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Marigold Flower (*Tagetes erecta*) Extract Microcapsules by Ultrasound-Assisted Extraction and Spray Drying: Antioxidant and Antimicrobial properties

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Abstract: Ultrasound-assisted extraction (UAE) of marigold flower using response surface methodology was conducted. The marigold flower extract (MFE) obtained at temperature (40 °C), time (15 min), and ethanol concentration (68% v/v), showed the highest total phenolic compounds, total flavonoid content, total carotenoid content, and antioxidant activity by DPPH assay and FRAP assay. MFE microcapsules using spray drying were prepared in 45% maltodextrin (MFE:MD; 1:1; 1:2) and 20% gum arabic (MFE:GA; 1:2; 1:3). MD (1:2 ratio) sample showed highest encapsulation yield (%) compared to other samples. However, 45% MD (1:1 ratio) sample had the highest encapsulation efficiency than the other samples ($p \leq 0.05$). 45% MD (1:1 ratio) and 20% GA (1:2 ratio) samples revealed the highest moisture content, while 45% MD (1:1 ratio) sample had the lowest water activity ($p > 0.05$). 45% MD (1:1, 1:2) samples showed the increased L* and a* values in comparison with 20% GA samples that exhibited increment in b* values ($p \leq 0.05$). Micrographs of 20% GA (1:3 ratio) and 45% MD (1:2 ratio) samples displayed spherical shape, and smooth surface without major pleats. Highest TPC was analyzed in 20% GA (1:2 ratio) sample compared to other microcapsules ($p \leq 0.05$). Therefore, UAE with RSM aided in the production of plant based functional food ingredients.

Keywords: response surface methodology; ultrasonication; marigold flower; polyphenols; microcapsules; antimicrobial activity

1. Introduction

Consumers demand towards functional food ingredients is increasing worldwide that could enhance the therapeutic properties of food products [1]. Functional food ingredients could serve as a reliable substitute to chemical additives in food and pharmaceutical industries to prevent non-communicable diseases [2]. Phytochemicals attained from plant sources such as stem, leaf, flower, and fruit waste including seed have been investigated to exhibit enormous bioactive properties [3]. Total phenolic profile of plant-based extracts has been reported with higher antioxidant and antimicrobial properties [4]. For the extraction of bioactive compounds, aqueous ethanol as safe solvents have been employed in conjunction with the non-thermal technologies. Moreover the novel extraction technologies might minimize the loss of active ingredient during extraction, preparation and fortification process for functional food development [5].

Flower petals are a rich source of bio-colorants and diversity of polyphenols. Marigold (Tagetes spp.) a specie of Asteraceae family abundant in carotenoids such as lutein, fatty acid esters and diesters [6]. Extract from marigold flower have been used since Middle Ages as a culinary and medicinal purposes. Marigold flowers and leaves contain physiologically active phytochemical essential oil employed in a variety of illnesses viz skin allergies, wounds, burns, kidney problems, and menstrual irregularities [7]. Marigold petals are yellow to orange colored due to the presence of flavonoid, essential oil, and other pigments employed in Ayurveda system of medicine against

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several physiological and neurological disorders [8]. Additionally, Tagetes species are yellowish being used as bio-colorants in culinary services [9].

Plant based biological materials can be extracted using conventional (Soxhlet, maceration, and hydro distillation) and novel methods (ultrasonication, enzyme, and microwave, pulsed electric field, supercritical fluid, and pressurized liquid extraction technologies) [10].Ultrasound assisted extraction (UAE) could minimize the processing hour and boost the extraction rate and production in comparison with conventional methods [11].UAE having frequencies between 20 kHz and 100 MHz has physical specifications such as ultrasonic power, temperature, and processing time with an impact not only on the extraction yield but also on the extract composition [12]. Response surface methodology (RSM) generally utilizes a second-order polynomial model commonly used to describe the interaction between the response variables and the independent variables. The second-order model includes both linear and quadratic terms of the independent variables such as extraction time, temperature and solvent concentration, as well as interaction terms [13].

Microencapsulation is a valuable technique for the food and beverage industries, helps protect sensitive ingredients such as vitamins, minerals, flavors, and active compounds from degradation caused by exposure to light, oxygen, moisture, and other environmental factors [14]. It can also enhance the stability, shelf life, and bioavailability of these ingredients. Among the various methods of microencapsulation, spray drying is a widely used technology because of its affordability, scalability, and versatility. Spray drying involves atomizing a liquid mixture containing the core material and the coating material into droplets, which are then dried by hot air to form small, dry, free-flowing particles [15]. Several studies have been published on spray dried microcapsules containing plant extracts. However, from the current status of scientific literature, there are no records available on the RSM optimized ultrasonic extraction of marigold flower petals. Therefore, marigold flower extract was prepared and characterized for physicochemical properties without and with encapsulation in different ratios of maltodextrin and gum arabic. Moreover, the bioactivity of MFE was measured and microstructural analysis was done for microcapsules. In general, this study focused on the development of functional MFE microcapsules for future food applications.

2. Materials and Methods

2.1. Sample Procurement and Preparation Marigold Flower Powder

Marigold flower samples from the fresh flower market in Bangkok, Thailand were bought in the month of August 2022, and were brought to Food Technology Laboratory, Chulalongkorn University. On arrival at the laboratory, marigold flower petals were gently rinsed with distilled water to get rid of dirt. The cleaned petals were placed in hot air oven (Memmert, DO 6062, Germany) for 10 hr at 60 °C to adjust the moisture content of < 5%. The dried sample was blended and screened using a sieve (50-mesh size) to obtain marigold flower powder (MFP) and stored in an aluminum-laminated bags at -20 °C for further analysis.

2.2. Response Surface Methodology, Optimization, and Preparation of Marigold Flower Extract

RSM technique equipped Box-Behnken Design (BBD) was applied for the maximum extraction condition on MFP samples using various independent variables as shown in Table 1. The three independent variables were ethanol concentration (A), temperature (B), and time (C) which presented three levels (-1, 0, 1) in numeral form as shown in Table 1. Briefly, MFP sample (3g) was mixed with 100 mL (60 %, 80 %, and 100 %) of ethanol and the beakers were placed in ultrasonic bath (Elmasonic bath) followed by heating at various temperatures (30, 40, 50 °C) and process times of 5, 10, 15 min, respectively (Table 2). A refrigerated centrifuge (Centrifuge Kubota, series 6000, Japan) was used for the separation of ethanolic extract from the solid residue of all the 17 samples from RSM analysis, set at 10,000 rpm for 10 min. Subsequently, rotary evaporator (Oilbath B-485, BUCHI, Switzerland) was used at 45 °C to obtain ethanol free marigold flower extract (MFE). 10 mL of distilled water was added to the residual MFE samples and held in brown colored glass bottles at 4 °C for further analysis.

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Table 1. The values for Box–Behnken design (BBD) using response surface methodology for optimized extraction of marigold flower extract.

Independent variables	Independent variable codes	Level		
		-1	0	1
Ethanol concentration (%)	A	60	80	100
Extraction temperature (°C)	B	30	40	50
Ultrasonication time (*min*)	C	5	10	15

Table 2. The functional properties of marigold flower extract (MFE) under different extraction conditions using response surface methodology.

	Independent variables			Responses				
Run	**Concentration (%)**	**Temperature (°C)**	**Time (min)**	**TPC (mg GAE/100g dry wt.)**	TFC (mg QE/100g dry wt.)	DPPH (mM Trolox/ 100g dry wt.)	FRAP (mM Trolox/ 100g dry wt.)	Carotenoid content (mg carotenoid/ 100g dry wt.)
1	80	50	15	72.38	95.73	610.50	4627.37	368.23
2	100	30	10	33.59	37.83	606.75	750.74	49.51
3	80	40	10	71.28	66.30	624.25	3364.21	288.62
4	80	40	10	70.29	47.68	625.50	2995.79	266.23
5	100	40	15	69.75	76.19	621.44	1722.11	137.96
6	80	40	10	74.14	49.36	643.62	2501.05	323.17
7	80	30	5	34.69	17.45	609.25	4585.26	414.67
8	80	40	10	72.71	57.83	644.87	2869.47	294.69
9	80	50	5	72.71	56.72	622.37	1869.47	312.94
10	100	50	10	74.14	55.73	627.37	7364.21	79.36
11	60	40	5	79.19	48.37	634.25	2880.00	292.48
12	100	40	5	91.94	92.14	623.31	1995.79	231.12
13	80	40	10	75.20	64.30	634.56	2932.63	266.23
14	60	50	10	69.31	55.24	615.81	9048.42	95.12
15	60	40	15	70.46	50.85	633.62	2427.37	55.04
16	60	30	10	31.39	14.78	581.12	1039.16	184.68
17	80	30	15	34.14	18.33	607.69	2422.32	319.30

Total phenolic compound (TPC), total flavonoid content (TFC), antioxidant activity by DPPH and FRAP assays and carotenoid content.

