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Sustainability Spotlight Statement

This study advances sustainable food innovation by valorizing pandan leaves—a widely available but underutilized botanical—through optimized ultrasound-assisted extraction and eco-friendly microencapsulation. By employing food-grade carriers and energy-efficient processes, it offers a scalable approach to producing stable, bioactive ingredients for functional food applications, reducing reliance on synthetic additives while promoting natural resource utilization and waste minimization.

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Sustainable Production of Bioactive Pandan Leaf Extract Microcapsules via Optimized Ultrasound-Assisted Extraction and Spray Drying

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Abstract

Pandan (*Pandanus amaryllifolius* Roxb.) leaves are a promising source of bioactive compounds, yet their application in functional foods remains limited due to the lack of optimized extraction and stabilization techniques. This study employed response surface methodology (RSM) to optimize ultrasound-assisted extraction (UAE) of pandan leaf extract (PLE), targeting maximal recovery of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH and FRAP assays). Optimal UAE conditions—60% ethanol, 40% amplitude, and 20-minute sonication—yielded extracts with significant antioxidant and antibacterial activities, with inhibition zones of 27 mm (*Bacillus cereus*), 29 mm (*Staphylococcus aureus*), and 18 mm (*Escherichia coli*). To enhance stability and facilitate incorporation into functional foods, the PLE was microencapsulated via spray drying using gum arabic (GA), resistant maltodextrin (RMD), and their mixture (GRMD) in 1:1 and 1:2 ratios. The GRMD (1:1) formulation exhibited superior encapsulation efficiency (93.03%), bioactive retention, low water activity, and high solubility. Over 90 days of storage, microcapsules packed in vacuum-sealed aluminum foil laminated bags retained the highest bioactivity, with final TPC, TFC, DPPH, and FRAP values of 157.91 mg GAE/100g db, 21.49 mg QE/100g db, 324.75 mM Trolox/100g db, and 444.84 mM Trolox/100g db, respectively ($p \leq 0.05$). This study demonstrates the potential of combining UAE and GRMD microencapsulation using spray drying to sustainably produce stable, bioactive pandan leaf extract microcapsules to use in functional food systems. Further studies are recommended to assess sensory acceptance, bioaccessibility, and scale-up potential.

Keywords: Antioxidant activity, Antibacterial properties, Bioactive compounds, Microencapsulation, Response surface methodology, Storage stability.

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1. Introduction

The growing consumer demand for functional foods enriched with bioactive compounds has spurred interest in plant-derived ingredients with health-promoting properties. Pandan (*Pandanus amaryllifolius Roxb*.), a tropical plant from the Pandanaceae family, is widely valued in Southeast Asia for its aromatic leaves, which impart a distinctive flavor to culinary dishes. Beyond its culinary applications, pandan leaves are recognized for their medicinal properties, including diuretic, cardiotonic, anti-diabetic, and skin-healing effects, attributed to bioactive compounds such as phenolic compounds, flavonoids, alkaloids, glycosides, and tannins [1]. Not only that, but the leaf is also valued for its ability to freshen the body, relieve fever, and indigestion. They contain a wide range of alkaloids as well as the unglycosylated protein, which has antiviral effect. These compounds exhibit antioxidant, antibacterial, and anticancer activities, making pandan a promising candidate for functional food development [2, 3].

Bioactive chemicals are naturally occurring, additional nutritional components that have biological properties. Bioactive compounds may occur in many foods and foodstuffs derived from plant, animal, and aquatic sources. These meals may offer advantages beyond supplying the required nutrition because of the bioactive compounds they contain. Because they are capable of controlling one or more significant metabolic processes and physiological activities, many substances have favourable health benefits. Bioactive compounds play essential roles in maintaining health and preventing disease [4].

Extracting bioactive compounds from plants traditionally involves methods like Soxhlet, maceration, and hydro-distillation, which are energy-intensive and time-consuming due to prolonged heating and solvent use. In contrast, ultrasound-assisted extraction (UAE) offers an efficient, sustainable alternative by enhancing mass transfer through acoustic cavitation, reducing extraction time and solvent consumption [5]. Operating at ultrasonic frequencies between 20 kHz and 100 MHz, UAE is recognized as an eco-friendly and energy-efficient alternative to traditional extraction techniques. To optimize extraction process, response surface methodology (RSM) is widely employed. RSM combines statistical and mathematical tools, using second-degree polynomials to model relationships among multiple interacting variables [6].

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However, the stability, bioactivity, and bioavailability of these bioactive substances can be compromised by storage conditions such as exposure to light, temperature, humidity, and oxygen. These biologically active substances could be encapsulated to regulate their release, stabilize their bioactivities, and increase their bioavailability. These components have the potential to replace a variety of synthetic supplements and additives. However, comprehensive analysis and study in the lab are needed to fully exploit these molecules. To enhance the stability and bioavailability of bioactive compounds, microencapsulation via spray drying is an effective strategy. Encapsulating agents like gum arabic (GA) and resistant maltodextrin (RMD) protect sensitive compounds from environmental degradation, improving their shelf life and controlled release in food applications [7, 8]. However, the literature lacks comprehensive studies on UAE optimization, microencapsulation, and storage stability of pandan leaf extract (PLE). Additionally, the influence of packaging materials and storage conditions on the retention of PLE bioactivity remains underexplored.

For bioactive compounds to exert beneficial biological effects, phenolic substances must be released from the food matrix and become bioaccessible in the gut. In vitro gastrointestinal digestion (GID) models stimulate this process by assessing the release and transformation of phenolics during digestion. Bioaccessibility refers to the fraction of a nutrient released from the food matrix during digestion that is available for absorption in the small intestine [9].

Pandan leaves contain several bioactive compounds, including phenolics, alkaloids, flavonoids, and tannins, which have shown antibacterial, anticancer, and antipyretic activities. Despite evidence of their richness in phenolic compounds, studies on pandan leaf extraction using UAE, encapsulation of the extract, and its bioaccessibility after gastrointestinal digestion remain limited.

This study aims to: (1) optimize UAE conditions for PLE using RSM to maximize TPC, TFC, and antioxidant activity (DPPH and FRAP); (2) evaluate the physicochemical and biological properties of PLE microcapsules prepared with GA, RMD, and their combination (GRMD); and (3) assess the storage stability of microcapsules under different packaging materials (aluminum foil laminated bags vs. HDPE) and conditions (vacuum vs. atmospheric).

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The findings will contribute to the development of PLE as a functional food ingredient with enhanced stability and bioactivity for industrial applications.

2. Materials and Methods

2.1 Sample Preparation

Fresh pandan leaf samples from a local supplier were ordered and brought to the laboratory at Chulalongkorn University in Bangkok, Thailand. The pandan leaves were washed with clean cold water to remove any dirt or dust, and dried in a hot air oven (Memmert, DO 6062, Memmert GmbH & Co. KG, Dchwabach, Germany) for 24 h at 60 °C to adjust the moisture content to < 5%. The powder was sieved through 50-mesh sieve and then stored in aluminum-laminated bags at −20 °C for further experiments.

2.2 Ultrasound-assisted Extraction (UAE) and Optimization of Pandan Leaf Extract

Pandan leaf extract (PLE) was extracted according to the method described by [10] with slight modifications. 3g of dried pandan leaf (PL) powder sample was mixed with 100 mL of solvent (mixture of ethanol and distilled water) and then conducted the extraction at various extraction conditions. The extract was centrifuged at 6000 rpm for 15 min at room temperature (Centrifuge Kubota, series 6000, Osaka, Japan) and separated by rotary evaporator (Oilbath B-485, BUCHI, Flawil, Switzerland) at 45 °C to get ethanol-free pandan leaf extract (PLE). The concentrated extract was adjusted to 10 mL with distilled water and stored in brown bottle at 4 °C for further analysis. Response surface methodology (RSM) with a Box–Behnken Design (BBD) was used in this research (Table 1) for the optimization of the extraction condition with different independent variables (A, ethanol concentration; B, amplitude; C, extraction time) at three levels (−1, 0, 1). The responses were the total phenolic compound (TPC), total flavonoid content (TFC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP). The operational conditions for UAE in pandan leaf extract (PLE) were optimized using BBD with 17 experimental runs generated by RSM, which included ethanol concentration (A: 40–80%), amplitude (B: 30–50 %), and time (C: 10–30 min) (Table 2).

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2.3 Antimicrobial Activity Determination of Dried Sample

2.3.1. Growth Conditions

Microbial cultures of *Staphylococcus aureus (S. aureus* ATCC 25923), *Escherichia coli (E. coli* ATCC 25922), and *Bacillus cereus* were obtained from Faculty of Science, Chulalongkorn University. Each microorganism's inoculum was generated by inoculating 10 mL of sterile growing medium (Muller Hinton Broth) (MHB) and Muller Hinton Agar (MHA). The culture test tubes were incubated at 37 °C for 18–24 h.

