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Sustainable production of bioactive pandan leaf extract microcapsules *via* optimized ultrasound-assisted extraction and spray drying

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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/100 g db, 21.49 mg QE/100 g db, 324.75 mM Trolox/100 g db, and 444.84 mM Trolox/100 g db, respectively. This study demonstrates the potential of combining UAE and GRMD microencapsulation using spray drying to sustainably produce stable, bioactive pandan leaf extract microcapsules for use in functional food systems. Further studies are recommended to assess sensory acceptance, bioaccessibility, and scale-up potential.

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Sustainability spotlight

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.

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, the leaf is also valued for its ability to freshen the body, relieve fever, and treat indigestion. They contain a wide range of alkaloids as well as unglycosylated proteins, which has an 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

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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 extraction, 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 the 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]

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 assays); (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). 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 transported to the laboratory at Chulalongkorn University in Bangkok, Thailand. The pandan leaves were washed with clean 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 sample was ground, 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 Ummat *et al.*[10] with slight modifications. 3 g of the dried pandan leaf (PL) powder sample was mixed with 100 mL of solvent (mixture of ethanol and distilled water) and then the extraction was conducted under various extraction conditions. The extract was centrifuged at 6000 rpm for 15 min at room temperature (Centrifuge Kubota, series 6000, Osaka, Japan) and the supernatant was evaporated by using a 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 a 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 conditions with different independent variables (A, ethanol concentration; B, amplitude; C, extraction time) at three levels (−1, 0, and 1). The responses were the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and ferric-reducing antioxidant power (FRAP) assay. 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).

Table 1 The values for the Box–Behnken Design (BBD) using response surface methodology (RSM)

		Level		
Independent variables	Independent variables codes	−1	0	1
Ethanol concentration (%)	A	40	60	80
Amplitude (%)	B	30	40	50
Extraction time (min)	C	10	20	30

Table 2 The functional properties of pandan leaf extract (PLE) under various extraction conditions[a]

	Independent variables			Responses			
Treatment	Time (min) (C)	Amplitude (%) (B)	Ethanol concentration (%) (A)	TPC (mg GAE/100 g db)	TFC (mg QE/100 g db)	DPPH (mM Trolox/100 g db)	FRAP (mM Trolox/100 g 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

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

2.3 Antimicrobial activity determination of optimized pandan leaf extract (PLE)

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 the Faculty of Science, Chulalongkorn University. Each microorganism's inoculum was generated by inoculating 10 mL of a 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 the 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 in 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 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 bactericidal concentration (MBC). The pathogenic bacterial inoculum was cultured at 37 °C for 16 h before being diluted to the turbidity of the McFarland 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^{-1}. The bacterial suspension (50 μL) was introduced to a sterilized 96-well plate containing 100 μL of MHB and 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 nutrient 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 the 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^{-1} and hot air flow rate 1.54 m^3 min^{-1} according to the study by Chheng *et al.*[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 under vacuum condition until further investigation.

2.5 Stability of the antioxidant capacity of microcapsules during storage

The best microcapsule which retained 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 30 °C, which reflects typical room temperature conditions, and relative humidity at around 60% for 3 months and the analysis was conducted every 15 days.

2.6 Determination of physicochemical properties of spray-dried PLE microcapsules

A 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 using a Scanning Electron Microscope (SEM) with magnification 1000 times as described by a published method.[7] The encapsulation efficiency (%) was evaluated by using a previously reported method.[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 using a previously reported method.[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 a pre-weighed plate and placed in an 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

1 g of PLE microcapsules 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×g for 20 min and collected for the analysis of bioactive compounds.[11] The procedure for the analysis is detailed as follows.

TPC of PL extract and microcapsules was determined using the Folin–Ciocalteau method, as presented by Jafari *et al.*[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 examined 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 the PLE sample was mixed with 1 mL of 2% aluminum chloride solution and then vortexed. After mixing, the solution was placed in a dark room for 30 min. The TFC value was determined at 430 nm using a 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 a test tube with 4.75 mL of DPPH solution and then kept in a 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/100 g db.