All 17 sample extracts prepared from RSM values of independent variables were subjected to analysis. The optimum extraction condition of MFE was characterized based on the highest bioactive compounds assayed by total phenolic compound, total flavonoid content, carotenoids content and antioxidant properties detailed in section 2.5.1. After phytochemical characterization, the MFE sample extracted from optimized condition at 68% ethanol concentration, 40 °C temperature, and 15 min of extraction time had the highest phytochemical content and antioxidant properties was subjected to antimicrobial analysis prior to microencapsulation process.

2.3. Antimicrobial Activity of RSM Optimized MFE

2.3.1. Growth Condition

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Microbial cultures including E. coli ATCC 25922, and S. aureus ATCC 25923, were obtained from Faculty of Science, Chulalongkorn University, and inoculated in 10 mL of sterile Muller Hinton Broth (MHB) to obtain inoculum for bacterial culture. The inoculated test tubes containing MHB were grown at 18-24 h at 37 °C. The initial load of test microorganism was approximately 6.0 log CFU/mL by measuring the optical density (Saénz, Tapia, Chávez, & Robert) at 600 nm.

2.3.2. Determination of Inhibition Zones of MFE by Minimum Inhibitory Concentration and Disk Diffusion Method

Antimicrobial activities of RSM optimized MFE were determined using the disc diffusion technique [16]. To obtain the final concentration, a stock extract solution was made by dissolving with relative solvents (ethanol). The sterile, blank discs were soaked with 20 µL of extract and then dried. These discs were placed on Mueller-Hinton agar plates that had previously contained 108 CFU/mL of the target bacteria. To test the inhibitory zone of the MFE samples, ethanol and chloramphenicol were utilized as negative control and positive control in each disc, correspondingly. The plates containing treated discs were incubated for 24 h at 37 °C. Zones of inhibition were determined as diameter and expressed in mm [17]. Antibiotic (chloramphenicol, 32 µg/mL) was used to compare antimicrobial potential of the MFE.

Optimized MFE sample (5 mg/ mL) was mixed with sterile distilled water. Two-fold dilutions of 5, 4, 3, 2, 1, 0.5, and 0.25 mg/mL from the previous solution were then made in sterilized water.

Bacterial inoculum was prepared at 37 °C for 16 hr Mc Farland 0.5 standard was used to assess turbidity of both the bacterial cultures. Additional serial dilution in sterile MHB were carried out to achieve a suitable suspension with at least 1x10-6 CFU/mL. The inoculum (50 µL) of cell cultures were provided to a sterile 96-well plate with 100 µL of MHB. Extract dilutions in 100 µL were added. A positive control (containing only microbial inoculum) was poured into each micro-plate well. The micro-plates were incubated at 37 °C for 24 hr. The OD value at 600 nm was measured with a microplate reader. MIC value indicated no visible growth of the tested bacterial strain.

2.4. Microencapsulation of RSM Optimized MFE in Maltodextrin (MD) and Gum Arabic (GA)

The RSM optimized (68% ethanol concentration, 40 °C temperature, and 15 min) MFE sample was microencapsulated via spray drying in 45% (w/v) maltodextrin (1:1; and 1:2 ratios of MFE to MD), and 20% (w/v) gum arabic (1:2; and 1:3 ratios of MFE to GA) (Table 4). Microcapsules were prepared by mixing optimized MFE sample with the coating material at different ratios. Solution for spray drying was prepared on a magnetic stirrer for 5 min and homogenized. The nozzle size (0-5 mm) of spray drying machine was employed in microencapsulation of MFE in MD and GA used as wall materials. The MD and GA solutions containing MFE were fed into the spray dryer (inlet air temperature of 155 °C and the outlet air temperature of 90 °C), to obtain MFE encapsulated powder in MD and GA. The powders were collected and kept at -20 °C in aluminum-laminated bags until further analysis.

Table 3. Analysis of variance (ANOVA) for determination of optimization model fit.

	TPC (mg GAE/100g dry wt.)				DPPH (mM Trolox/100 g dry wt.)			
Source	**Sum of Squares**	**df**	**Mean square**	**P-value**	**Sum of squares**	**df**	Mean squares	p-value
Model	5361.08	9	595.68	0.0001	2879.70	9	319.97	0.16
A	45.44	1	45.44	0.17	24.72	1	24.72	0.69
B	2992.49	1	2992.49	0.0001	634.57	1	634.57	0.08
C	126.51	1	126.51	0.04	31.75	1	31.75	0.66
AB	1.74	1	1.74	0.77	49.44	1	49.44	0.58
AC	45.30	1	45.30	0.17	0.39	1	0.39	0.96
BC	0.01	1	0.01	0.98	26.59	1	26.59	0.69
A^2	14.69	1	14.69	0.41	129.55	1	129.55	0.38

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B^2	2129.0	1	2129.0	0.0001	1901.32	1	1901.32	0.01
C^2	44.24	1	44.24	0.17	3.11	1	3.11	0.89
Residual	132.75	7	18.96		1040.41	7	148.63	
Lack of Fit	116.64	3	38.88	0.03	663.45	3	221.15	0.21
Pure Error	16.11	4	4.03		376.95	4	94.24	
Cor Total	5493.84	16			3920.11	16		
R^2				0.97				0.73
Adj-R^2				0.94				0.39

	FRAP (mM Trolox/100g dry wt.)				TFC (mg QE/100g dry wt.)			
Source	**Sum of squares**	**df**	**Mean squares**	**p-value**	**Sum of squares**	df	Mean squares	p-value
Model	4.43+07	9	4.92+06	0.42	7147.90	9	794.21	0.04
A	1.59+06	1	1.59+06	0.56	1073.51	1	1073.51	0.05
B	2.49+07	1	2.49+07	0.04	3829.80	1	3829.80	0.0028
C	2157.21	1	2157.21	0.98	87.20	1	87.20	0.52
AB	4.87+05	1	4.87+05	0.74	127.21	1	127.21	0.44
AC	800.5+54	1	800.5+54	0.97	84.96	1	84.96	0.52
BC	6.05+06	1	6.05+06	0.27	363.47	1	363.47	0.21
A^2	2.61+05	1	2.61+05	0.81	13.85	1	13.85	0.79
B^2	7.89+06	1	7.89+06	0.21	1366.44	1	1366.44	0.03
C^2	3.61+06	1	3.61+06	0.38	267.97	1	267.97	0.27
Residual	2.92+07	7	4.18+06		1326.53	7	1326.53	
Lack of Fit	2.89+07	3	9.62+06	0.0003	1040.66	3	346.89	0.08
Pure Error	3.80+05	4	95124.65		285.86	4	71.47	
Cor Total	7.36+07	16			8474.43	16		
R^2				0.60				0.84
Adj-R^2				0.09				0.64

	Carotenoid content (mg carotenoid/ 100g dry wt.)			
Source	**Sum of squares**	**df**	Mean squares	p-value
Model	1.89+05	9	20967.33	0.01
A	2091.99	1	2091.99	0.38
B	1582.19	1	1582.19	0.44
C	17176.18	1	17176.18	0.03
AB	3565.06	1	3565.06	0.26
AC	5205.23	1	5205.25	0.18
BC	5674.03	1	5674.03	0.16
A^2	1.366+05	1	1.37+05	0.0001
B^2	127.09	1	127.09	0.82
C^2	21519.70	1	21519.70	0.02
Residual	16505.75	7	2357.96	
Lack of Fit	14275.60	3	4758.53	0.03
Pure Error	2230.15	4	557.54	
Cor Total	2.052+05	16		
R^2				0.92
Adj-R^2				0.82

Ethanol concentration (A), Temperature (B), Time (C).