2.3.2. Determination of Inhibition Zones by Disc Diffusion Method

The antibacterial activity of optimized PLE was evaluated using the disc diffusion method described by a published method [8]. Muller Hinton Agar (MHA) was placed to a Petri dish along with the inoculum and gently stirred. After solidification, the disks were soaked in 20 µL of extract and allowed for 30 minutes to spread it on the inoculated agar plates. The inhibition zone was determined using ethanol and chloramphenicol as negative and positive controls, respectively. The plates containing the disks were then incubated for 24 h at 37°C. Following incubation, the inhibition zone for each sample extract was measured and expressed in mm.

2.3.3. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The pathogenic bacterial inoculum was cultured at 37°C for 16 h before being diluted to the turbidity of the Mc Farland 0.5 standard. Serial dilutions in sterile Muller Hinton Broth (MHB) were performed to achieve a satisfactory suspension with approximately 1×10^{-6} CFU/mL. Bacterial suspension (50 µL) was introduced to a sterilized 96-well plate containing 100 µL of MHB then extract dilutions of 100 µL were added. A positive control (containing purely microbial inoculum) was injected into each microplate well. The micro-plates were incubated at 37 °C for 24 h. The OD value at 600 nm was measured with a microplate reader. The MIC value indicated no observable growth of the tested bacterial strain.

Streaks were collected from the two lowest concentrations of the PLE extract plates that indicated invisible growth (from the inhibition zone of the MIC plates) and sub-cultured onto

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nutritional agar (NA) plates. The plates were incubated at 37 °C for 24 h before being evaluated for bacterial growth at the corresponding plant extract concentration. MBC was defined as the concentration of plant extract that indicated no bacterial growth on freshly infected agar plates.

2.4 Microencapsulation Experiment using Resistant Maltodextrin (RMD) and Gum Arabic (GA)

The RSM-optimized PLE sample was microencapsulated using spray drying technique. RMD, GA, and a mixture of RMD and GA (GRMD) were mixed with PLE (20% w/v in 1:1 and 1:2 w/w ratios) and stirred with a magnetic stirrer at 45–50 °C for 10 minutes to dissolve. The mixed solution was placed in a spray-dryer with rotary atomizer (Mobile Minor Niro-Atomizer, Søborg, Denmark) with an inlet temperature of 160 °C and an outlet temperature of 90 °C, feed temperature below 10 °C, and feed rate 5 mL/min and hot air flow rate 1.54 m^3/min according to the study of [11]. The PLE-encapsulated powders in RMD (RMDM), GA microencapsulated powder (GAM), and GRMD microencapsulated powder (GRMDM) were stored in aluminum-laminated bags and HDPE bags at ambient temperature until further investigation.

2.5 Stability of antioxidant capacity of microcapsules during storage

The best microcapsule which maintained the highest antioxidant activity was selected for the storage stability under various packaging types and conditions. The microcapsules were packaged using High Density Polyethylene (HDPE) and aluminum foil laminated bag sealed under vacuum and atmospheric conditions. The samples were stored at ambient temperature at approximately 25 °C, which reflects typical room temperature conditions, and relative humidity at around 60% for 3 months and conducted the analysis every 15 days.

2.6 Determination of Physicochemical Properties of Spray Dried PLE Microcapsules

The water activity analyzer (MODEL MS1, Novasina, Switzerland) was used to determine the water activity (a_w) of PLE microcapsules. Moisture content (%) was determined using a moisture analyzer (Mettler Toledo, Greifensee, Switzerland). A chroma meter Minolta CR-400 color meter with CIE LAB system (L*, a*, and b*) was used to determine the color values. The microstructure of PLE microcapsules was analyzed by Scanning Electron

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Microscope (SEM) with magnification 1000 times as described by a published method [7]. The encapsulation efficiency (%) was evaluated by [12]. The yield in percent of microencapsulation was calculated by dividing the total fraction in powder by total fraction in feed solution. Water solubility was measured by [13], 1 g of sample powder was mixed with 100 mL of distilled water and stirred at 400 rpm for 4 min using a magnetic stirrer at room temperature. The sample was centrifuged at 4000× g for 4 min. Afterwards, 25 mL of the precipitate was transferred to the pre-weighed plate and placed it in the oven at 105 °C for 5 h. The solubility was determined by dividing the weight of the dried supernatant by the initial powder weight.

2.7 Determination of Bioactive Compounds (TPC and TFC) and Antioxidant Activity (DPPH and FRAP) in PLE Microcapsules

1g of PLE microcapsule was mixed with 10 mL of distilled water and vortexed for 3 min and kept in a hot shaking water bath (30 °C for 30 min). Then, the sample was centrifuged at 4000 rpm for 20 min and collected for the analysis of bioactive compounds [11]. The procedure for the analysis was detailed as follows.

TPC of PL extract and microcapsules were determined using the Folin-Ciocalteau method, as presented by [7]. 0.5 mL of PLE was diluted in 10 mL of distilled water and then combined with 0.5 mL of 10% Folin–Ciocalteau's phenol reagent. After the incubation time of 5 min, 2 mL of 10% (v/v) sodium carbonate was added into the sample solution, vortexed and kept at room temperature for 10 min. The samples were determined at 765 nm wavelength using a spectrophotometry method with gallic acid as the standard.

The aluminum tri-chloride method was used to determine TFC of PL extract and microcapsules [14]. 1 mL of PLE sample was mixed with 1 mL of 2% aluminum chloride solution and then vortexed. After mixing, the solution was placed in the dark room for 30 min. The TFC value was determined at 430 nm using spectrophotometer with quercetin as the standard.

The antioxidant activity was measured by the inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl). 250 µL of sample was added to the test tube with 4.75 mL of DPPH solution and then keep it in the dark room at room temperature for 15 min. The absorbance of the sample was evaluated at 515 nm using methanol as the blank. The difference in absorbance ($A_{difference}$) can be expressed as mM Trolox/ 100g db.

$$A_{difference} = A_{initial} - A_{final}$$

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The antioxidant activity by FRAP assays of the samples was determined according to [15]. Sample (50 µL) was added to the test tube and mixed with 950 µL of FRAP solution, which was then kept at room temperature for 4 min. The absorbance difference ($A_{difference}$) was calculated at 593 nm and expressed in mM Trolox/ 100g db.

$$A_{difference} = A_{final} - A_{initial}$$

2.8 Statistical Analysis

The optimum condition from UAE experiment was obtained using the Design Expert 11 program, the Box-Behnken design showing three-dimensional (3D) model graph. All analyses were measured in triplicates (n = 3) and interpreted with SPSS (Statistical Package for Social Sciences) version 20.0 statistical software. The data were demonstrated using ANOVA and Turkey's test at a significance level of $p \leq 0.05$.

3 Results and Discussion

3.1. Optimization of Bioactive Compounds (total phenolic compounds, total flavonoid compounds, and antioxidant activity) using RSM

Phenolic compounds are the most common secondary metabolites, varying by phylum. According to Table 2, extraction duration of 20 minutes, 80% ethanol concentration, and 50% amplitude resulted in the highest TPC value of PLE, whereas 10 minutes, 60% ethanol concentration, and 50% amplitude resulted in the lowest TPC value. Time, amplitude, and ethanol concentration significantly influenced TPC extraction from PLE ($p \leq 0.05$). ANOVA results (Table 3) show significant linear, quadratic, and interaction effects (A, B, C), with nonsignificant lack of fit and R^2 values between 0.69 to 0.71 indicating moderate to good model fit. The TPC response was modeled by a second-order polynomial considering these variables.

$$TPC = +981.15350 - 5.55963\ Time - 5.42104\ Amplitude - 16.35311\ Conc. + 0.010250\ Time * Amplitude + 0.044411\ Time * Conc. + 0.053125\ Amplitude * Conc. + 0.075491\ Time^2 + 0.023419\ Amplitude^2 + 0.115873\ Conc^2$$

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Figure 1(A-C) depicts 3D surface plots of TPC responses under various extraction conditions. TPC increased with longer sonication times and higher ethanol concentration (40-80%), while amplitude decline initially boosted TPC yield. Longer sonication enhances phenolic release, consistent with [16]. The ethanol-water mixture improves extraction efficiency due to solvent polarity effects, as noted by [17]. Low concentration of ethanol aid phenolic solubility, whereas high concentration of ethanol may denature the protein structure and limit extraction.

Table 2 shows the highest total flavonoid content (TFC) at 10 min extraction time, 40% amplitude, and 40% ethanol concentration, while the lowest TFC occurred at 20 min, 30% amplitude, and 40% ethanol. Table 3 indicates the quadratic model fits well (R^2 = 77.56) and predicts TFC accurately (R^2_{Adj} = 48.72). The second order polynomial model describes the effect of amplitude, time, and ethanol concentration on TFC.

