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ORIGINAL ARTICLE

Optimisation of spray drying operating conditions of *Morinda citrifolia* L. fruit extract using response surface methodology

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Response surface methodology

Abstract A conventional solvent extract of *Morinda citrifolia* L. fruit was spray dried using adjuvant maltodextrin (5 wt.%). Spray drying was carried out according to the D-optimal design, and the independent variables selected were temperature and M_{core}/M_{wall}. The spray drying process was optimised by using response surface methodology (RSM) for four different responses: moisture content (MC), DPPH scavenging activity, total phenolic content (TPC), and total flavonoid (TF). The effects of temperature and of the core to wall material ratio were found to be significant for all responses. The optimal spray drying condition for maltodextrin as binding material was found to be 1:1.5 (M_{core}/M_{wall}, volume ratio of *M. citrifolia* L. extract to additive solution) at 95 °C. The experimental values of the response variables correspond well to the predicted values. The microparticles obtained in this study represent an interesting food additive for incorporation into functional foods due to the presence of antioxidants.

1. Introduction

Morinda citrifolia L. (*Rubiaceae*), noni a Polynesian medicinal plant has been used in the Pacific islands for more than 2000 years (Dixon et al., 1999; Pawlus et al., 2005). It is a small evergreen shrub or tree growing in tropical and subtropical areas worldwide. Originally native to southeastern Asia, the noni plant was spread to Australia, Hawaii, French Polynesia Islands, and other tropical areas through possible water-dispersal of buoyant seeds, or by being transported by early migrants or voyagers (Degener, 1929; Setchell, 1924). They are also referred as Indian Mulberry, Mengkudu, Hai Ba Ji, Ba Ji Tian, Nono or Nonu, Cheese fruit and Nhau (Deng et al., 2010). The bark, stem, root, leaf and fruit have been used traditionally as folk remedies for many diseases. Especially, as a popular ethno medicine among indigenous Polynesians, noni

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fruits were traditionally used for the improvement in health for those with various health problems, such as cancer, infection, arthritis, diabetes, asthma and pain (Wang et al., 2002). It is primarily used to stimulate the immune system and thus to fight bacterial, viral, parasitic and fungal infections. It is also used to prevent the formation and proliferation of tumours, including malignant ones (Dixon et al., 1999; Earle, 2001). In 2002, the noni juice was also accepted in the European Union as a novel food (Dussossoy et al., 2011).

About 200 phytochemicals have been identified from the noni plant, including anthraquinones, flavonoids, and polysaccharides (Pawlus et al., 2005). The chemical constituents of *M. citrifolia* have been isolated from the fruits, such as fatty acid derivatives (Dalsgaard et al., 2006; Wang et al., 1999, 2000). Recently Kim et al. (2010) isolated two novel fatty acid glucosides 1,6-di-O-octanol-β-D-glucopyranose and 6-O-(β-D-glucopyranosyl)1-O-decanoyl-β-D-glucopyranose from *M. citrifolia* L. fruit. Pharmacologically synergetic effects among the components in noni fruits may account for its diversified health benefits (Deng et al., 2010). In noni fruits, compounds such as scopoletin, rutin, ursolic acid, β-sitosterol, asperuloside and damnacanthal are considered to be predominant (Wang et al., 2002; Levand and Larson, 1979; Pawlus and Kinghorn, 2007; Potterat, 2007). Noni fruit extract has also been claimed to have anti-inflammatory (Mckoy et al., 2002) and antioxidative activities (Ikeda et al., 2009) in several *in vitro* and *in vivo* test systems (Mahattanadul et al., 2011; Yang et al., 2010). In our recent study, the antioxidant potentials of various medicinal plant species were reviewed (Krishnaiah et al., 2011a). *M. citrifolia* L. fruit extract is a good source of natural antioxidants. Hence it is processed into microparticles by spray drying technique.