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Table 4. Regression coefficient of the predicted second order polynomial models (BBD)for bioactive properties.

	Bioactive properties									
Factor	**TPC**	**p-values**	**TFC**	**p-values**	**DPPH**	**p-values**	**FRAP**	p-values	Carotenoid	p-values
Intercept	72.73		57.10		634.56		2932.63		287.79	
Linear										
A	2.38	0.17	11.58	0.05	1.76	0.70	-445.26	0.56	-16.17	0.38
B	19.34	<0.0001	21.88	0.0028	8.91	0.08	1764	0.04	-14.06	0.44
C	-3.98	0.04	3.30	0.52	-1.99	0.66	-16.42	0.98	-46.34	0.03
Cross product										
AB	0.66	0.77	-5.64	0.44	-3.52	0.58	-348.95	0.74	29.85	0.26
AC	-3.37	0.17	-4.61	0.52	-0.31	0.96	44.74	0.97	36.07	0.18
BC	0.051	0.98	9.53	0.21	-2.58	0.69	1230.21	0.27	37.66	0.16
Quadratic										
A^2	1.87	0.41	1.81	0.79	-5.55	0.38	249.10	0.81	-180.13	0.0001
B^2	-22.49	<0.0001	-18.01	0.03	-21.25	0.009	1368.89	0.21	-5.49	0.82
C^2	3.24	0.17	7.98	0.27	-0.86	0.89	-925.42	0.38	71.49	0.02

2.5. Physiochemical Properties of MD and GA Microcapsules Loaded with MFE

The encapsulation yield (%) of MFE microcapsules was determined as described by Ramakrishnan [18]. Water activity (aw) analyzer (model MS1, Novasina, Switzerland) was used to determine aw of MFE microcapsules. A moisture analyzer was determined the moisture content (%). The aluminum cups were dried at 105 °C for 2-3 hr and put in desiccator until the temperature of the container is equal to the room temperature and then weighed. The procedure was repeated until the weight obtained was constant. For sample analysis, 0.2 g of sample was put into the aluminum cup and dried at 105 °C for 4-5 hr. Aluminum cups were transferred from hot air oven to the desiccator, cooled down and final weight was measured after several repetitions. The moisture content was figured out based on the following equation 3:

Moisture (%) = (B-A)/ B ⊚ 100 (3)

where 'B and A' are the sample weights before and after drying, respectively.

CIE LAB system (L *, a *, and b *) using chroma meter Minolta CR-400 color meter was employed at room temperature to assess color values of MFE microcapsules. Scanning Electron Microscope (SEM) and energy dispersive X-ray spectrometer were applied to examine the surface characteristics of microcapsules (JEOL, JSM-IT300 Oxford, X-Max N 20) at 30 kV magnifications at 1000 times .

The encapsulation efficiency (%) was evaluated by Saénz [19]. 0.1 g of MFE microcapsule powder was weighed and dissolved in 1 mL of mixed solution (Ethanol: Acetic acid: Water) at a ratio of (50: 8: 42) and vortexed for 1 min. Sample was centrifuged with 10,000 rpm for 5 min and then separated the residue using Whatman No. 1. Surface bioactive compounds were calculated by 0.1 g of MFE encapsulated samples were weighed and dissolved in the mixed solution (ethanol and methanol at a ratio of 1:1) and vortexed for 1 min. The encapsulation efficiency of GA and MD based microcapsules was calculated by equation 4

Efficiency % = TO – SO/ TO * 100 (4)

Where, TO is the total bioactive compounds, and SO is the surface bioactive compounds.

The water solubility was conducted as described by Sarabandi [20]. Briefly, Sample powder (1 g) was put into of distilled water (100 mL) and mixed at 400 rpm for 4 min using a magnetic stirrer. Samples were centrifuged at 4000×g for 4 min. 25 mL of the supernatant was placed in a pre-weighed plate and placed in oven at 105 °C for 5 hr. The solubility was obtained by dividing the weight of the dried supernatant by the initial powder weight.

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2.5.1. Determination of Bioactive Compounds (TPC and TFC), Antioxidant Activity (DPPH and FRAP) and Carotenoids of MFE without and with Encapsulation

MFE (0.5 mL) and MFE microcapsule (1 g) were added into 10 mL of distilled water. MFE microcapsule was mixed with distilled water (10 mL) and vortexed for 3 min. The sample was placed on a shaker at 30 °C for 30 min. After centrifugation at 4000 rpm for 20 min, MFE microcapsule supernatant was collected. MFE solution and MFE supernatant solution were subjected to the analysis of TPC, TFC, carotenoid content, DPPH and FRAP detailed as follows.

Folin–Ciocalteau method as presented by Jafari et al. [16] was used to determine TPC of the MFE samples without and with microencapsulation. 0.5 mL of each MFE and supernatant of MFE microcapsule were diluted in 10 mL of distilled water in an amber vial and mixed with 10% Folin–Ciocalteau's phenol reagent (0.5 mL). After incubation time for 5 min was over, 10% (v/v) sodium carbonate (2 mL) was poured in MFE samples and kept in dark room for 10 min. Finally, readings of all the samples were taken at 765 nm with spectrophotometry method and values were calculated as mg GAE/100 g dry db using gallic acid as standard.

TFC was done according to aluminum tri-chloride as described by Jafari [16]. 1 mL of MFE and supernatant of MFE microcapsule samples were pipetted and added to 1 mL of 2% AlCl3 and subjected to incubation for 10 min in a dark room. The absorbance of the samples was recorded at 430 nm in a spectrophotometer and calculated as mg QE/ 100 g dry db using quercetin as standard.=.

Carotenoid content of MFE and MFE microcapsules was analyzed according to Biswas et al. [21] with some modifications. 250 µL of MFE and supernatant of MFE microcapsule samples were pipetted and mixed with 5 mL of acetone and the sample mixtures were centrifuged at 1370×g for 10 min. After that the supernatant was collected and the remaining samples were subjected to the extraction again using acetone (5 mL). Finally, both supernatants were collected after filtration. Readings of all the samples were noted at 450 nm with spectrophotometry method using acetone as a blank and revealed as mg carotenoid/100 g db.

The antioxidant activity by DPPH method of MFE and MFE microcapsules was described by Brand-Williams et al. [22]. 250 µL of MFE and supernatant of MFE microcapsule samples were pipetted and blended with 4.75 mL of DPPH and the mixture solution was kept in a dark room for 15 min for incubation. The absorbance of samples (A final) was measured at 515 nm in a spectrophotometer using distilled water as a blank. The difference of the absorbance values (Adiff.) was indicated as mM Trolox/ 100 g dry wt. (equation 1).

$$A\ difference = A\ initial - A\ final. \quad (1)$$

The antioxidant activity by FRAP method of MFE and MFE microcapsules was described by Benzie and Strain [23]. 50 µL of MFE and supernatant of MFE microcapsule samples were pipetted and mixed with 950 µL of FRAP and for 4 min in a dark room. The absorbance of samples (A final) was measured at 593 nm in a spectrophotometer using distilled water as a blank. The difference of the absorbance values (Adiff.) calculated mM Trolox/ 100 g dry wt. (equation 2).

$$A\ difference = A\ final - A\ initial \quad (2)$$

2.7. Statistical Analysis

Using the Design Expert 11 program, the Box-Behnken design were implemented to approximate the optimum condition from the UAE experiment (Stat-Ease, Inc., U.S.A.). Three-dimensional (3D) model designs were also created using Design Expert 11 software. All physical and chemical analyses were conducted in triplicates (n=3), and data were interpreted with SPSS version 20.0 statistic software. Duncan's multiple range test was applied to demonstrate significant differences ($p \leq 0.05$) among the microencapsulated samples in a one-way variance analysis (one-way ANOVA).