TFC = - 461.63200 – 2.95347 Time + 17.02739 Amplitude + 9.81033 Conc. + 0.187862 Time * Amplitude + 0.042213 Time * Conc. – 0.014885 Amplitude * Conc. – 0.189818 $Time^2$ – 0.252490 $Amplitude^2$ – 0.085641 $Conc^2$

Figure 1 (D-F) shows 3D response plots for total flavonoid content (TFC). Excessive extraction time and intensity can degrade flavonoids beyond optimal levels. Ultrasound enhances extraction by disrupting cell walls and improving solvent penetration. TFC increased with ethanol concentration up to 60% (v/v), then declined at higher concentrations, consistent with previous studies [18]. Moderate ethanol levels facilitate phenolic dissolution, while high ethanol can cause protein degradation, reducing extraction efficiency [7].

Natural antioxidants from medicinal and aromatic plants, rich in polyphenols and flavonoids, are increasingly valued for their health benefits [19]. Ethanol concentration showed significant quadratic effect on total flavonoid content. The second-order polynomial model describes how amplitude, time and ethanol concentration affect antioxidant activity (DPPH) in pandan extract.

DPPH = + 4654.75000 + 4.24040 Time + 5.27545 Amplitude – 98.66261 Conc. – 0.421875 Time * Amplitude + 0.290513 Time * Conc + 0.596875 Amplitude * Conc + 0.107076 $Time^2$ – 0.323951 $Amplitude^2$ + 0.608800 $Conc.^2$

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Figures 1 (G-I) display 3D response plots of time, amplitude, and ethanol concentration on DPPH antioxidant activity. Antioxidant activity increased with longer extraction time, higher amplitude, and ethanol concentration above 60% (v/v). Low ethanol and short extraction led to insufficient extraction, while moderate conditions caused some antioxidant degradation. Higher ethanol levels and longer extraction preserved more antioxidants, boosting DPPH values, consistent with [20].

The linear effect of ethanol concentration, and the cross-interaction effects between amplitude and ethanol concentration variables, followed by the remaining quadratic effect of ethanol concentration applied to PLE samples on FRAP values was statistically significant ($p \leq 0.05$) (Table 3). The equilibrium of the second-order polynomial model indicating the effect of amplitude, time and ethanol concentration on the FRAP values of PLE samples was as follows:

$$\text{FRAP} = +5620.23612 - 44.25127\ \text{Time} - 81.67666\ \text{Amplitude} - 98.31977\ \text{Conc.} + 0.973700\ \text{Time} * \text{Amplitude} + 0.127354\ \text{Time} * \text{Conc.} + 1.02631\ \text{Amplitude} * \text{Conc.} + 0.087893\ \text{Time}^2 + 0.091954\ \text{Amplitude}^2 + 0.541703\ \text{Conc.}^2$$

The lack of fit test for antioxidant activity by FRAP assay was significant ($p \leq 0.05$), indicating a good model fit (Table 3). High R^2 and adjusted R^2 values showed strong agreement between predicted and experimental data. Figures 1(J-L) illustrate that increasing ethanol concentration from 60% to 80% enhanced antioxidant activity, while changes in amplitude had less effect. These findings align with [20], who reported optimal antioxidant extraction with 60 to 80% (v/v) ethanol and adequate extraction time to prevent bioactive degradation.

3.2. Antimicrobial Activity by Disc Diffusion Method, Minimum Inhibitory Concentration (MIC), and Minimum Bacterial Concentration (MBC)

The optimum condition of UAE by RSM results, 60% ethanol concentration, 40 % amplitude, and 20 min extraction time. Based on the optimum result, antibacterial activity of extracted sample by the disk diffusion method and minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) on Gram-positive bacteria (*Bacillus cereus* (*B. cereus*) and *Staphylococcus aureus* (*S. aureus*)) and Gram-negative bacteria (*Escherichia coli* (*E. coli*)) were investigated. Evaluation of antibacterial activity of these plant extracts was recorded in

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Table 4. The results revealed that PLE extract had the ability to inhibit the microbiological growth of food poisoning bacteria with different efficiency. The crude extract inhibited *B. cereus with* inhibition zone of 29 mm, followed by inhibition zone of 27 mm with *S. aureus* and the minimum inhibition zone of 18 mm with *E. coli* plate (Table 4). According to the results, *E. coli* was the most sensitive microorganisms to the crude PLE extract, as determined by the MIC values (Table 4). Gram negative bacterial represent as barriers to foreign substances because they have an outer membrane, strain characteristics and periplasmic region that Gram-positive bacteria may lack these properties. Consistently, the study of [21] demonstrated that *S. aureus* (Gram-positive) has a greater antimicrobial capacity than *E. coli* (Gram-negative). Gram-negative bacteria can cause resistance through alteration in the outer membrane, such as by modifying the hydrophobic character or causing mutations in porins.

MBC was measured by the absence of bacterial growth of the tested strains-streaked form inhibition zone corresponding to their lowest MIC. PLE extract showed potentially bacterial activity against the tested pathogenic bacteria (*B. cereus*, *S. aureus, E. coli*) with MBC of 1.87 mg/mL, 3.75 mg/mL, 7.50 mg/mL. The results of MIC and MBC of the effective plant extracts suggested that PLE sample can be used to control and prevent food borne bacteria and food poisoning diseases. Bacterial strains included in this study were chosen for their importance in food spoilage and food poisoning. The antimicrobial activity observed in our investigation, as well as in previous studies, can be attributed to the presence of phenolic compounds in the tested samples, which are known for their strong antibacterial properties. [22] suggested that antimicrobial components of the plant extracts (terpenoid, alkaloid and phenolic compounds) interact with enzymes and proteins of the microbial cell membrane leading to the disruption. This disruption may trigger a proton flux toward the cell exterior, ultimately causing cell death, or it may inhibit enzymes essential for amino acid biosynthesis.

3.3 Determination of Total Phenolic Compound (TPC), Total Flavonoid Content (TFC), Antioxidant Activity of DPPH and FRAP Assays of GA- and RMD- Based PLE Microcapsules

The determination of total phenolic compound (TPC), total flavonoid content (TFC), and antioxidant activity is of considerable importance in the pharmaceutical, cosmetics and food

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industries. This is because antioxidants play a key role in preventing and treating diseases linked to oxidative stress, as well as protecting products from oxidation and deterioration. The results of the microcapsules exhibited total phenolic compound are ranged from 287.94±1.67 to 292.71±1.26 mg GAE/100g db. The microcapsules generated with the combination of RMD and GA in a 1:1 encapsulation ratio had the greatest TPC value, followed by RMD 1:1 ratio and GA 1:1, GA 1:2, RMD 1:2, and RMD + GA 1:2 (Table 5). Using PLE microcapsules with a mixture of wall materials (GRMD) produced more phenolic compounds in comparison with each microcapsule alone (GA and RMD), exhibiting that they had synergistic effects and provided superior protection to these compounds during the spray-drying process [7]. Similar results were also reported that the effectiveness of using mixture of maltodextrin and different coating material for the phenolic values of microcapsules from spray drying and freeze-drying. This research revealed that mixture of maltodextrin and gum arabic (1:1) had the higher phenolic release rate than the use of individual coating agent [23].

Flavonoids are secondary metabolites mostly found in plants, fruits, and seeds that influence the color, fragrance, and flavor characteristics. Flavonoids have the ability to reduce the accumulation of reactive oxygen species (ROS) via scavenger ROS when they are formed. As a result, these antioxidant compounds play an essential function in plant stress tolerance and beneficial for human health, due to their anti-inflammatory and anti-microbial characteristics. Total flavonoid content can be affected by the type of coating material used and the encapsulation ratio. The values of TFC were ranging from 50.27±1.09 to 63.56±1.07 mg QE/100g db shown in Table 5. The results indicated that higher encapsulation ratio reduced the TFC content of PLE extract microcapsules. This could be attributed to the fact that antioxidant activity decreases when coating material concentration increases. Increasing the amount of maltodextrin and gum arabic, which do not contain any active radicals, results in lowering DPPH free radical scavenging activity [24].

The antioxidant capacity of the encapsulated powders containing PLE was evaluated using DPPH and FRAP assays and the results are shown in Table 5. In accordance with the study of total phenolic compound, the solution obtained from the microcapsules using combination of resistant maltodextrin and gum arabic showed the highest DPPH and FRAP values with 709.12±5.26 mM Trolox equivalent/100 g db and 739.93±2.19 mM Trolox equivalent/100 g db respectively. Many studies highlight that there is a positive correlation between phenolic content

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and antioxidant activity [24]. Many studies have proven that maltodextrins are one of the most efficient and cost-effective wall materials for encapsulating bioactive phytochemicals, even though their poor emulsifying capacity limits their use to the encapsulation of hydrophobic and volatile com- pounds. Therefore, blending maltodextrins with gum arabic and cyclodextrins had better protective effects and antioxidant properties than the other wall materials [25].