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

The antioxidant activity by FRAP assays of the samples was determined according to Benzie and Strain[15] with a slight modification. The sample (50 μL) was added to a test tube and mixed with 950 μL of FRAP solution, which was then incubated in the at the darkness at room temperature for 30 min. The absorbance was measured at 593 nm against methanol as a blank, and the antioxidant activity was expressed as mM Trolox/100 g db.

2.8 Statistical analysis

The optimum conditions from the UAE experiment were obtained using the Design Expert 11 program and the Box–Behnken design showing a three-dimensional (3D) model graph. All analyses were performed in triplicate ($n = 3$) and interpreted with SPSS (Statistical Package for Social Sciences) version 20.0 statistical software. The data were analyzed 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 content, total flavonoid content, and antioxidant activity) using RSM

Phenolic compounds are the most common secondary metabolites, varying by phylum. According to Table 2, an 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, and C), with nonsignificant lack of fit and R^2 values between 0.69 and 0.71 indicating moderate to good model fit. The TPC response was modeled using 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 ×

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

Source	Sum of squares	df	Mean squares	*p*-value	Sum of squares	df	Mean squares	*p*-value
TPC (mg GAE/100g db)					**TFC (mg QE/100g db)**			
Model	11 188.47	9	1243.16	0.2187	10 857.65	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	15 941.49	16			13 998.45	16		
R^2			0.7018				0.7756	
Adj R^2			0.3185				0.4872	
DPPH (mM Trolox/100 g db)					**FRAP (mM Trolox/100 g db)**			
Model	4.757 + 05	9	52 860.52	0.2242	8.157 + 05	9	90 631.83	0.0028
A-time	71 018.87	1	71 018.87	0.1633	29 521.38	1	29 521.38	0.1087
B-amplitude	36 281.45	1	36 281.45	0.3024	36 262.32	1	36 262.32	0.0811
C-conc	57 329.03	1	57 329.03	0.2045	3.645×10^5	1	3.645×10^5	0.0003
AB	7119.14	1	7119.14	0.6371	37 923.67	1	37 923.67	0.0757
AC	15 754.30	1	15 754.30	0.4871	3027.57	1	3027.57	0.5746
BC	57 001.56	1	57 001.56	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	2.460×10^5	0.0230	1.948 + 05	1	1.948×10^5	0.0022
Residual	2.050 + 05	7	29 282.56		61 175.38	7	8739.34	
Lack of fit	1.233 + 05	3	41 116.28	0.2543	60 304.27	3	20 101.42	0.0004
Pure error	81 629.09	4	20 407.27		871.11	4	217.78	
Total	6.807 + 05	16			8.769×10^5	16		
R^2			0.6989				0.9302	
Adj R^2			0.3117				0.8405	

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

conc. + 0.053125 amplitude × conc. + 0.075491 time² + 0.023419 amplitude² + 0.115873 conc.²

Fig. 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 an earlier report.[16] The ethanol–water mixture improves extraction efficiency due to solvent polarity effects, as noted by Kobus-Cisowska *et al.*[17] Low concentrations of ethanol aid phenolic solubility, whereas high concentrations of ethanol may denature the protein structure and limit extraction.

Table 2 shows that the highest total flavonoid content (TFC) occurred 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 that the quadratic model fits well ($R^2 = 77.56$) and predicts TFC accurately (Adj $R^2 = 48.72$). 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 time² − 0.252490 amplitude² − 0.085641 conc.²

Fig. 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 levels 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 leaf extract.

