2 and LP + WP1 microcapsules exhibited monophasic cumulative release profiles, while other PP + WP and LP + WP combinations demonstrated a biphasic cumulative release, which agreed with the findings reported by Kairam et al. (2020) for the microencapsulation of flaxseed and garlic oil hydrogel beads. The gradual release observed in the presence of SGF could be attributed to the potential coalescence or flocculation effects resulting from the sudden exposure to gastric enzymes at a low pH (Wang et al., 2018). Our findings were consistent with the studies conducted by Wang et al. (2018), Kairam et al. (2020), and Kurek and Pratap-Singh (2020), in which authors concluded the initial fast release followed by a delayed release of encapsulated oil microcapsules using plant-animal proteins as the wall matrix. In general, the release behavior of microcapsules is influenced by several factors, including the properties of the wall material (protein), core material (oil), and their ratio. The protein and oil materials can affect the permeability and release kinetics of the encapsulated oils. The differences in release profile could be attributed to diffusion of the encapsulated oil through the microcapsule wall, swelling of the wall, and erosion of the wall material. PP or LP with WP have different properties that can affect the stability and permeability of the microcapsule wall. Overall, the protein/oil material ratio of 1:1 provided controlled release over time.

Mānuka essential oil release kinetics were evaluated based on the experimental cumulative % oil release vs time and fitted with four kinetic models (i.e., zero order, first order, Higuchi, and Korsmeyer–Peppas models) as shown in Table 3. The findings revealed an insignificant decrease for the release rate constant ( $k$ ) across all four models, indicating the variability in the release rate of oil from the protein matrix. However, it is worth noting that the other model parameter(s) exhibited no definitive trend with respect to change in time or protein/oil material ratio. Generally, ' $k$ ' can change over time due to various factors, including the protein/oil material ratio, core material (oil) properties, release mechanism, and environmental conditions. For example, the ' $k$ ' may decrease over time as the diffusion pathway becomes more restricted (Ariyanto & Yoshii, 2019).

Recently, many computational modeling studies have emerged as an innovative approach to gain a deeper understanding of the coefficients in kinetic models. To determine the most appropriate kinetic model, a combination of higher  $R^2$  (coefficient of determination) and model selection criterion (MSC, which must be  $\geq 2$  to 3 for a good fit) values were considered. The values of  $R^2$  and MSC were observed in the range of 0.676–0.991 and 0.974–4.505, respectively for all four kinetic models. Among the kinetic models, the zero order exhibited a superior fit with high  $R^2$  values ( $\geq 0.966$ ) and MSC values of  $\geq 3.225$  for the PP + WP3, LP + WP1, LP + WP2, and LP + WP3 microcapsules. For the PP + WP1 and PP + WP2 microcapsules, the first order and Korsmeyer–Peppas model, respectively, demonstrated a good fit (Table 3). However, it was found that the Korsmeyer–Peppas model provided the best fit ( $R^2 =$

**Table 3**  
Parameters of kinetic models for the mānuka essential oil release<sup>1</sup>

Samples	Kinetic models												
	Zero order			First order			Higuchi model			Korsmeyer–Peppas model			
	R <sup>2</sup>	k	MSC	R <sup>2</sup>	k	MSC	R <sup>2</sup>	k	MSC	R <sup>2</sup>	k	n	MSC
PP + WP1	0.843	13.887	1.702	0.917	0.232	2.334	0.806	29.340	1.489	0.874	19.954	0.769	1.852
PP + WP2	0.936	7.097	2.602	0.882	0.085	1.985	0.676	14.417	0.974	0.991	3.298	1.479	4.505
PP + WP3	0.966	8.676	3.233	0.954	0.112	2.930	0.789	17.998	1.404	0.963	8.629	1.003	3.079
LP + WP1	0.977	14.476	3.627	0.876	0.227	1.935	0.758	29.757	1.268	0.983	11.680	1.136	3.868
LP + WP2	0.984	11.771	3.991	0.922	0.168	2.396	0.771	24.249	1.321	0.988	9.823	1.114	4.260
LP + WP3	0.970	10.763	3.225	0.961	0.151	3.090	0.818	22.440	1.551	0.966	11.967	0.933	3.150

<sup>1</sup>R<sup>2</sup>, coefficient of determination; k, release rate constant; n, diffusional release exponent (0.50, Fickian diffusion; 0.50 < n < 1, anomalous (non-Fickian) diffusion; 1, case-II transport; >1, super case-II transport); MSC, model selection criterion (≥2 to 3 indicates a good fit). For sample codes, refer to section 2.3.

0.966–0.988 and MSC = 3.150–4.260) for describing the release of mānuka essential oil using LP + WP combinations. Based on the models, oil release rate of LP + WP and PP + WP3 combinations was found to be independent of the oil concentration, while PP + WP1 combinations exhibited the concentration-dependent (i.e., release rate decreases exponentially over time) diffusion. Moreover, LP + WP combinations and PP + WP2 release mechanism was governed by the wide range of release profiles, which was confirmed by the Korsmeyer–Peppas model's release exponent (n). The findings of this study agreed with previous research that concluded the bioactive compounds release profiles can be effectively predicted using the zero order and Korsmeyer–Peppas models (Nasri et al., 2020). Thus, zero order, first order, and the Korsmeyer–Peppas models were recommended as the most suitable model for characterizing the release of mānuka essential oil based on its superior fit to the experimental data.

#### 4. Conclusions

The present study was designed to evaluate plant-animal protein mixed systems as a wall material for microencapsulation of mānuka essential oil and studied its physicochemical properties and *in vitro* release profile. Mānuka essential oil microcapsules had low moisture content, low water activity, and good solubility. The color of the microcapsules was affected by the ratio of the wall materials. The microcapsules prepared with a 1:1 protein/oil material ratio exhibited high encapsulation efficiency for both protein mixed systems (PP + WP and LP + WP). The microcapsules demonstrated low lipid oxidation values, indicating high oxidative stability and antioxidant activity. The release behavior of the encapsulated oil was described by the zero order and Korsmeyer–Peppas models, which varied depending on the type and concentration of the protein mix used. Overall, the protein mixed systems (pea/lupine with whey proteins) successfully encapsulated mānuka essential oil, providing controlled release and high oxidative stability. Further research on the long-term storage studies under various storage conditions (temperature, humidity, light exposure), *in vivo* release behavior, and transitioning from laboratory-scale production to pilot-scale or industrial-scale manufacturing processes would be necessary for market acceptance and commercialization. These findings offer valuable insights into the potential application of plant-animal protein mixed systems as innovative carriers for the encapsulation of bioactive compounds, which will support the growing demand for flexitarian diets in the consumer market.

#### CRedit authorship contribution statement

**Kandi Sridhar:** Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pascaline Hamon:** Writing – review & editing, Validation, Software. **Jordane Ossemond:** Writing – review & editing, Validation. **Said Bouhallab:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Conceptualization. **Thomas**

**Croguennec:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Denis Renard:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Valérie Lechevalier:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, 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.

#### Data availability

Data will be made available on request.

#### Acknowledgments

This project, MicroEncapsulation Legume prOteiNs (MELON), has received funding from the European Union's Horizon 2020 research and programme under the Marie Skłodowska-Curie grant agreement No 899546.

#### Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2024.114419>.

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