3.4 Physicochemical properties of microcapsules

Moisture content and water activity, a_w, play an essential characteristic to forecast food quality and security in terms of microbiological and biochemical processes, as determined by its value [26]. The powder's stability and storage abilities have an impact on the moisture content and water activity. Table 5 shows how different types of coating materials and encapsulating agent ratios affect on the moisture content and water activity, a_w, of PLE microcapsules after spray drying. The microcapsules produced in the present study exhibited a moisture content ranging from 3.41 ± 0.02 % to 4.98 ±0.02 % and water activity with the range between 0.14 ± 0.01 and 0.25 ± 0.05 (Table 5). The a_w values obtained are below the maximum limit of 0.6 needed to ensure the stability of the powders during storage. According to [27], such low moisture and a_w levels are crucial for maintaining the stability of spray-dried powders by limiting microbial activity and oxidative reactions.

The color of spray dried PLE microcapsules of various types and ratios of encapsulating agents were investigated. The results from Table 5 displayed the color values L* (lightness), a* (green - red), and b* (blue - yellow), which ranged from 66.56 ± 0.05 to 85.17 ± 0.04, -4.47 ± 0.03 to -9.95 ± 0.03, and 14.11 ± 0.04 to 30.46 ± 0.03, respectively. PLE microcapsules with RMD at a 1:1 ratio had the highest brightness (85.17 ± 0.04), while those with GA at a 1:2 ratio had the lowest brightness (66.56 ± 0.05). Furthermore, a* denotes greenness (-a*) and red (+a*), and all samples with a* that are negative denote the sample's greenness. The values of a* and b* declined as the concentration of encapsulating material increased, resulting in decreasing in yellow and green tonalities.

The encapsulation efficiency of PLE microcapsules ranged from 78.27 ± 0.04 to 93.03 ± 0.03. Microcapsules with the mixture of resistant maltodextrin and gum arabic 1:1 had the highest encapsulation efficiency of 93.03 ± 0.03 % followed by gum arabic (1:1) with the value of 91.91±0.02. This study was consistent with [23] who observed that using single coating material

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had lower encapsulation efficiency comparing to the mixture of maltodextrin and gum arabic (1:1). For the extraction yield, it was found that the percentage of yield obtained from pandan leaf extract microcapsules was between 63.36 ± 0.34 and 78.44 ± 0.48. The production yield was highest in the GRMD combination (78.44 ± 0.48 %), followed by RMD (70.03 ± 0.13 %) and GA (69.54 ± 0.48 %). Higher yield in GRMD can be attributed to better film-forming properties of the combination, leading to efficient encapsulation and recovery.

The solubility of PLE microcapsules ranged from 73.41 to 92.55 %. Combinations of the carriers RMD and GA (1:1 ratio) proved to be the best combinations and showed the highest solubility of the PLE microcapsules, while the lowest solubility was obtained when GA was used as single carrier. [28] concluded that mixture of MD and GA is preferrable as the carrier for the solubility due to their differences in chemical structures. Maltodextrin contains numerous hydroxyl groups that can stimulate the dissolution, whereas GA has excellent emulsifying capabilities, a highly branched structure, and significant solubility.

The scanning electron micrographs at a magnification of 1000× of GA, RMD, and GRMD based atomized PLE microcapsules are displayed in Figure 2. It was found that microcapsule using GA at a ratio of (1:1) pronounced more dents and crimps compared to those formulated with RMD. On the other hand, microcapsules with GA 1:2 ratio displayed more spherical and smoother surface. Increasing the thickness of the encapsulation layer lead to more uniform outer surface and rounded morphology. Resistant maltodextrin at ratios of (1:1, 1:2) (Figure 14c, d) exhibited slightly wrinkled surfaces compared to the other samples, likely due to its lower solubility. The wrinkling may result from uneven moisture loss during drying process. Microcapsules produced with RMD alone tended to have more wrinkled and shriveled surfaces whereas combining RMD with gum arabic improved the overall morphology and surface smoothness. Furthermore, when mixture of RMD and other coating material showed more noticeable spherical morphology compared to the use of RMD alone; as shown in Figure 2. The current study's observations in morphological characteristics of microcapsules align with those of a recent finding reported by [25] who demonstrated the particle size distribution was more homogenous in the microcapsules formulated with blends of maltodextrin with gum arabic, pectin, and soy protein compared to the individual use.

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3.5 Effect of packaging and storage conditions changes on biological properties (TPC, TFC, DPPH and FRAP) of spray dried pandan leaf extract microcapsules

Biological properties from plant extract can be degraded easily due to the environmental conditions (light, oxygen, temperature). The storage conditions and types of the package have been affected the compositions of phytochemicals and antioxidant properties [29]. Appropriate storage conditions and packaging are possible to maintain the extract's bioactive components and increase its shelf life without destroying them. This study investigated the effect of packaging materials (aluminum foil laminated bag and HDPE bag sealed vacuum and atmosphere conditions) on the TPC values of PLE microcapsules. It was found that total phenolic content (TPC) decreased significantly in all samples over 90 days, from 292.71 ± 1.27 mg GAE/100 g db to 157.91 ± 1.68 mg GAE/100g db in vacuum-packed aluminum foil bags and to 131.54 ± 1.09 mg GAE/100 g db in vacuum-packed HDPE bags. The better retention in aluminum foil packaging is likely due to its superior barrier against oxygen and moisture, which shows phenolic degradation during storage. Over 90 days, a significant decline in TPC was observed for all samples regardless of packaging or storage atmosphere. All samples showed a significant reduction in total phenolic compounds during storage primarily due to oxidative degradation. Exposure to oxygen, light, temperature fluctuations, and moisture can accelerate the breakdown of phenolic compounds. These environment factors promote enzymatic and non-enzymatic reactions that degrade bioactive compounds over time. This research is in line with the study of [29], it was also observed that flavonoid loss in PHDP was significant because of the chemical changes in phenolic compounds, including oxidation reactions. Decomposition, Epimerization and polymerization, pH, and exposure to oxygen and light may degrade the temperature-related phenolic compounds of samples during storage [30].

Flavonoids are important bioactive compounds which are often sensitive to the storage conditions, i.e., the pH, temperature, light, oxygen, and water activity, so the use of packaging can improve the storage efficiency of bioactive, flavors, and microorganisms [31]. In Table 6, it was revealed that the pandan leaf extract microcapsules with GRMD (1:1) had an initial total flavonoid content of 57.04±1.06 mg QE/100g db and the final TFC values was 23.48±0.12 mg GAE/100g db on 90 days storage. More so, the TFC values steadily decrease with increasing the

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455 storage time. This degradation of bioactive compounds is probably caused by oxidation and
456 polymerisation processes [32].

457 Analysis of antioxidant activity analysis by DPPH is based on the principle of measuring
458 the ability to remove free radicals. The antioxidant activity (AA) values obtained for each
459 packaging bag and storage condition were evaluated at the beginning of storage time (day 0) in
460 Table 6. The pandan leaf extract microcapsules stored in aluminum foil laminated bag sealed
461 vacuum and atmospheric for 90 days at ambient temperature had a significant loss of antioxidant
462 by DPPH, from 324.75 ± 2.50 to 314.75 ± 4.39 (mM Trolox/100g db) and the samples stored in
463 HDPE under vacuum packaging and atmospheric packaging from 318.50 ± 1.91 to 298.50 ± 4.51
464 (mM Trolox/100g db) along the shelf life was observed. The highest DPPH was obtained in the
465 sample packed in aluminum foil bag with 709.12 ± 5.26 mM Trolox/100g db followed by HDPE
466 packaging under vacuum condition with an antioxidant effect. For FRAP values, all samples
467 began with an identical initial value of 739.39 ± 2.19 mM Trolox/100 g db at day 0 and then
468 consistent decline was occurred across all packaging types and conditions over time in Table 6.
469 This study was in line with the study of [33], it was studied the shelf life of tamarillo powder at 28
470 days and found that the amount of flavonoid, phenolic compounds and antioxidant effects tend to
471 decrease. Increasing shelf life decrease the stability of bioactive compounds due to the
472 decomposition of anthocyanin and carotenoids.