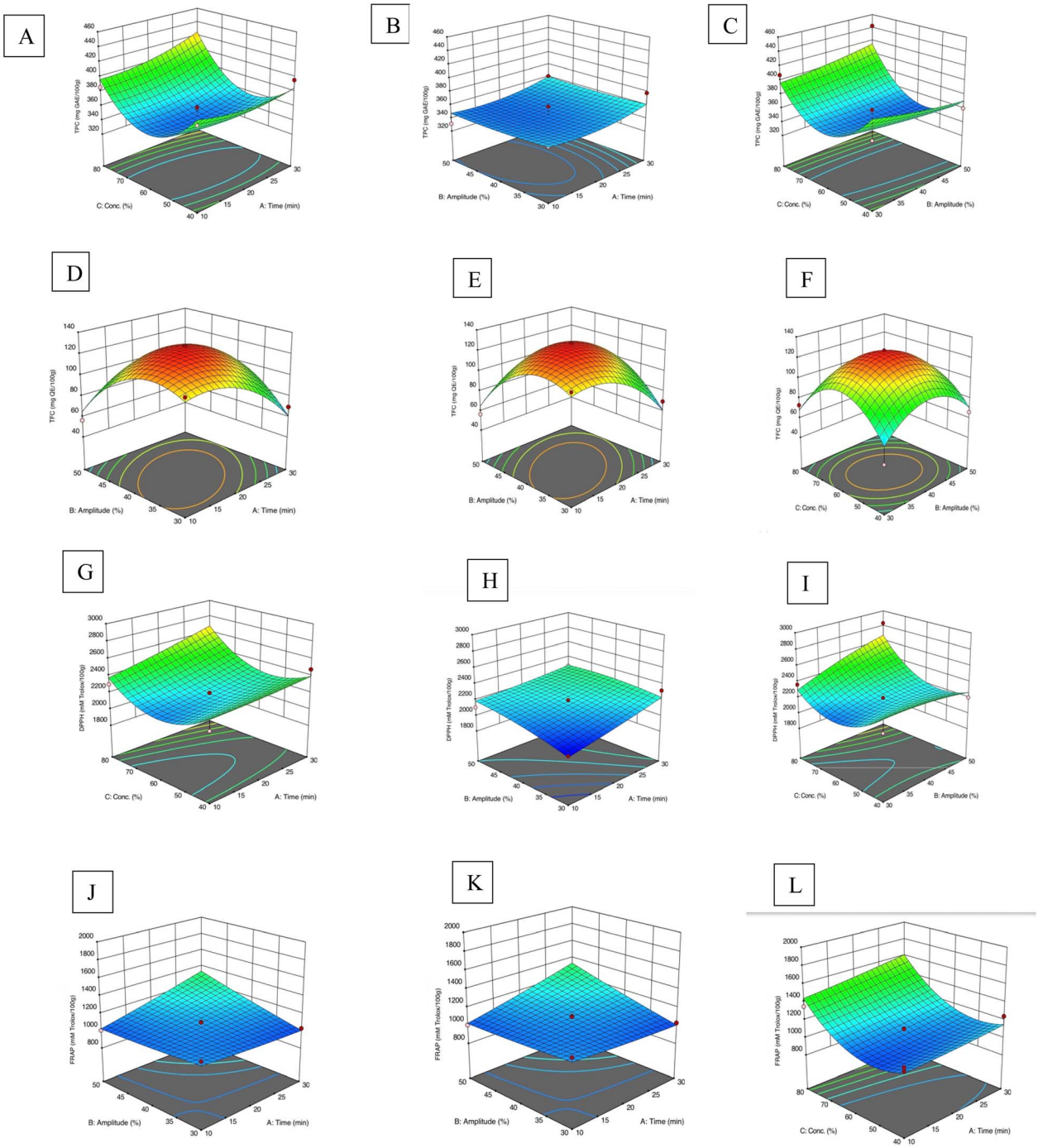

Fig. 1 Three-dimensional plots of response surface methodology of total phenolic content, total flavonoid content, antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays; (A, D, G, and J) ethanol concentration and time; (B, E, H, and K) amplitude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

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}

Fig. 1(G–I) display 3D response plots of the effect 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 concentration and short extraction time led to insufficient extraction, while moderate conditions caused some antioxidant degradation. Higher ethanol levels and longer

Table 4 The diameter of the inhibition zone in cm and Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of three bacterial strains of optimized pandan leaf extract (PLE)[a]

Strain of bacteria	Extract concentration	Inhibition zone (mm)	MIC (mg mL^{-1})	MBC (mg mL^{-1})
Escherichia coli	60%	18	3.75	7.50
Bacillus cereus	60%	29	0.94	1.87
Staphylococcus aureus	60%	27	1.87	3.75

[a] MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration.