The microencapsulation technique of spray-drying is an effective way to protect drug or food ingredients against deterioration and volatile losses. The main objective of encapsulation is to protect the core material from adverse environmental conditions, such as undesirable effects of light, moisture and oxygen, thereby contributing to an increase in the shelf life of the product, and promoting a controlled liberation of the encapsulate (Shahidi and Han, 1993). The protective mechanism consists of the formation of a membrane wall that encloses droplets or particles of the encapsulated material. There are currently a variety of means available to prepare such microparticles (Fang and Bhandari, 2010). The choice of a particular method depends on the type of microparticles desired. The properties of the wall structures, and the microparticle size and shape are important considerations. However, in the food and drug industry, spray-drying is still the most popular method for forming microparticles because it is very easy to industrialise and allows for continuous production (Su et al., 2008). Thus, many researchers have used the spray drying technique for the production of microparticles of fruit extract (Tonon et al., 2010; Saenz et al., 2009; Kha et al., 2010; Goula and Adamopoulos, 2010). In our previous study, optimal operating conditions of spray-dried *M. citrifolia* L. fruit extract using κ-carrageenan and maltodextrin were determined (Krishnaiah et al., 2011b). In addition, optimisation of spray drying conditions of *M. citrifolia* L. fruit extract using κ-carrageenan by response surface methodology was studied (Krishnaiah et al., 2009, 2011c).

For microencapsulation by spray-drying, maltodextrin is a good choice as wall material due to their low cost, bland flavour, high water solubility (up to 75%), and low viscosity when in solution (Turchiuli et al., 2005). Maltodextrins are mainly used to reduce stickiness and agglomeration problems during storage, thereby improving product stability (Bhandari et al., 1993).

The response surface methodology (RSM) has been demonstrated to be a powerful tool for determining the effects of each factor and the interactions among them, and this allows process optimisation to be conducted effectively (Bas and Boyaci, 2007). The response surface procedures involve experimental strategy, mathematical methods, and statistical inference, which, when combined, enable users to make an efficient empirical exploration of the system in which they are interested (Myers, 1976). The experimental strategy enables the analyst to explore the response surface with equal precision, in any direction. The experimental design initially limits the region under investigation. Subsequent to the initial investigation, the experimental design enables the analyst to explore the response surface in a systematic manner in the direction that offers to be the most promising for improvement (Bono et al., 2004).

RSM can be applied to any system that has the following key elements: (1) a criterion of effectiveness, which is measurable on a continuous scale (extraction time), and (2) quantifiable independent variables (both controllable and uncontrollable) that affect the system's performance (such as the extraction process, solvent, and drying method). Given these conditions, RSM offers techniques for finding the optimum response of the system in an efficient manner (Powers, 1989). The major advantage of the RSM is that the amount of data needed for evaluation, analysis and optimisation significantly reduces the number of experiments required. RSM is a faster and more economical method for gathering research results than the classic one-variable at a time or full-factor experimentation. The Design-Expert 8.0.2 RSM software package has been used for this purpose. RSM generated the experimental design table using the D-optimal method.

The objective of the present study is to elucidate the effect of adjuvant maltodextrin in the spray-drying of *M. citrifolia* L. fruit extract and to find the optimal processing parameters of spray-drying to create microencapsulated powder *M. citrifolia* L. extract with the best free radical scavenging activity, the highest total phenolic content, the highest total flavonoid content, and the lower moisture content by applying RSM.

2. Materials and methods

2.1. Chemicals

Methanol and ethyl acetate (HPLC grade) were obtained from J.T. Baker (USA). Folin Ciocalteau reagent and tannic acid (TA) were purchased from Merck, Germany. The chemicals 2,2-diphenyl picryl hydrazyl (DPPH), aluminium trichloride ($AlCl_3$), and sodium carbonate were obtained from Sigma Chemicals (St. Louis, USA). A total of 5 kg of *M. citrifolia* L. fruit that had not yet reached the ripening stage was obtained from Kota Kinablu, Sabah, Malaysia. All other chemicals were of reagent grade and were used without further purification.