473 *3.6 Effect of packaging and storage conditions changes on moisture content and water activity*
474 *and color changes of spray dried pandan leaf extract microcapsules*

475 The moisture content of spray-drying samples is an important factor in the study of the
476 shelf life of natural extract products, since moisture contributes to the movement of molecules,
477 thus accelerating the oxidative reaction of phenolic compounds during storage. PLE
478 microcapsules have initial moisture content of 3.85 %. When the shelf life of storage increased,
479 the moisture content of the microcapsules stored under vacuum and atmospheric conditions
480 showed the increased value up to 6.71%, as shown in Table 7. In terms of water activity, the
481 microcapsules packed in HDPE bag under atmospheric condition showed higher content (0.57)
482 compared to the samples stored under vacuum conditions (0.53). The observed increase in
483 moisture content and water activity, even in packaged samples, may be attributed to the lack of

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controlled storage temperature and relative humidity, which can promote moisture migration through packaging with limited water vapor barrier properties or micro-leakage; in addition, temperature fluctuations may induce condensation inside the package, accelerating moisture uptake [34]. Despite these variations, all samples maintained water activity level (< 0.6) across all storage conditions, which is sufficient to inhibit the microbial growth, although it may still lead to gradual degradation of bioactive compounds [35]. Color changes, especially in the L*, a* and b* values, were observed across all conditions, as shown in Figure 3(a, b, c). The decrease in L* (lightness) and increase in a* (redness/greenness) and b* (yellow- ness/blueness) suggests oxidative changes in the pigments present in PLE powder, which is consistent with studies on plant-based powder stability during storage [36].). The color difference (ΔE*) was lower for the samples packed in the aluminum foil bag under vacuum condition (2.13) compared to those stored in HDPE packed powder under atmospheric condition (2.94), as shown in Figure 3d. The difference is likely due to the higher permeability of the packaging material to oxygen and water vapor. Additionally, residual air trapped inside the package may cause oxidation that led to color changes over time. These findings are also consistent with the study of [37] which reported that ΔE* value of papaya sample powder is also increased after 7 weeks of storage, this may be contributed to the oxidation reaction that may cause color changes during storage.

4 Conclusion

This study successfully optimized the ultrasound-assisted extraction (UAE) of pandan leaf extract (PLE) using response surface methodology (RSM), identifying 60% ethanol, a 20-min extraction time, and 40% amplitude as the optimal conditions for maximizing total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity. The optimized PLE demonstrated robust antibacterial activity against *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*. Microencapsulation of PLE with a 1:1 blend of gum arabic and resistant maltodextrin (GRMD) via spray drying produced stable microcapsules with high encapsulation efficiency (93.03%), excellent solubility, and improved retention of bioactive compounds. Storage trials revealed that vacuum-sealed aluminum foil laminated packaging outperformed conventional HDPE packaging in preserving antioxidant properties, though a notable decline in bioactive compounds was observed over 90 days. Future research should focus on: a) Investigating the impact of refrigerated storage on the retention of bioactive compounds in spray-

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dried pandan leaf powders to enhance shelf-life stability, b) Exploring the use of multiple wall materials, including milk proteins alongside gum arabic and resistant maltodextrin, to evaluate potential synergistic effects on encapsulation efficiency and bioactive retention, c) Assessing the scalability of these findings for industrial production, emphasizing key parameters such as drying yield, moisture content, and bioactive compound retention to optimize process efficiency, product quality, and commercial viability, and d) Exploring the impact of refrigerated storage on the retention of bioactive compounds in spray-dried pandan leaf powders. These efforts will further enhance the stability, functional value, and industrial applicability of GRMD-encapsulated PLE as a sustainable bioactive ingredient for functional food applications.

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Credit Author Statement

Nilar Oo: Investigation, Formal analysis, Data curation and Writing –original draft. Saranya Jansamutr: Data curation and Writing –original draft. Saeid Jafari: Writing – review & editing. Kitipong Assatarakul: Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing –original draft and Writing –review & editing.

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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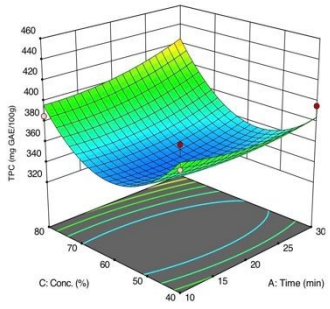

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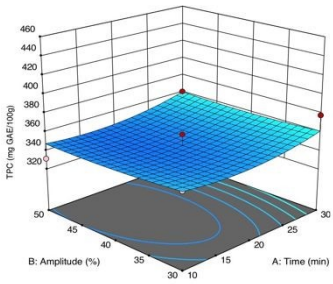

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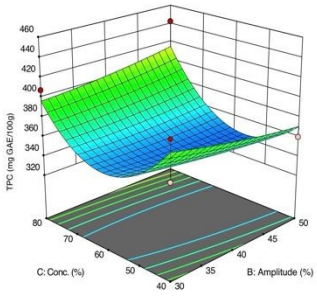

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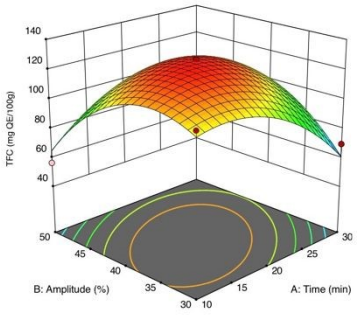

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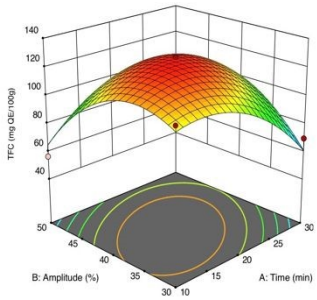

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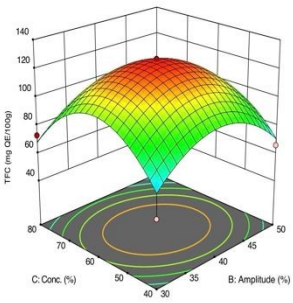

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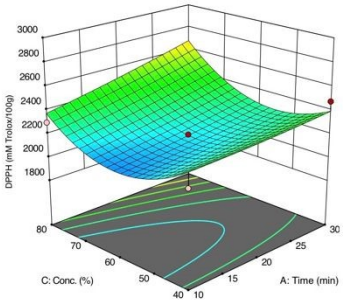

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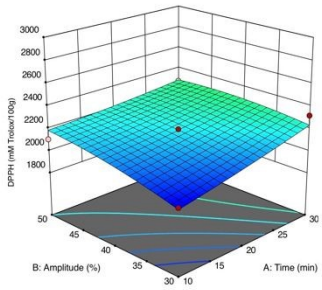

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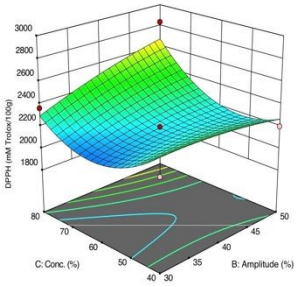

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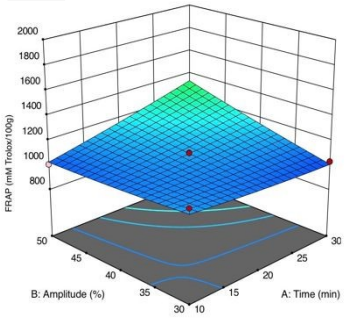

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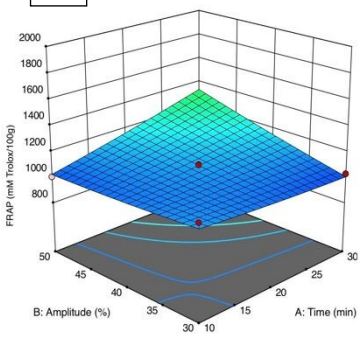

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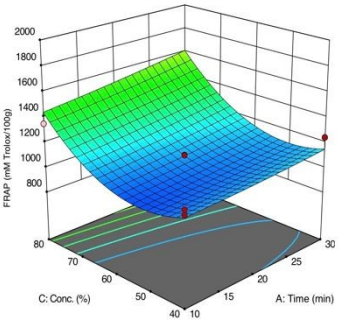

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Figure 1. Three-dimensional plots of response surface methodology of total phenolic compounds, total flavonoid antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays; (A, D, G, J) ethanol concentration and time; (B, E, H, K) amplitude and time; (C, F, I, L) amplitude and ethanol concentration of PLE. The red dot represents design point above predicted value. The pink dot represents design point below predicted value.