Table 5 Physicochemical properties and biological properties (TPC, TFC, DPPH, and FRAP assays) of PLE microcapsules[a]

	Encapsulation type and concentration (w/v)					
Parameter	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 content (mg GAE/100 g 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/100 g 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/100 g 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/100 g 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}

[a] GA = gum arabic, RMD = resistant maltodextrin, GRMD = mixture of gum arabic and resistant maltodextrin. Three replications were used for each microcapsule per analysis. Different letters (a, b, c, and d) within the same row indicate statistically significant differences ($p \leq 0.05$).

extraction time preserved more antioxidants, boosting DPPH values, consistent with an earlier report.[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 were 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:

FRAP = +5620.23612–44.25127 time – 81.67666 amplitude – 98.31977 conc. + 0.973700 time * amplitude + 0.127354 time * conc. + 1.02631 amplitude * conc. + 0.087893 time^2 + 0.091954 amplitude^2 + 0.541703 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. Fig. 1(J–L) illustrate that increasing the ethanol concentration from 60% to 80% enhanced antioxidant activity, while changes in amplitude had less effect. These findings align with the study by Liyana-Pathirana and Shahidi,[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 the disc diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC)

The optimum conditions of UAE from RSM results are 60% ethanol concentration, 40% amplitude, and 20 min extraction time. Based on the optimum result, antibacterial activity of the extracted sample by the disk diffusion method and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) on Gram-positive bacteria (*B. cereus* and *S. aureus*) and Gram-negative bacteria (*E. coli*) were investigated. Evaluation of antibacterial activity of these plant extracts is shown in Table 4. The results revealed that PLE extract had the ability to inhibit the microbiological growth of food poisoning bacteria with different efficiencies. The crude extract inhibited *B. cereus* with an inhibition zone of 29 mm, followed by an

inhibition zone of 27 mm with *S. aureus* and the minimum inhibition zone of 18 mm with *E. coli* plates (Table 4). According to the results, *E. coli* was the most sensitive microorganism to the crude PLE extract, as determined by the MIC values (Table 4). Gram negative bacteria act as barriers to foreign substances because they have an outer membrane, strain characteristics and periplasmic region that Gram-positive bacteria may lack. Consistently, the study by De Zoysa *et al.*[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 from the inhibition zone corresponding to their lowest MIC. The PLE showed potential bacterial activity against the tested pathogenic bacteria (*B. cereus*, *S. aureus*, and *E. coli*) with MBC of 1.87 mg mL^{-1}, 3.75 mg mL^{-1}, and 7.50 mg mL^{-1}, respectively. The results of MIC and MBC of the effective plant extracts suggested that the 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 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] The results 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 content (TPC), total flavonoid content (TFC), antioxidant activity by DPPH and FRAP assays of GA- and RMD- based PLE microcapsules