3. Results and Discussion

3.1. Optimization of Ultrasound-Assisted Extraction (UAE) of Bioactive Compounds from Marigold Flower Using RSM

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Plant based products including fruits, vegetables and their byproducts that are discarded as waste contain abundant amounts of polyphenols showing antioxidant, and antimicrobial activities. The antioxidant potential of plant based phenolic compounds aid in the reduction of free radicals via hydrogen donation mechanism, to prevent the malfunctioning of life processes in the human body [24]. As indicated in Table 2, the highest TPC value in marigold flower extract (MFE) was attained in 12th run sample set at 5 min, 40 ℃ and 100% ethanol concentration, compared to the 16th sample set at 10 min, 30 ℃ and 60% ethanol concentration. Three independent variables including time, temperature, and ethanol concentration showed as linear effect on extraction of TPC from MFE with marked significant ($p \leq 0.05$). Table 3 presents ANOVA results of significant differences in linear, quadratic and their interaction terms as indicated by A, B, C on the response values (Y). The model's fit values indicated the genuine form of computed response surface plot. However, lack of fit was insignificant in all the three models (Table 3). The R2 values in the range of 0.60-0.97, revealed that all the three models suited best to the response. The experimental results obtained in TPC following the predicted models were in agreement with the results of Yıkmış [25]. Table 4 displays the regression coefficients of the predicted second order polynomial models for TPC, TFC, carotenoid content and antioxidant activity by DPPH and FRAP assays using BBD. The second-order polynomial model's equilibrium, suggesting the findings of MFE samples with varied conditions of time, temperature, and ethanol concentration are given as follows:

TPC = -339.07602 – 0.423366 A+ 19.64846 B– 0.740277 C+ 0.003297 A* B- 0.033654 A*C+ 0.001099 B* C+ 0.004670 A2 – 0.224864 B2 + 0.129665 C2

Figure 1A-C demonstrates 3D graphic surface plots for the TPC obtained during RSM optimization of MFE samples using A, B, and C independent variables (Table 1). In this Figure 1A, the temperature and time combination yielded lowest TPC values at 30 ℃. The higher increments in TPC values were attained with the increase in UAE processing time and temperature. Therefore, temperature goes up the diffusion coefficient, which accelerates the diffusion rate and TPC values. Figure 1B TPC results of MFE as a function of temperature, and ethanol content. The experimental results from the 3D graphics demonstrated that rising ethanol content from 60% to 100% (v/v) decrease the TPC values. Furthermore, the effects of ethanol concentration and time on TPC values of MFE samples are revealed in Figure 1C which indicated that increasing processing time intend to increase extraction efficiency of TPC. UAE process is an efficient technology for the extraction of high-quality intracellular compounds in a short period of time Wen et al. [26].

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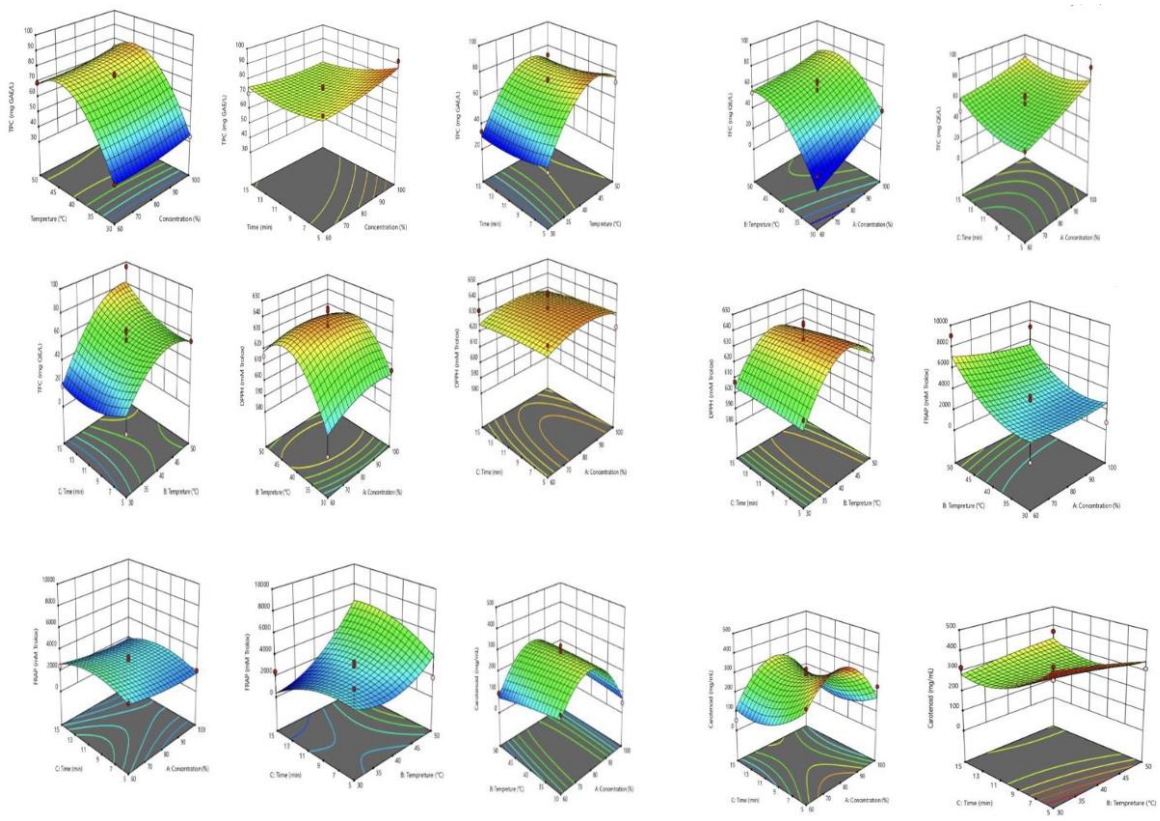

Figure 1. Three dimensional plots of response surface methodology of total phenolic compounds, total flavonoid compounds, antioxidant activity by DPPH and FRAP assays, and carotenoid content as a function of significant interaction between temperature and ethanol concentration(1ST row); time and ethanol concentration (2nd row); time and temperature (3rd row) of marigold flower extract.

The findings from Table 2 revealed that the minimum TFC values were evaluated at the condition (10 min, 30 °C and 60% ethanol), while the maximum TFC value was obtained at a condition of 80% ethanol (v/v), 50 °C and 15 min. Temperature, time, and ethanol concentration showed statistically significant linear impacts on TFC values in MFE samples ($p \leq 0.05$) (Table 3). The linear effect of ethanol and temperature and the quadratic effect of temperature had significant effect on TFC values. The equilibrium of the second-order polynomial model, expressing the effect of temperature, time, and ethanol concentration on TFC value using RSM analysis, was characterized as follows according to the following equation:

TFC = -361.50901 + 1.44252 A+ 16.94897 B– 9.66077 C– 0.028197 A* B– 0.046088 A* C+ 0.190649 B* C+ 0.004534 A2 – 0.180147 B2 + 0.319107 C2

TFC's lack of fit test was insignificant ($p>0.05$), which expressed as the proposed regression equation generated less errors when comparing findings from the experiments and that independent variables had substantial effects on the outcomes. The coefficient of determination (R2 = 84.35), as well as the adjusted coefficient of determination (R2 Adj = 64.22) (Table 3), showed a high degree of fit and appropriateness in predicting experimental results.