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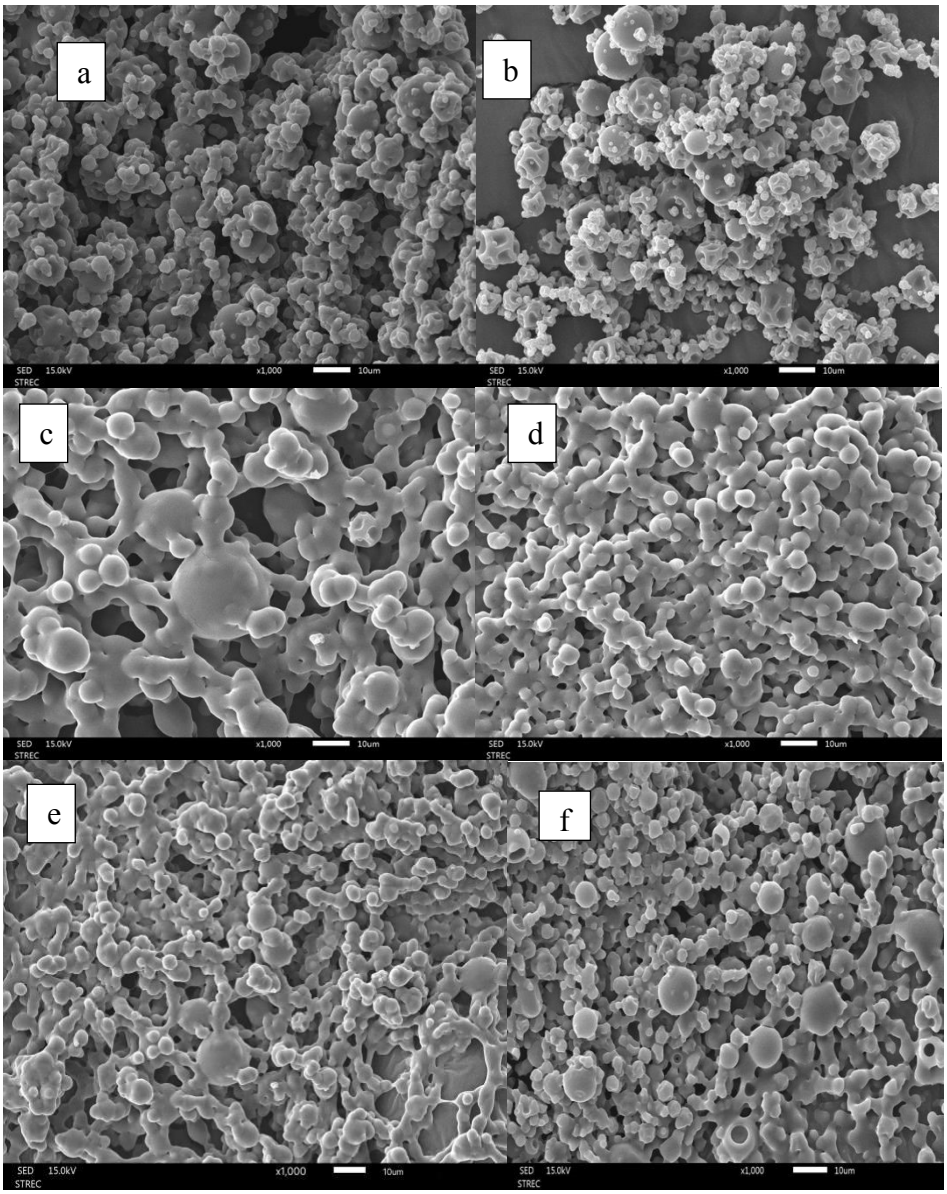

Figure 2. Photo with scanning electron microscope expanding 1000 times of the PLE microcapsule. (a) PLE + GA (1:1ratio), (b) PLE + GA (1:2 ratio), (c) PLE + GRMD (1:1 ratio), (d) PLE + GRMD (1:2 ratio), (e) PLE + RMD (1:1 ratio), and (f) PLE + RMD (1:2 ratio)

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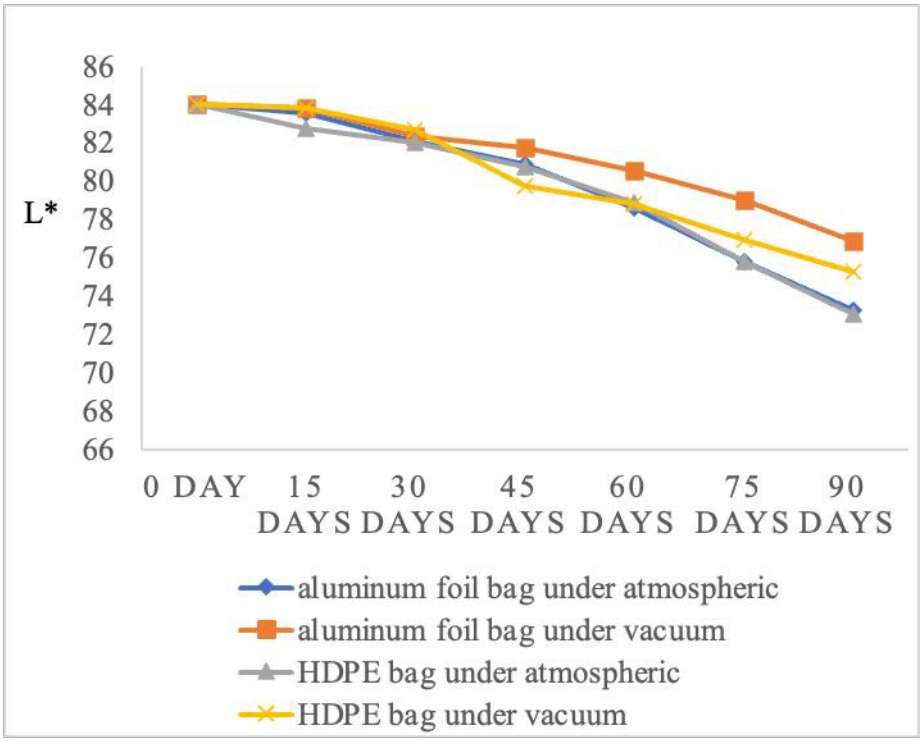

Figure 3a. L* of PLE microcapsules during storage

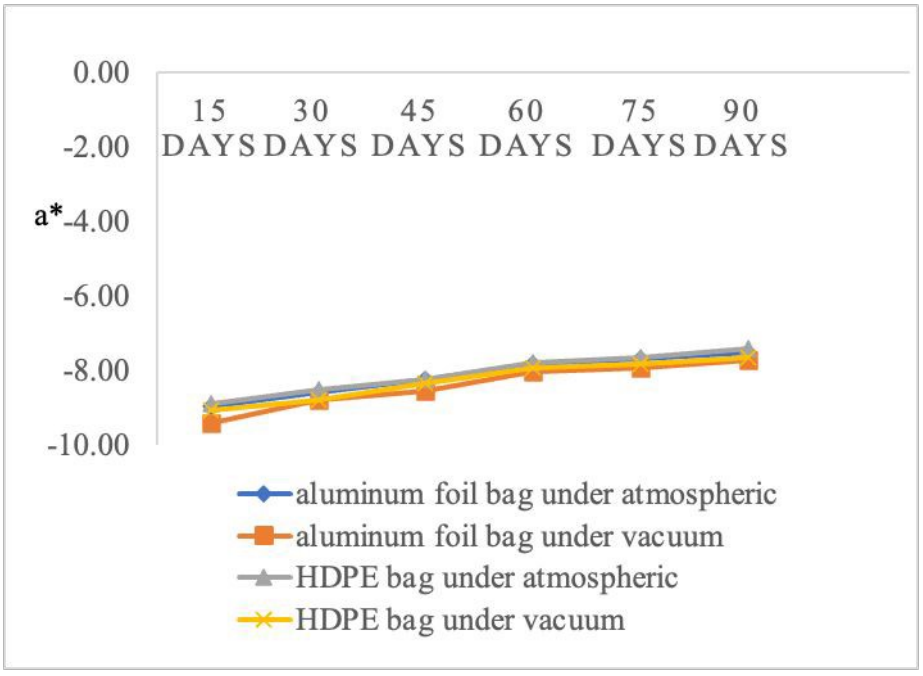

Figure 3b. a* of PLE microcapsules during storage

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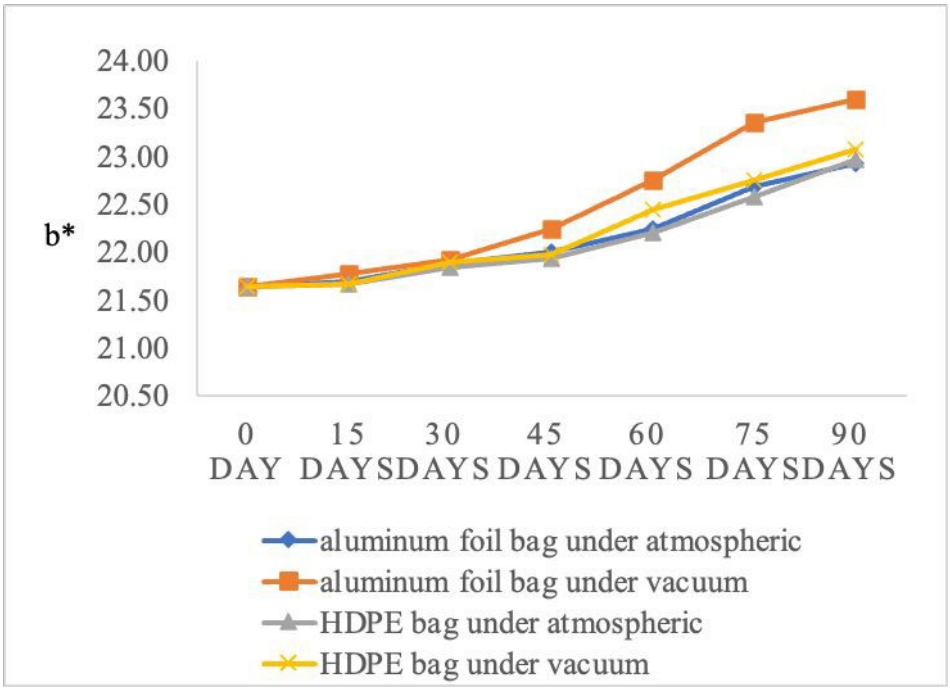