The determination of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity is of considerable importance in the pharmaceutical, cosmetics and food industries. This is because antioxidants play a key role in preventing and treating diseases linked to oxidative stress, as well as in protecting products from oxidation and deterioration. The results of the microcapsules showed that total phenolic content ranged from 287.94 ± 1.67 to 292.71 ± 1.26 mg GAE/100 g db. The microcapsules generated with the combination of RMD and GA in a 1 : 1 encapsulation ratio had the greatest TPC value, followed by ratios of RMD 1 : 1 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), showing that they had synergistic effects and provided superior protection to these compounds during the spray-drying process.[7] Similar results were also reported regarding the effectiveness of using a mixture of maltodextrin and different coating materials for the phenolic values of microcapsules from spray drying and freeze drying. This research revealed that a mixture of maltodextrin and gum arabic (1 : 1) had a higher phenolic release rate than the use of an 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 are 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 ranged from 50.27 ± 1.09 to 63.56 ± 1.07 mg QE/100 g db as shown in Table 5. The results indicated that higher encapsulation ratios reduced the TFC content of PLE 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 content, the solution obtained from the microcapsules using a 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 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 compounds. 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 pandan leaf extract (PLE) microcapsules

Moisture content and water activity, a_w, play an essential role in forecasting food quality and security in terms of microbiological and biochemical processes, as determined by their values.[26] The microcapsule'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 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 in 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 microcapsule (powder) during storage. According to Abdullah *et al.*,[27] such low moisture content and a_w

levels are crucial for maintaining the stability of spray-dried powders by limiting microbial activity and oxidative reactions.

The colors of spray dried PLE microcapsules of various types and ratios of encapsulating agents were investigated. The results in Table 5 display the color values L^* (lightness), a^* (green – red), and b^* (blue – yellow), which range from 66.56 $\pm$ 0.05 to 85.17 $\pm$ 0.04, -4.47 ± 0.03 to -9.95 ± 0.03, and 14.11 $\pm$ 0.04 to 30.46 $\pm$ 0.03, respectively. PLE microcapsules with RMD at a 1 : 1 ratio had the highest brightness (85.17 $\pm$ 0.04), while those with GA at a 1 : 2 ratio had the lowest brightness (66.56 $\pm$ 0.05). Furthermore, a^* denotes greenness ($-a^*$) and redness ($+a^*$), 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 yellow and green tonalities.

The encapsulation efficiency of PLE microcapsules ranged from 78.27 $\pm$ 0.04 to 93.03 $\pm$ 0.03. Microcapsules with the mixture of resistant maltodextrin and gum arabic 1 : 1 had the highest encapsulation efficiency of 93.03 $\pm$ 0.03% followed by gum arabic (1 : 1) with the value of 91.91 $\pm$ 0.02. This study was consistent with the study by Ballesteros *et al.*[23] who observed that using single coating material had lower encapsulation efficiency compared to the mixture of maltodextrin and gum arabic (1 : 1). For the extraction yield, it was found that the