In Figure 1D, the effects of time and temperature on the amount of TFC of the MFE samples indicated that TFC values of the MFE sample were the minimum at 30 °C. TFC values increase with increasing extraction time because longer extraction time can increase the mass transfer velocity and then release the bioactive compounds from plant matrix by destroying the plant cells. Figure 1E, described that TFC values was slowly increased until 100% ethanol concentration (v/v). In Figure 1F, it showed that the lowest extraction time for TFC value was 10 min then it increased continuously, and the maximum value was at 15 min. Ethanol is a polar solvent that is used to extract bioactive chemicals from plant matter. Factors that affect the extraction efficiency of phenolic compounds also have an impact on the extraction of flavonoid compounds, according to Salehi et al.[27] . The second-

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order polynomial model, describing the effect of temperature, time and ethanol content in equilibrium on antioxidant activity by DPPH assay of MFE using RSM analysis, was described as follows:

DPPH = +84.32812 + 3.04102 A+ 19.81250 B+ 2.60156 C– 0.017578 A*B– 0.003125 A*C– 0.051563 B* C– 0.013867 A2 – 0.212500 B2 – 0.034375 C2

Table 2 shows that the lowest antioxidant activity by DPPH assay was found at 60 % ethanol concentration (v/v), 30 °C, and 10 min, while the highest amount was revealed at 40 °C and 80 % ethanol concentration (v/v) for 10 min. According to ANOVA, the quadratic effect of temperature utilized in MFE samples on DPPH values were statistically significant ($p \leq 0.05$). Whereas the linear effects of temperature, time and ethanol concentration and the relation between temperature and time, time and ethanol concentration, temperature and ethanol concentration and the quadratic effect of concentration and time applied to MFE samples on antioxidant activity by DPPH assay was statistically insignificant ($p > 0.05$). Because the lack of fit test for DPPH values was insignificant ($p > 0.05$), the model was well fitted. The coefficient of determination (R2 = 73.46) and adjusted coefficient of determination (R2 Adj = 39.34) demonstrated a high degree of fit and appropriateness in predicting experimental results (Table 3). High R2 values indicated that the quadratic model was very effective at fitting the data, and adjusted R2 (R2 Adj) indicated that the predicted and experimental results of the model were in good agreement.

In addition, the surface plots of three-dimensional responses for the antioxidant activity by DPPH assay of MFE samples are demonstrated in Figure 1G. Figure 1G indicates the effect of extraction time and temperature on the antioxidant activity by DPPH assay and antioxidant activity increased with increasing time. Figure 1H depicts the effect of ethanol concentration as well as temperature on DPPH. The antioxidant activity of DPPH improved when the temperature was raised to 40 °C, but declined when the temperature was raised to 50 °C. When the ethanol concentration was raised by more than 80% (v/v) in Figure 1I, the antioxidant activity by DPPH assay begins to decline. This research is also in line with Liyana-Pathirana & Shahidi [28] who discovered that utilizing ethanol concentrations of 60 to 80 % (v/v) resulted in better antioxidant activity than using the same ethanol concentration with a longer extraction period evidenced by antioxidant activity by DPPH assay. It is also approved that the extraction period must be long enough to avoid bioactive components degrading and resulting in reduced antioxidant activity [29]

The linear impact of temperature on antioxidant activity by FRAP assay in MFE samples were statistically significant ($p \leq 0.05$). On the other hand, the quadratic effects and relationship of time, temperature, and ethanol concentration implemented to MFE samples on FRAP values were statistically insignificant ($p > 0.05$) (Table 3). The equilibrium of the second-order polynomial equation describing the effect of temperature, time, and ethanol concentration on the antioxidant activity of MFE samples as measured by FRAP values was as follows:

FRAP = +24493.26316 – 56.58947 A– 1025.17895 B– 282.90526 C– 1.74474 A* B+ 0.447368 A* C+ 24.60421 B* C+ 0.622763 A2 + 13.68895 B2 – 37.01684 C2

The quadratic model's high R2 values indicated the data that fitted well under experimental conditions, and the modified coefficient of determination revealed the model's adequacy in predicting experimental findings as well as its high degree of fit. The minimum antioxidant activity measured by FRAP value was discovered at 100% ethanol concentration and 30 °C for 10 min, whereas the greatest FRAP value was obtained at 50 °C and 60% ethanol concentration for 10 min. Surface plots of three-dimensional responses for the effects of temperature and time, temperature and ethanol concentration, and ethanol concentration and time were shown in Figure 1. The results from Figure 1J demonstrated that antioxidant activity didn't show any significance changes when the temperature rises from 30 °C to 40 °C. However, when the temperature rises over 40 °C, the antioxidant activity by FRAP assay increased. Figure 1K demonstrated that increasing ethanol concentration caused a decline of antioxidant activity by FRAP values. Additionally, Figure 1L indicates the effect between ethanol concentration and time showed that the minimum value of antioxidant activity was calculated at 10 min with 100% ethanol concentration (v/v).

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According to the analysis, the linear effects of time and the quadratic effects of ethanol concentration and time applied to MFE samples on carotenoid were statistically significant ($p \leq 0.05$) (Table 3). The remaining quadratic and the interaction between temperature, time and ethanol concentration utilized to MFE samples on carotenoid content were not statistically significant ($p > 0.05$) (Table 3). The equilibrium of the second-order polynomial model, describing the effect of temperature, time, and ethanol concentration on total carotenoid content (TCC) of MFE using RSM analysis, was described as follows:

TCC = -1115.00719 + 61.66381 A– 16.48538 B– 125.44919 C+ 0.149270 A* B+ 0.360736 A* C+ 0.753262 B* C– 0.450316 A2 – 0.054940 B2 + 2.85963 C2

The lowest TCC was discovered at 100% ethanol concentration and 30 °C for 10 min, while the greatest TCC value was recorded for 5 min at 30 °C and 80% ethanol concentration. Surface plots of three-dimensional responses for the effects of temperature and time, temperature and ethanol concentration, and ethanol concentration and time were shown in Figure 1. The effect of extraction time and temperature on TCC is seen in Figure 1M, where the TCC value increased as the extraction period increased. Because long extraction periods produce more cell wall breaking due to ultrasound. The duration of the extraction process is crucial in the extraction of carotenoids because prolonging the processing time of the solvent with the solids may promote the diffusion of the compounds and resulting in carotenoids being more easily discharged from the matrix into the extraction medium. Similar to that, ethanol concentration is crucial to getting the most carotenoids back. Figure 1N demonstrated that increasing ethanol concentration up to 80% result in increased carotenoid content and then decrease when increased ethanol concentration to 100%. This is possibly because ultrasound waves spread more widely in aqueous solutions, using a solvent with water can lead to more radicals' production because of the ultrasound-induced dissociation of water. The extraction efficiency of the target chemicals may reduce because the oxidative reaction and the extraction reaction can coexist [30]. In Figure 1O, it showed that the lowest extraction time for TCC value was 10 min then it gradually increased until 15 min.

The second-order polynomial equation and 3D plot results clearly demonstrated that the quadratic regression equation was possible to demonstrate 3D response surface plots and estimate TPC, TFC, antioxidant activity (DPPH and FRAP), and carotenoid concentration in MFE samples (Figure 1A-O). Visually, the curvature of response surfaces can be noticed, which reflects the degree of effect of independent variables in the study value. Temperature (B), time (C), and ethanol concentration (A) found a substantial effect on the biological properties and antioxidant activity of the MFE. Different shapes reflect various interactions between the factors being studied. If the contour plot was elliptical, the interactions between the corresponding variables were very important; nevertheless, a circular contour plot suggested that there were no significant interactions between variables [31]. From RSM results, the optimum condition of ultrasound-assisted extraction for all antioxidants (total phenolic compound, total flavonoid content, total carotenoid content) and antioxidant activity (DPPH and FRAP assay) was 68% (v/v) ethanol concentration, 40 °C temperature and 15 min extraction time from optimized data of RSM. Based on the optimum results of antioxidant constituents, antimicrobial activity by disc diffusion method and minimum inhibitory concentration [32] on Gram positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (Escherichia coli) were further investigated.