Figure 3c. b* of PLE microcapsules during storage

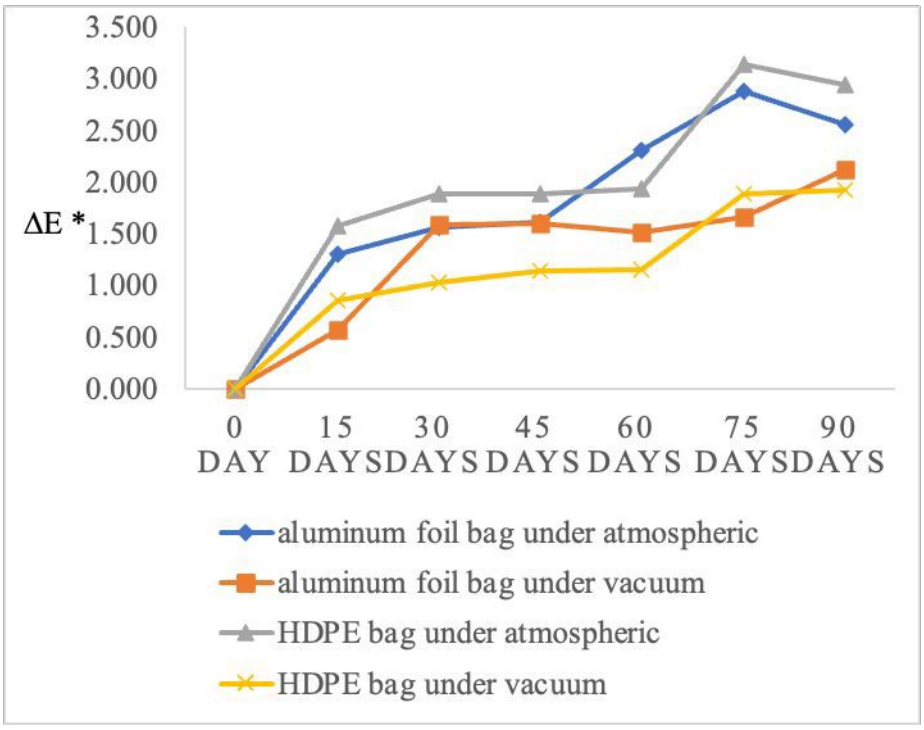

Figure 3d ΔE* of PLE microcapsules during storage

Table 1. The values for the Box-Behnken design (BBD) using response surface methodology (RSM).

Independent Variables	Independent Variables Codes	Level -1	0	1
Ethanol concentration (%)	A	40	60	80
Amplitude (%)	B	30	40	50
Extraction time (min)	C	10	20	30

9 **Table 2.** The functional properties of pandan leaf extract (PLE) under various extraction conditions.

Treatment	Independent Variables			Responses			
	Time (min) (C)	Amplitude (%) (B)	Ethanol Concentration (%) (A)	TPC (mg GAE/100g db)	TFC (mg QE/100g db)	DPPH (mM Trolox/100g db)	FRAP (mM Trolox/100g db)
1	20	40	60	341.60	126.85	2195.50	1097.19
2	10	40	40	426.40	66.33	2608.00	1139.30
3	20	30	40	367.70	53.80	2209.25	1141.05
4	20	40	60	341.04	125.86	2185.50	1101.58
5	30	40	80	397.50	58.53	2524.25	1539.30
6	30	50	60	360.50	88.68	2273.00	1296.32
7	20	40	60	342.69	126.55	2196.75	1091.05
8	10	40	40	385.00	128.15	2204.25	1098.95
9	20	40	60	357.60	124.80	2184.75	1095.44
10	10	50	60	331.00	56.39	2104.25	1005.09
11	20	50	40	360.00	65.93	2200.50	927.01
12	20	50	80	442.40	73.26	2833.00	1913.86
13	10	30	60	352.80	112.45	1980.50	1127.02
14	10	40	80	385.80	56.32	2298.00	1350.70
15	20	30	80	407.60	73.03	2364.25	1306.84

16	30	40	40	395.7	72.35	2475.5	1241.93
17	30	30	60	378.2	69.60	2318.00	1028.77

Total phenolic compound (TPC), total flavonoid content (TFC), antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays

Table 3. Analysis of Variance (ANOVA) of responses for total phenolic compounds (TPC), total flavonoid content (TFC), and antioxidant activity by DPPH and FRAP assays

	TPC (mg GAE/100g db)				TFC (mg QE/100g db)			
Source	Sum of Squares	df	Mean Squares	p-value	Sum of Squares	df	Mean Squares	p-value
Model	11188.47	9	1243.16	0.2187	10857.65	9	1206.41	0.1029
A-Time	246.26	1	246.26	0.5660	214.41	1	214.41	0.5117
B-Amplitude	19.22	1	19.22	0.8711	75.71	1	75.71	0.6935
C-Conc.	1099.40	1	1099.40	0.2438	163.41	1	163.41	0.5652
AB	4.20	1	4.20	0.9395	1411.69	1	1411.69	0.1194
AC	368.16	1	368.16	0.4855	332.64	1	332.64	0.4177
BC	451.56	1	451.56	0.4416	35.45	1	35.45	0.7868
A^2	236.40	1	236.40	0.5737	1494.61	1	1494.61	0.1107
B^2	22.75	1	22.75	0.8599	2644.49	1	2644.49	0.0456
C^2	8911.20	1	8911.20	0.0085	4867.89	1	4867.89	0.0132
Residual	4753.02	7	679.00		3140.80	7	448.69	
Lack of Fit	3706.90	3	1235.63	0.0839	1226.71	3	408.90	0.5328
Pure Error	1046.12	4	261.53		1914.09	4	478.52	
Total	15941.49	16			13998.45	16		
R^2			0.7018				0.7756	
Adj R^2			0.3185				0.4872	

	DPPH (mM Trolox/100g db)				FRAP (mM Trolox/100g db)			
Model	4.757+05	9	52860.52	0.2242	8.157+05	9	90631.83	0.0028
A-Time	71018.87	1	71018.87	0.1633	29521.38	1	29521.38	0.1087
B-Amplitude	36281.45	1	36281.45	0.3024	36262.32	1	36262.32	0.0811
C-Conc.	57329.03	1	57329.03	0.2045	3.645E+05	1	3.645E+05	0.0003
AB	7119.14	1	7119.14	0.6371	37923.67	1	37923.67	0.0757
AC	15754.30	1	15754.30	0.4871	3027.57	1	3027.57	0.5746
BC	57001.56	1	57001.56	0.2056	1.685+05	1	1.685+05	0.0032
A^2	475.60	1	475.60	0.9022	320.45	1	320.45	0.8536
B^2	4353.24	1	4353.24	0.7113	350.75	1	350.75	0.8469
C^2	2.460+05	1	2.460E+05	0.0230	1.948+05	1	1.948E+05	0.0022
Residual	2.050+05	7	29282.56		61175.38	7	8739.34	
Lack of Fit	1.233+05	3	41116.28	0.2543	60304.27	3	20101.42	0.0004
Pure Error	81629.09	4	20407.27		871.11	4	217.78	
Total	6.807+05	16			8.769E+05	16		
R^2			0.6989				0.9302	
Adj R^2			0.3117				0.8405	

Total phenolic compound (TPC), total flavonoid content (TFC), antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP). df: degree of freedom

Table 4. The Diameter of the Inhibition Zone in cm and Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) of Three Bacteria Strains of Ethanol Extract of Pandan Leaf

Strain of bacteria	Extract concentration	Inhibition zone (mm)	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	60%	18	3.75	7.50
Bacillus cereus	60%	29	0.94	1.87
Staphylococcus aureus	60%	27	1.87	3.75

MIC = minimum inhibitory concentration, MBC = minimum bacterial concentration

36 **Table 5.** Physicochemical Properties and Biological Properties (TPC, TFC, DPPH, and FRAP assays) of Microcapsule