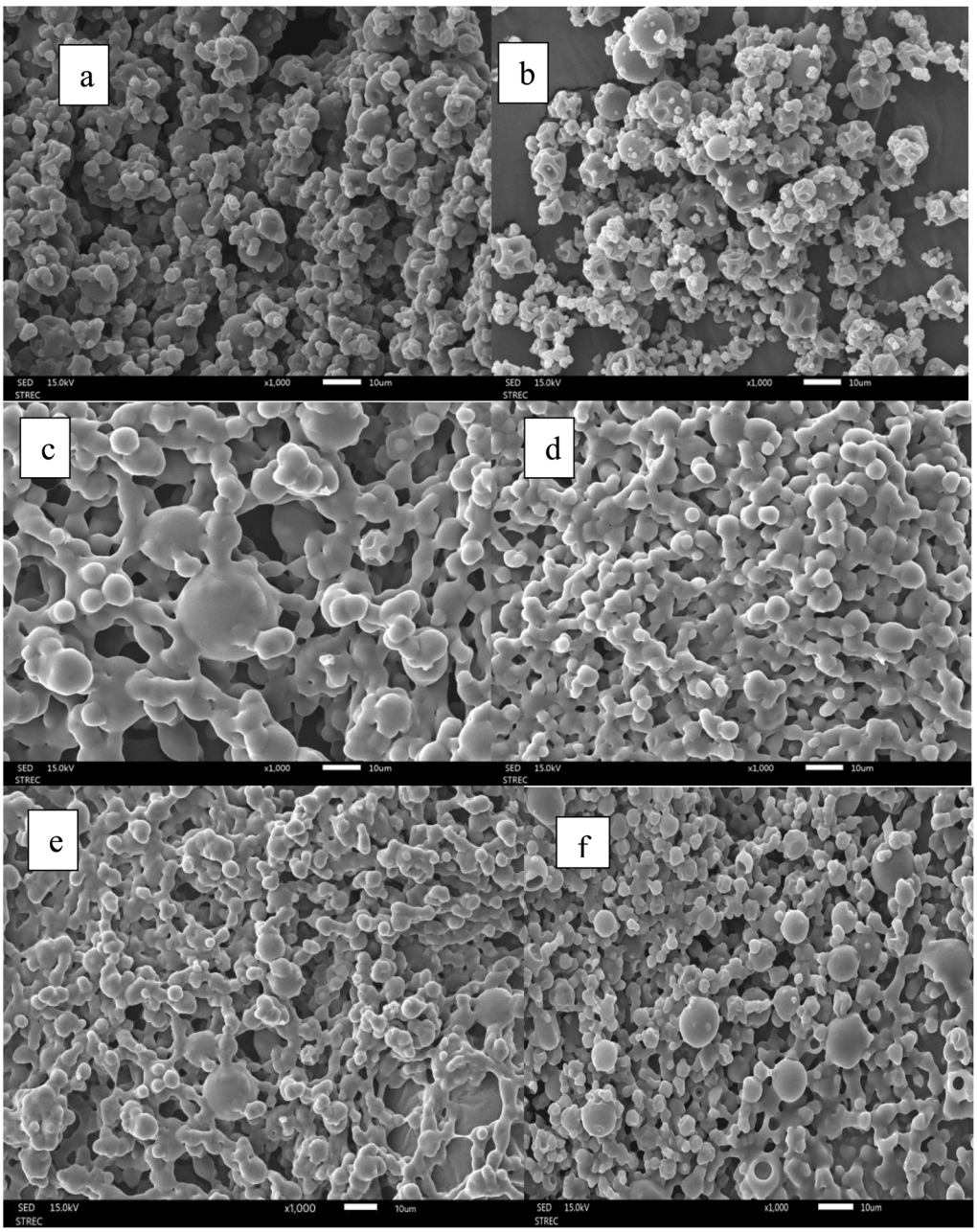

Fig. 2 Scanning electron microscopy images of the PLE microcapsules at 1000 times magnification. (a) PLE + GA (1 : 1 ratio), (b) PLE + GA (1 : 2 ratio), (c) PLE + GRMD (1 : 1 ratio), (d) PLE + GRMD (1 : 2 ratio), (e) PLE + RMD (1 : 1 ratio), and (f) PLE + RMD (1 : 2 ratio).

percentage of yield obtained from pandan leaf extract microcapsules was between 63.36 ± 0.34 and 78.44 ± 0.48. The production yield was the 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] It was concluded that a mixture of MD and GA is preferrable as the carrier for the solubility due to differences in their 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 Fig. 2. It was found that microcapsules using GA at a ratio of 1 : 1 showed more dents and crimps compared to those formulated with RMD. On the other hand, microcapsules with a 1 : 2 ratio of GA displayed a more spherical and smoother surface. Increasing the thickness of the encapsulation layer leads to more uniform outer surface and rounded morphology. Resistant maltodextrin at ratios of 1 : 1 and 1 : 2 (Fig. 2c and 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

Table 6 Storage stability of bioactive compounds (TPC and TFC) and antioxidant activity (DPPH and FRAP assays) in PLE microcapsules[a]

	Package type			
	Aluminum foil laminated bag		HDPE bag	
Duration (days)	Packed under atmospheric condition	Packed under vacuum condition	Packed under atmospheric condition	Packed under vacuum condition
Total phenolic content **(mg GAE/100 g 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/100 g 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 assay (mM Trolox/100 g 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 assay (mM Trolox/100 g 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}

[a] Total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays. Different letters (a, b, c,…) within the same column indicate statistically significant differences ($p \leq 0.05$).