3.2. Analysis of Bioactive Properties of MFE

MFE samples extracted from 68% and 80% ethanol (v/v) along with chloramphenicol, and absolute ethanol were analyzed for zone of inhibition and minimum inhibitory concentration (MIC) in S. aureus and E. coli. The diameter of the inhibition zones for the tested bacteria using MFE samples against positive controls such as chloramphenicol and absolute ethanol are shown in Figure 2. The maximal inhibition zone of 9.66 mm was measured in Escherichia coli inoculated plate at i-spot treated with 68% extracted MFE sample (Figure 2A), whereas the minimum inhibition zone of 8.33 mm was measured in Staphylococcus aureus at i-spot as marked in Figure 2B. Additionally, MFE sample extracted in 68% ethanol that showed the highest inhibition zones in the tested bacteria was

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analyzed for MIC. It was found that MIC values of E. coli and S. aureus were 0.25 mg/ mL and 0.5 mg/ mL, respectively. Consistently, the study of De Zoysa et al. [32] demonstrated that S. aureus (Gram-positive) has greater antimicrobial capacity than *E. Coli* (Gram-negative). Gram-negative bacteria can cause resistance through alteration in the outer membrane, such as modifying the hydrophobic character or mutations in porins [33].

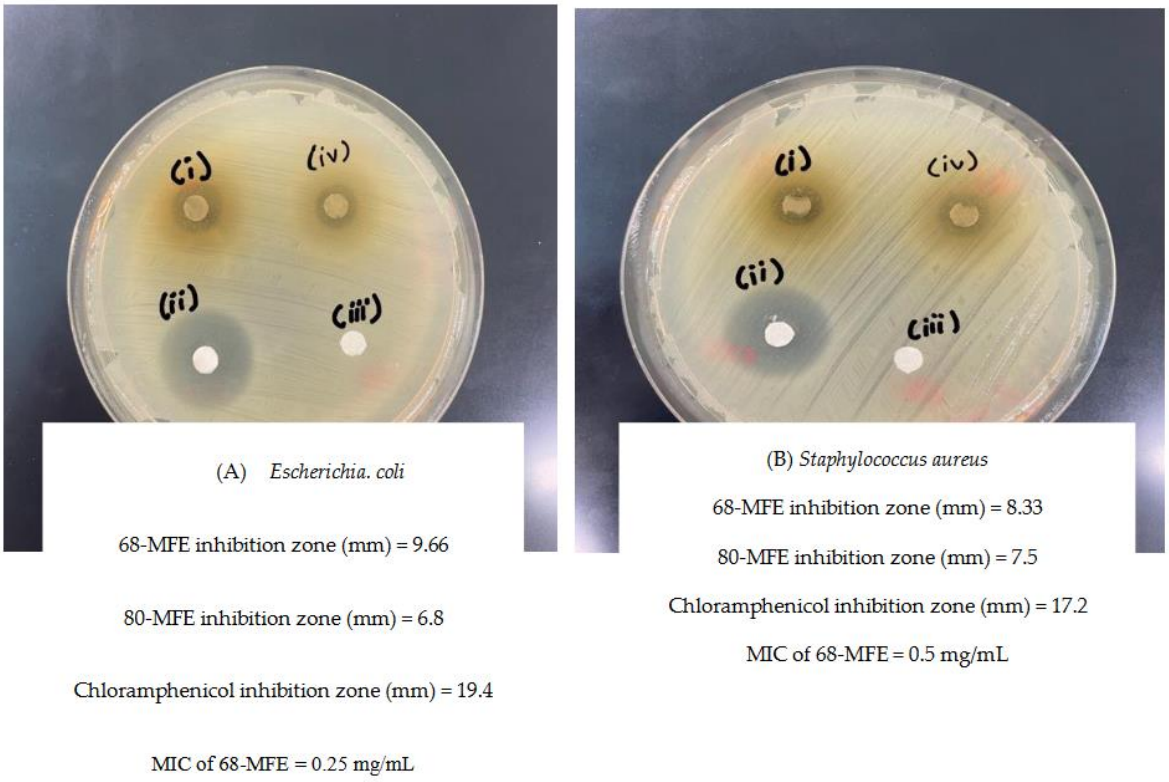

Figure 2. Antimicrobial activity analyzed by clear zone of pathogen inhibition, and minimum inhibitory concentration (MIC), of marigold flower extract (MFE) against (A) *Escherichia. coli*,(B) *Staphylococcus aureus*. The spots (i, ii, iii, iv) indicated68-MFE: MFE extracted in 68% ethanol;80-MFE: MFE extracted in 80% ethanol, chloramphenicol,and absolute ethanol inhibition zones, respectively.

3.3. Impact of Microencapsulation Process on Physicochemical Properties and Microstructure of MFE Microcapsules

3.3.1. Physicochemical Properties of GA and MD-Based MFE Microcapsules

The percentage of yield obtained from MFE microcapsules from spray drying was between 53.59 ± 0.70 and 79.2 ± 0.56 ($p \leq 0.05$) as shown in Table 5. It was noted that the use of 45% MD (1:2) sample gave the highest yield percentage (79.2%), compared to GA samples ($p \leq 0.05$). In addition, GA provided the lowest yield percentage (53.59%). Furthermore, the results indicated that the moisture content was in the range between 3.19 ± 2.05 to 4.35 ± 0.13, while the water activity was between 0.10 ± 0.04 and 0.15 ±0.07. Water activity for dried food product should be lower than 0.6 and moisture content should be less than 8%. The color values of MFE microcapsules after spray drying for L* (lightness), a* (green, red), and b* (blue, yellow). L* Indicates brightness from 0-100, which 0 means black, and 100 means white (very bright). The color values L*, a*, and b* ranged from 58.16 ± 3.81 to 73.18 ± 1.51; -3.17 ± 0.13 to -5.99 ± 0.14; and 19.02 ± 0.73 to 30.45 ± 1.59, respectively. Where 45% MD (1:1) sample containing MFE described the highest brightness with 73.18 ± 1.51 in comparison with the 20% GA (1:2) sample showed the least lightness (L* = 58.16 ± 3.81). The encapsulation efficiency of the MFE microcapsules ranges from 78.05 ± 1.04 to 88.00 ± 1.15. It was found that the microcapsules using maltodextrin showed the highest encapsulation efficiency (88.00%) while the using gum arabic as encapsulant resulted in (78.05%). The solubility of MFE microcapsules ranged between 88.41 ± 2.91 and 92.98 ± 4.22%. Using maltodextrin as a coating material showed a highest encapsulation efficiency

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due to its properties in terms of good solubility and low viscosity at high concentration. In addition, increasing in coating material ratio resulted in a higher encapsulation efficiency. It is possible due a thickness of microcapsule could result in the protection improvement of core material [13].

Table 5. Physicochemical and bioactive properties of gum Arabic and maltodextrin microcapsules loaded with marigold flower.

Parameters	Treatments			
	20% GAM (1:2)	**20% GAM (1:3)**	45% MDM (1:1)	45% MDM (1:2)
Yield (%)	53.59±0.70^{d}	56.15±0.73^{c}	61.15±0.58^{b}	79.20±0.56^{a}
Moisture content (%)	4.42±0.18^{a}	3.45±0.13^{b}	4.35±0.13^{a}	3.19±2.05^{b}
Water activity	0.12±0.03^{ab}	0.15±0.07^{a}	0.12±0.03^{ab}	0.10±0.04^{b}
Color values				
L*	61.46±1.55^{c}	58.16±3.81^{d}	73.18±1.51^{a}	71.27±1.39^{b}
a*	-4.47±0.17^{b}	-3.17±0.13^{a}	-5.99±0.00^{c}	-5.23±0.11^{c}
b*	30.45±1.59^{a}	25.09±1.53^{b}	24.46±0.61^{c}	19.02±0.73^{d}
Encapsulation efficiency (%)	80.79±1.12^{b}	80.49±1.85^{b}	88.00±1.15^{a}	78.05±1.04^{c}
Solubility (%)	88.41±2.91^{bc}	92.73±3.20^{ab}	89.31±1.31^{b}	92.98±4.22^{a}
TPC (mg GAE/100g dry wt.)	627.91±24.86^{a}	508.13±9.52^{b}	435.24±8.32^{c}	304.10±7.06^{d}
TFC (mg QE/100g dry wt.)	389.56±9.58^{a}	376.73±10.37^{b}	282.90±1.53^{c}	209.87±4.60^{d}
Carotenoid content (mg carotenoid/ 100g dry wt.)	208.45±2.36a	162.75±1.69^{b}	55.22±1.94^{c}	44.62±2.63^{d}
DPPH (mM Trolox/100 g dry wt.)	1756.66±28.99^{a}	1469.70±31.02^{b}	1217.83±22.68^{c}	794.50±15.61^{d}
FRAP (mM Trolox/100g dry wt.)	4837.89±27.85^{a}	4308.07±26.49^{b}	3076.49±16.08^{c}	2665.96±21.91^{d}

Three replications were used for each microcapsule per each analysis. MFE encapsulated in 45% MD (1:1, w/v); MFE encapsulated in 45% MD(1:2, w/v); MFE encapsulated in 20% GA: (1:2, w/v); (MFE encapsulated in 20% GA (1:3, w/v). MDM; maltodextrin microcapsule, GAM; gum Arabic microcapsule.