Parameter	Encapsulation type and concentration (w/v)					
	20% GA 1:1	20% GA 1:2	20% RMD 1:1	20% RMD 1:2	20% GRMD 1:1	20% GRMD 1:2
Total phenolic compound (mg GAE/100g db)	291.24±3.53^{ab}	289.41±4.57^{b}	291.97±5.03^{ab}	288.68±2.90^{bc}	292.71±1.26^{a}	287.94±1.67^{c}
Total flavonoid content (mg QE/100g db)	63.08±1.55^{ab}	63.56±1.07^{a}	59.17±0.96^{b}	53.22±0.93^{c}	57.04±1.05^{bc}	50.27±1.09^{d}
Antioxidant by DPPH (mM Trolox/100g db)	422.67±12.22^{c}	421.42±2.89^{c}	424.30±1.44^{bc}	371.83±15.12^{d}	709.12±5.26^{a}	499.33±4.16^{b}
Antioxidant by FRAP (mM Trolox/100g db)	599.58±1.53^{c}	584.84±3.98^{d}	591.16±1.49^{cd}	583.79±0.96^{d}	739.93±2.19^{a}	680.63±1.18^{b}
Moisture content (%)	4.98±0.02^{a}	4.07±0.05^{b}	3.78±0.02^{c}	3.52±0.09^{cd}	3.85±0.03^{bc}	3.41±0.02^{d}
Water activity	0.21±0.03^{b}	0.17±0.02^{c}	0.25±0.05^{a}	0.22±0.03^{ab}	0.20±0.02^{bc}	0.14±0.01^{d}
Color values						

L*	73.79±0.05^{bc}	66.56±0.05^{c}	85.17±0.04^{a}	75.46±0.03^{b}	84.01±0.03^{ab}	73.18±0.05^{bc}
a*	-4.47±0.03^{a}	-5.09±0.04^{ab}	-9.95±0.03^{cd}	-8.17±0.02^{b}	-9.90±0.04^{c}	-8.47±0.05^{bc}
b*	30.46±0.03^{a}	24.46±0.04^{b}	18.65±0.03^{cd}	14.11±0.04^{d}	21.64±0.05^{bc}	19.65±0.06^{c}
Encapsulation efficiency (%)	91.91±0.02^{ab}	88.23±0.01^{bc}	88.08±0.07^{bc}	78.27±0.04^{c}	93.03±0.03^{a}	89.90±0.04^{b}
Yield (%)	63.36±0.34^{d}	69.54±0.48^{bc}	67.50±0.60^{c}	70.03±0.13^{b}	70.60±0.38^{b}	78.44±0.48^{a}
Solubility (%)	75.49±0.04^{cd}	73.41±0.02^{d}	82.41±0.03^{b}	79.23±0.06^{c}	92.55±0.02^{a}	91.62±0.04^{ab}

GA = gum Arabic, RMD = resistant maltodextrin, GRMD = mixture of gum arabic and resistant maltodextrin

Three replications were used for each microcapsule per each analysis.

Different letters (a, b, c, d) within same row indicate statistically significant differences ($p \leq 0.05$).

50 **Table 6**. Storage Stability of Bioactive Compounds (TPC and TFC) and Antioxidant Activity (DPPH and FRAP) in Microcapsules

Duration (days)	Package type			
	Aluminum foil laminated bag		HDPE bag	
	Packed under atmospheric conditions	Packed under vacuum conditions	Packed under atmospheric conditions	Packed under vacuum conditions
	Total phenolic compound (mg GAE/100g db)			
0	292.71±1.27^{a}	292.71±1.27^{a}	292.71±1.27^{a}	292.71±1.27^{a}
15	278.71±2.77^{ab}	283.19±3.29^{b}	272.20±3.29^{ab}	277.69±2.20^{ab}
30	245.82±2.20^{b}	256.81±2.20^{bc}	223.85±3.29^{b}	240.33±4.40^{b}
45	216.15±3.29^{bc}	227.14±2.19^{c}	198.57±1.10^{bc}	212.86±3.29^{bc}
60	182.09±2.29^{c}	191.98±2.19^{d}	167.80±3.29^{c}	179.89±2.19^{c}
75	154.61±2.77^{cd}	175.49±1.10^{de}	141.43±1.10^{cd}	149.12±1.68^{d}
90	138.13±3.29^{d}	157.91±1.68^{e}	121.65±2.29^{d}	131.54±1.09^{d}
	Total flavonoid content (mg QE/100g db)			
0	57.04±1.06^{a}	57.04±1.06^{a}	57.04±1.06^{a}	57.04±1.06^{a}
15	47.76±0.12^{b}	49.51±0.15^{b}	41.72±0.23^{b}	43.02±0.08^{b}

30	34.93±0.12^{c}	36.08±0.08^{c}	31.88±0.15^{c}	32.95±0.12^{c}
45	33.56±0.12^{cd}	35.16±0.15^{cd}	28.44±0.08^{cd}	29.13±0.19^{d}
60	32.56±0.16^{cd}	33.71±0.08^{cd}	26.53±0.36^{cd}	28.59±0.16^{d}
75	27.76±0.09^{d}	28.59±0.08^{d}	23.48±0.12^{d}	26.69±0.16^{de}
90	18.90±0.39^{e}	21.49±0.23^{e}	18.44±0.08^{e}	19.13±0.12^{e}
	Antioxidant by DPPH (mM Trolox/100g db)			
0	709.12±5.26^{a}	709.12±5.26^{a}	709.12±5.26^{a}	709.12±5.26^{a}
15	648.50±5.91^{b}	679.75±5.05^{ab}	577.25±4.02^{ab}	592.25±6.96^{b}
30	548.50±7.22^{c}	567.25±5.45^{b}	511.00±6.88^{b}	552.25±5.05^{c}
45	493.50±10.68^{d}	518.50±5.00^{c}	487.25±5.20^{c}	503.50±9.71^{d}
60	434.75±8.78^{e}	462.25±9.46^{d}	423.50±5.05^{d}	452.25±6.96^{e}
75	407.25±2.50^{ef}	431.00±2.60^{de}	394.75±3.61^{e}	421.00±2.60^{f}
90	314.75±4.39^{f}	324.75±2.50^{e}	298.50±4.51^{f}	318.50±1.91^{f}
	Antioxidant by FRAP (mM Trolox/100g db)			
0	739.93±2.19^{a}	739.93±2.19^{a}	739.93±2.19^{a}	739.93±2.19^{a}
15	704.84±1.05^{b}	720.63±1.21^{ab}	695.37±2.11^{b}	700.63±2.19^{b}

30	681.68±8.99^{bc}	695.37±10.80^{b}	666.95±4.21b^{c}	685.89±1.61^{bc}
45	663.79±5.79^{c}	673.26±8.95^{bc}	632.21±2.78^{c}	658.53±1.61^{c}
60	568.00±3.15^{cd}	594.32±3.79^{c}	500.63±1.21^{d}	524.84±1.05^{d}
75	461.68±1.05^{d}	503.79±1.21^{d}	396.42±1.61^{e}	434.32±2.11^{e}
90	424.84±1.61^{e}	444.84±8.42^{e}	335.37±2.65^{f}	354.32±2.10^{f}

Total phenolic compound (TPC), total flavonoid content (TFC), antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP).

Different letters (a, b, c, d) within same column indicate statistically significant differences ($p \leq 0.05$).

73 **Table 7** Effect of packaging conditions on moisture content and water activity of PLE microcapsules during storage

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Duration (days)	Package type			
	Aluminum foil laminated bag		HDPE bag	
	Packed under atmospheric conditions	Packed under vacuum conditions	Packed under atmospheric conditions	Packed under vacuum conditions
	Moisture content %			
0	3.85±0.05^{e}	3.85±0.09^{e}	3.85±0.06^{e}	3.85±0.02^{f}
15	3.98±0.11^{d}	3.92±0.05^{d}	4.38±0.05^{d}	4.38±0.06^{e}
30	4.21±0.04^{cd}	4.13±0.05^{cd}	4.91±0.04^{cd}	4.77±0.02^{de}
45	4.35±0.06^{c}	4.22±0.03^{c}	5.05±0.05^{c}	4.92±0.04^{d}
60	4.71±0.02^{bc}	4.65±0.10^{bc}	5.57±0.02^{bc}	5.32±0.05^{c}
75	5.18±0.03^{b}	4.81±0.04^{b}	5.81±0.04^{b}	5.77±0.08^{b}
90	5.85±0.06^{a}	5.32±0.02^{a}	6.38±0.06^{a}	6.71±0.05^{a}
	Water activity			
0	0.20±0.03^{e}	0.20±0.01^{d}	0.20±0.01^{e}	0.20±0.01^{e}

15	0.22±0.01^{d}	0.22±0.01^{cd}	0.31±0.01^{de}	0.28±0.01^{de}
30	0.27±0.01^{cd}	0.25±0.01^{c}	0.35±0.01^{d}	0.33±0.01^{c}
45	0.29±0.01^{c}	0.29±0.01^{bc}	0.45±0.01^{c}	0.41±0.01^{c}
60	0.32±0.01^{b}	0.31±0.01^{b}	0.51±0.01^{bc}	0.46±0.01^{bc}
75	0.35±0.01^{ab}	0.34±0.01^{ab}	0.53±0.01^{b}	0.49±0.01^{b}
90	0.37±0.01^{a}	0.35±0.01^{a}	0.57±0.01^{a}	0.53±0.01^{a}

Different letters (a, b, c, d) within same column indicate statistically significant differences ($p \leq 0.05$).

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Data availability Statement

The data supporting the findings of this study are available from the corresponding author upon request.