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, the mixture of RMD and other coating material showed more noticeable spherical morphology compared to the use of RMD alone; as shown in Fig. 2. The current study's observations of morphological characteristics of microcapsules align with those of a recent finding reported by Navarro-Flores *et al.*,[25] who demonstrated that the particle size distribution was more homogeneous in the microcapsules formulated with blends of maltodextrin with gum arabic, pectin, and soy protein compared to the individual use.

3.5 Effect of packaging and storage condition on biological properties (TPC, TFC, antioxidant activity by DPPH and FRAP assays) of spray-dried pandan leaf extract (PLE) microcapsules

Biological properties from plant extract can be degraded easily due to environmental conditions (light, oxygen, and temperature). The storage conditions and types of the package affect the compositions of phytochemicals and antioxidant properties.[29] Appropriate storage conditions and packaging are possible to retain 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 under 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/100 g 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 the storage atmosphere. All samples showed a significant reduction in total phenolic content 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 by El-Mesery *et al.*,[29] who also observed that flavonoid loss in fresh Chinese tomato packed in perforated high-density polyethylene (PHDP) bag during the storage 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 and flavor compounds, and microorganisms.[31] Table 6, reveals that the PLE microcapsules with GRMD (1 : 1) had an initial total flavonoid content of 57.04 ± 1.06 mg QE/100 g db and the final TFC value was 23.48 ± 0.12 mg GAE/100 g db on 90-day storage. Moreover, the TFC values steadily decrease with increasing the storage time. This degradation of bioactive compounds is probably caused by oxidation and polymerisation processes.[32]

Analysis of antioxidant activity by DPPH assay is based on the principle of measuring the ability to remove free radicals. The

Table 7 Effect of packaging conditions on moisture content and water activity of PLE microcapsules during storage[a]

	Package type			
	Aluminum foil laminated bag		HDPE bag	
Duration (days)	Packed under atmospheric condition	Packed under vacuum condition	Packed under atmospheric condition	Packed under vacuum condition
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}

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

antioxidant activity (AA) values obtained for each packaging bag and storage condition were evaluated at the beginning of storage time (day 0) and are presented in Table 6. The PLE microcapsules stored in aluminum foil laminated bag sealed under vacuum and atmospheric conditions for 90 days at ambient temperature had a significant loss of antioxidant activity by DPPH assay, from 324.75 ± 2.50 to 314.75 ± 4.39 mM Trolox/100 g db and the samples stored in HDPE under vacuum packaging and atmospheric packaging showed a loss from 318.50 ± 1.91 to 298.50 ± 4.51 mM Trolox/100 g db along the shelf life. The highest DPPH was obtained in the sample packed in an aluminum foil bag with 709.12 ± 5.26 mM Trolox/100 g db followed by HDPE packaging under vacuum conditions. For FRAP values, all samples began with an identical initial value of 739.39 ± 2.19 mM Trolox/100 g db at day 0 and then consistent decline occurred across all packaging types and conditions over time as shown in Table 6. This study was in line with the study by Ramakrishnan *et al.*,[33] who studied the shelf life of tamarillo powder at 28 days and found that the amount of flavonoids and phenolic compounds and antioxidant effects tend to decrease. Increasing shelf life decreases the stability of bioactive compounds due to the decomposition of anthocyanin and carotenoids.