3.3.2. Total Bioactive Analysis of GA and MD-Based MFE Microcapsules

The TPC, TFC, TFC, and antioxidant activity by DPPH and FRAP assay of MFE microcapsules obtained from different proportions of GA and MD are presented in Table 5. The amounts of TPC in 20% GAM (1:2), 20% GAM (1:3), 45% MDM (1:1), and 45% MDM (1:2) samples, were 304.10 ± 7.06, 435.24 ± 8.32, 508.13 ± 9.52, and 627.91 ± 24.86 mg GAE/100 g db, respectively. The highest TPC was obtained in 20% GAM (1:2) sample, compared to the 45% MDM (1:2) sample that showed lowest values in all the microencapsulated samples. The total flavonoid content of all the microencapsulated samples was in the range of 209.87 ± 4.60 to 389.56 ± 9.58 mg QCE/100 g db. 20% GAM (1:2) sample had the highest flavonoid content (389.56 ± 9.58 mg QCE/100 g db), while 45% MDM (1:2) sample had the lowest amount of TFC (209.87 ± 4.60 mg QCE/100 g db). The TCC of all the microencapsulated samples was in the range of 44.62 ± 2.63 to 208.45 ± 2.36 mg carotenoid/ 100 g db. The highest TCC was attained in 20% GAM (1:2) sample (208.45 ± 2.36 mg carotenoid/100 g db), in comparison with the 45% MDM (1:2) sample that showed the lowest TCC. In current study, the greater amount of total phenolic compound was found in GA samples as encapsulant. It is possible due to the high polyphenols affinity binding to GA and gum arabic is highly soluble with good emulsification and good film formation which preserve polyphenols during processing [34].

The antioxidant activity of MFE microcapsule samples measured by DPPH and FRAP assay were in the range of 794.50 ± 15.61-1756.66 ± 28.99 mM Trolox equivalent/100 g db and 2665.96 ◎ 21.91 - 4837.89 ◎ 27.85 mM Trolox equivalent/100 g db, respectively. The highest DPPH and FRAP values were evidenced in 20% GAM (1:2) sample and 45% MDM (1:2) sample had the lowest antioxidant values. The highest antioxidant activity by DPPH and FRAP assay was found in the sample used GA

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as encasulant. GA is able to promote Maillard reaction which its products are identified to improve antioxidant activity [35].

3.3.2. Microstructural Analysis of GA and MD-Based MFE Microcapsules

The scanning electron micrographs at a magnification of 1000⊚ of GA and MD-based atomized microcapsules containing MFE are displayed in Fig. 3. It was revealed that 20% GAM (1:3, w/v) samples had the spherical and dent or crimp free shape of microcapsules (Figure 3D). In contrast 20% GAM (1:2, w/v) sample containing lower ratio of GA as wall material produced the microcapsules with surface constriction as indicated in Fig. 3C. However, the 45% MDM (1:2, w/v) sample showed lesser pleats and dents than the 45% MDM (1:1, w/v) sample as shown in Figure 3A-B. Additionally, MD microcapsules at varying ratios (1:1 and 1:2) of MFE to MD were different in terms of capsule appearance and size when compared to GA microcapsules. Contraction and deformation of spray-dried particulate are associated with temperature and liquid dispersion, because longer drying times cause the configuration to change shape, shrink (resulting in roughness), and crumble (which results in breakage) [36]. The formation of concavities in the gum arabic powders may be the result of the particle shrinking that takes place due to the rapid evaporation during the drying process [37]. In the study of Cano-Higuita et al.[36], the microcapsules made entirely gum arabic had smooth surfaces but some teeth on the surface that exhibited shrinkage, whereas the microcapsules made from maltodextrin and modified starch had somewhat round surfaces with wrinkles on the surface but no fractures.

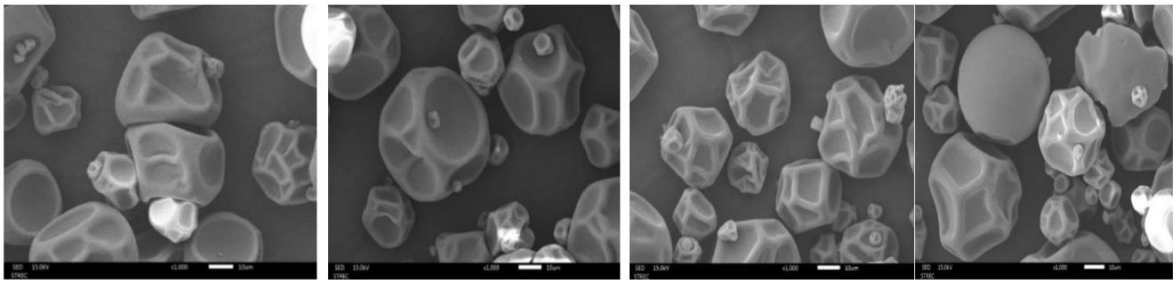

Figure 3. Scanning electron micrographs of marigold flower extract (MFE) microcapsules visualized at 1000⊚ magnification. From left to right: MFE encapsulated in 45% MDM (1:1, w/v); MFE encapsulated in 45% MDM (1:2, w/v); MFE encapsulated in 20% GAM: (1:2, w/v); MFE encapsulated in 20% GAM (1:3, w/v). MDM; maltodextrin microcapsule, GAM; gum Arabic microcapsule.

5. Conclusions

MFE prepared via RSM optimization of ethanol concentration (68%) (v/v), temperature (40 °C) and UAE time (15 min) revealed the highest bioactive compounds and antioxidant activities. Among all the tested samples, MFE sample extracted using 68% ethanol showed the highest zone of inhibition (9.66 mm and 8.33 mm) and MIC values (0.25 and 0.5 mg/ mL) against *E. coli* and *S. aureus*, respectively. MFE sample was encapsulated in 45% MD and 20% GA and the highest yield (79.20%) was attained in 45% MD (1:2) sample. Additionally, the moisture content, water activity, encapsulation efficiency, and solubility was higher in 45% MD (1:2) sample, compared to other samples. However, the highest L*, a* and b* values were visualized in 45% MD (1:1 ratio), 20% GA (1:3), and 20% GA (1:2), respectively. The MFE microcapsules 20% GA (1:1) demonstrated the highest values of TPC (627.91 mg GAE/100g db), TFC (389.56 mg QCE/100g db), TCC (208.45 mg carotenoid/100g db) and antioxidant activity by DPPH (1756.66 mM TE/100g db) and FRAP (4837.89 mM TE/100g db) assays in comparison with other microcapsules. Moreover, the morphology of MFE microcapsules visualized under SEM displayed lesser constriction or dents with bigger microcapsule size in 45% MD, and spherical shaped microcapsules were seen in 20% GA samples. In conclusion, the ratio of GA and MD played a vital role in the formulation of functional microcapsules containing MFE using spray drying technology. These bioactive microcapsules will be value addition to the bio-circular economy and an essential ingredient for functional food development.