3.6 Effect of packaging and storage condition on moisture content, water activity and color changes of spray dried pandan leaf extract (PLE) microcapsules

The moisture content of microcapsule samples is an important factor in the study of the shelf life of natural extract products, since moisture contributes to the movement of molecules, thus accelerating the oxidative reaction of phenolic compounds during storage. PLE microcapsules have an initial moisture content of 3.85%. When the shelf life of storage increased, the moisture content of the microcapsules stored under vacuum and atmospheric conditions showed an increased value up to 6.71%, as shown in Table 7. In terms of water activity, the PLE microcapsules packed in HDPE bag under atmospheric condition showed higher content (0.57) compared to the samples stored under vacuum condition (0.53). The observed increase in moisture content and water activity, even in packaged samples, may be attributed to the lack of 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 retained a water activity level of <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 Fig. 3a–c. The decrease in L^* (lightness) and increase in a^* (redness/greenness) and b^* (yellowness/blueness) suggests oxidative changes in the pigments present in PLE microcapsule, 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

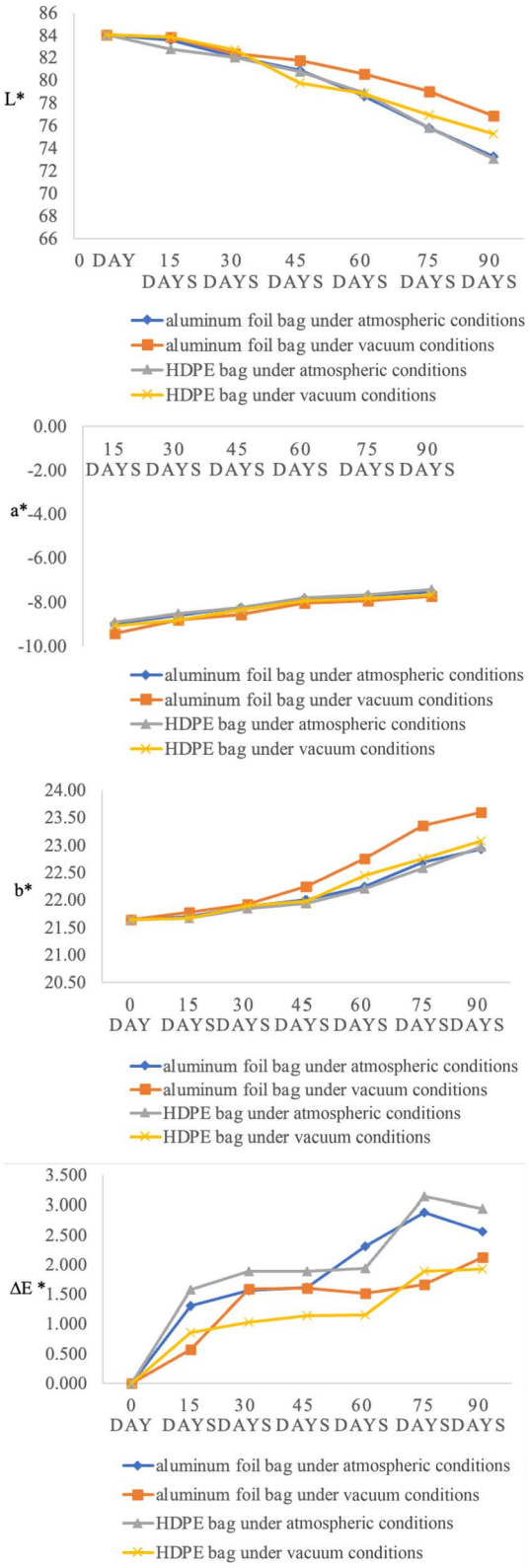

Fig. 3 (a) L^* of PLE microcapsules during storage. (b) a^* of PLE microcapsules during storage. (c) b^* of PLE microcapsules during storage. (d) ΔE^* of PLE microcapsules during storage.

aluminum foil bag under vacuum condition (2.13) compared to those stored in HDPE bag under atmospheric condition (2.94), as shown in Fig. 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 by Wong and Lim,[37] who reported that the ΔE^* value of papaya sample powder is also increased after 7 weeks of storage; this may be attributed 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 (DPPH and FRAP assays). 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-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.

Author contributions

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.

Conflicts of 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

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

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