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References

1. Janarny, G., Ranaweera, K., & Gunathilake, K. (2021). Antioxidant activities of hydro-methanolic extracts of Sri Lankan edible flowers. Biocatalysis and Agricultural Biotechnology, 35, 102081.
2. Walia, A., Gupta, A. K., & Sharma, V. (2019). Role of bioactive compounds in human health. Acta Scientific Medical Sciences, 3, 25-33.
3. Drabińska, N., Jeż, M., & Nogueira, M. (2021). Variation in the Accumulation of Phytochemicals and Their Bioactive Properties among the Aerial Parts of Cauliflower. Antioxidants, 10, 1597. doi:10.3390/antiox10101597
4. Ahmad Shiekh, K., Odunayo Olatunde, O., Zhang, B., Huda, N., & Benjakul, S. (2021). Pulsed electric field assisted process for extraction of bioactive compounds from custard apple (Annona squamosa) leaves. Food Chemistry, 359, 129976. doi:https://doi.org/10.1016/j.foodchem.2021.129976
5. Spinelli, S. (2016). Study of microencapsulated bioactive compounds in food products.
6. Kaimainen, M., Järvenpää, E., & Huopalahti, R. (2015). Enzyme-assisted oil extraction of lutein from marigold (Tagetes erecta) flowers and stability of lutein during storage.
7. Ćetković, G., Djilas, S., Čanadanović-Brunet, J., & Tumbas Šaponjac, V. (2004). Antioxidant properties of marigold extracts. Food Research International, 37, 643-650. doi:10.1016/j.foodres.2004.01.010
8. Chauhan, A. S., Chen, C.-W., Singhania, R. R., Tiwari, M., Sartale, R. G., Dong, C.-D., & Patel, A. K. (2022). Valorizations of Marigold Waste for High-Value Products and Their Industrial Importance: A Comprehensive Review. Resources, 11(10). doi:10.3390/resources11100091
9. Priyanka, D., Shalini, T., & Navneet, V. K. (2013). A brief study on marigold (Tagetes species): a review. International Research Journal of Pharmacy, 4(1), 43-48.
10. Usman, I., Hussain, M., Imran, A., Afzaal, M., Saeed, F., Javed, M., . . . A. Saewan, S. (2022). Traditional and innovative approaches for the extraction of bioactive compounds. International Journal of Food Properties, 25(1), 1215-1233. doi:10.1080/10942912.2022.2074030
11. Azmir, J., Zaidul, I. S. M., Rahman, M., Sharif, K., Mohamed, A., Sahena, F., . . . Omar, A. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117(4), 426-436.
12. Otles, S. (2016). Handbook of food analysis instruments: CRC Press.
13. Insang, S., Kijpatanasilp, I., Jafari, S., & Assatarakul, K. (2021). Ultrasound-assisted extraction of functional compound from mulberry (Morus alba L.) leaf using response surface methodology and effect of microencapsulation by spray drying on quality of optimized extract. Ultrasonics Sonochemistry, 105806.
14. Calderón-Oliver, M., & Ponce-Alquicira, E. (2022). The Role of Microencapsulation in Food Application. Molecules, 27(5). doi:10.3390/molecules27051499
15. Šeregelj, V. N., Ćetković, G. S., Čanadanović-Brunet, J. M., Tumbas-Šaponjac, V. T., Vulić, J. J., & Stajčić, S. S. (2017). Extraction and encapuslation of bioactive compounds from carrots. Acta periodica technologica, 48, 261-273.
16. Jafari, S., Karami, Z., Shiekh, K. A., Kijpatanasilp, I., Worobo, R. W., & Assatarakul, K. (2023). Ultrasound-Assisted Extraction of Bioactive Compounds from Cocoa Shell and Their Encapsulation in Gum Arabic and Maltodextrin: A Technology to Produce Functional Food Ingredients. Foods, 12(2). doi:10.3390/foods12020412
17. Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Frontiers in Microbiology, 9.

18. Ramakrishnan, Y., Adzahan, N. M., Yusof, Y. A., & Muhammad, K. (2018). Effect of wall materials on the spray drying efficiency, powder properties and stability of bioactive compounds in tamarillo juice microencapsulation. Powder Technology, 328, 406-414. doi:https://doi.org/10.1016/j.powtec.2017.12.018
19. Saénz, C., Tapia, S., Chávez, J., & Robert, P. J. F. c. (2009). Microencapsulation by spray drying of bioactive compounds from cactus pear (Opuntia ficus-indica). 114(2), 616-622.
20. Sarabandi, K., Peighambardoust, S. H., Sadeghi, A., & Samaei, S. (2019). Effect of different carriers on microstructure and physical characteristics of spray dried apple juice concentrate. Journal of Food Science and Technology, 55, 3098–3109
21. Biswas, A. K., Sahoo, J., & Chatli, M. K. (2011). A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. LWT - Food Science and Technology, 44(8), 1809-1813. doi:https://doi.org/10.1016/j.lwt.2011.03.017
22. Brand-Williams, W., Cuvelier, M.E., & Burset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft & Technologie - Food Science and Technology, 28(1), 25-30.
23. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry, 239(1), 70-76.
24. Air, M. B. P. U. B. (2018). Total phenolic, flavonoid content and antioxidant activity of Clinacanthus nutans leaves by water-based ultrasonic assisted extraction. Malaysian Journal of Analytical Sciences, 22(4), 659-666.
25. Yıkmış, S. (2019). Optimization of uruset apple vinegar production using response surface methodology for the enhanced extraction of bioactive substances. Foods, 8(3), 107.
26. Wen, C., Zhang, J., Zhang, H., Dzah, C. S., Zandile, M., Duan, Y., . . . Luo, X. (2018). Advances in ultrasound assisted extraction of bioactive compounds from cash crops–A review. Ultrasonics Sonochemistry, 48, 538-549.
27. Salehi, B., Venditti, A., Sharifi-Rad, M., Kręgiel, D., Sharifi-Rad, J., Durazzo, A., . . . Martins, N. (2019). The Therapeutic Potential of Apigenin. International Journal of Molecular Sciences, 20(6). doi:10.3390/ijms20061305
28. Liyana-Pathirana, C. M., & Shahidi, F. (2006). Antioxidant properties of commercial soft and hard winter wheats (Triticum aestivum L.) and their milling fractions. Journal of the Science of Food and Agriculture, 86(3), 477-485.
29. Sungthong, B., & Phadungkit, M. (2015). Anti-tyrosinase and DPPH radical scavenging activities of selected Thai herbal extracts traditionally used as skin toner. Pharmacognosy Journal, 7(2).
30. Umair, M., Jabbar, S., Nasiru, M. M., Lu, Z., Zhang, J., Abid, M., . . . Zhao, L. (2021). Ultrasound-assisted extraction of carotenoids from carrot pomace and their optimization through response surface methodology. Molecules, 26(22), 6763.
31. Jiao, S., Li, Y., Wang, Z., Sun-Waterhouse, D., Waterhouse, G. I., Liu, C., & Wang, X. (2020). Optimization of enzyme-assisted extraction of bioactive-rich juice from Chaenomeles sinensis (Thouin) Koehne by response surface methodology. Journal of Food Processing and Preservation, 44(9), e14638.
32. De Zoysa, M. H. N., Rathnayake, H., Hewawasam, R. P., & Wijayaratne, W. M. D. G. B. J. I. j. o. m. (2019). Determination of in vitro antimicrobial activity of five Sri Lankan medicinal plants against selected human pathogenic bacteria. 2019.
33. Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules, 25(6). doi:10.3390/molecules25061340
34. Kalušević, A., et al. (2017). "Microencapsulation of anthocyanin-rich black soybean coat extract by spray drying using maltodextrin, gum Arabic and skimmed milk powder." Journal of Microencapsulation, 34(5), 475-487.
35. Pitalua, E., et al. (2010). "Antioxidative activity of microcapsules with beetroot juice using gum Arabic as wall material." Food and Bioproducts Processing, 88(2-3), 253-258.
36. Cano-Higuita, D. M., Vélez, H. A. V., & Telis, V. R. N. (2015). Microencapsulation of turmeric oleoresin in binary and ternary blends of gum Arabic, maltodextrin and modified starch. Ciência e Agrotecnologia, 39, 173-182.
37. Bernstein, A., & Noreña, C. P. Z. (2015). Encapsulation of red cabbage (Brassica oleracea L. var. capitata L. f. rubra) anthocyanins by spray drying using different encapsulating agents. Brazilian Archives of Biology and Technology, 58, 944-952.