2.2. Preparation of fruit extracts

M. citrifolia L. fruit was washed with tap water, then with distilled water. The fruit was peeled, and the core (pulp and seed)

was cut into small species. The skin, pulp, and seed were sun-dried for two days. Then, the sample was kept at 60 °C in a hot-air oven for one day to remove moisture content until a constant weight is obtained. The dried fruit was then finely powdered using a mixer. A total of 250 ml of ethyl acetate was added to 25 g of the powdered sample (10 wt.%), and the extraction was performed in a water shaker bath at 35 °C for three days. The supernatant was then separated from the residue by filtration using Whatman No. 1 filter paper. The extracted solution was stored in a closed container and kept at 4 °C before being analysed.

2.3. Spray-drying of the *M. citrifolia* L. fruit extract

Maltodextrin (5 wt.%) was mixed with *M. citrifolia* L. fruit extract at different volume ratios (1:1, 1:1.6, and 1:4) as per the experimental design conditions of RSM, and then stirred to form an aqueous solution. The resulting mixture was then spray-dried using a lab plant spray-dryer SD-05 (pilot scale) with co-current flow (the spray-dried product and the drying air flow were in the same direction). The drying chamber had a diameter of 215 mm and a height of 500 mm. The main components of the system were the feed system of the *M. citrifolia* L. fruit extract consisting of a peristaltic pump, a fluid atomiser (inlet orifice diameter of 0.5 mm), and an air compressor, as well as a feed system for drying the gas, consisting of a blower and an air filter. Finally, a temperature control system and a product control system (cyclone) were also employed. The feed flow rate was kept at 315 ml/hr. The flow rate of the drying air was fixed at 60 m^3/hr, and the atomising air remained at a pressure of 1.1 bar. After spray-drying, the powders were collected through a high efficiency cyclone in a glass container, transferred to a glass vial, and stored in desiccators at ambient temperatures.

Several spray-drying runs were carried out, as per the D-optimal experimental design, to investigate the effects of the inlet drying gas temperature for different volume ratios of *M. citrifolia* L. fruit extract to excipient. Further analyses were performed to determine the moisture content, the antioxidant activity, the total phenolic content, and the total flavonoid content of the microencapsulated powder.

2.4. Moisture content

The moisture content of the spray-dried microparticles was determined by the oven drying method (Rattes and Oliveira, 2007). Samples of the microparticles with predetermined masses were placed in an oven (Memmert), heated to 102 °C, and weighed on an analytical balance (Mettler Toledo PB153-S/FACT, Switzerland) until a constant mass was observed. The product moisture content was determined from the weight loss by averaging three measurements.

2.5. DPPH radical scavenging activity

DPPH is a stable free radical that reacts with compounds that are able to donate a hydrogen atom. Thus, the hydrogen donating abilities of spray-dried *M. citrifolia* L. fruit extract was determined from the change in the absorbance at 515 nm by the Blois (1958) method with slight modifications as that of Praveen and Awang, 2007. The modification is no pH adjustment due to the usage of organic medium, methanol. For free radical scavenging measurements, samples in methanol solution were prepared by dissolving 10 mg of spray-dried powder in 30 ml of methanol and centrifuging for 10 min using a Sartorius Sigma 3–18 K centrifuge. Aliquots of supernatant were added to 3 ml of 0.025 g/l DPPH in methanol. The change in absorbance was measured after 40 min at room temperature using a 4802 UV–Vis double-beam spectrophotometer. Methanol was used as the reference. DPPH (0–100 mg/l) was used to obtain a standard calibration curve. All measurements were made in triplicate.

The DPPH radical scavenging activity was calculated as a percentage according to the following equation:

$$\text{DPPH scavenging activity } (\%) = \left[1 - \frac{\text{Abs}_{515}\text{sample}}{\text{Abs}_{515}\text{DPPH solution}}\right] \times 100$$

2.6. Total phenolic content

The total phenolic content (TPC) was determined according to the Folin Ciocalteau method (Slinkard and Singleton, 1977), with slight modifications as that of Praveen and Awang, 2007. The modification is an estimation of total phenolic content in terms of tannic acid equivalent. Briefly, samples in methanol solutions were prepared by dissolving 10 mg of spray-dried powder in 30 ml of methanol and centrifuging for 10 min using a Sartorius Sigma 3–18 K centrifuge. The supernatant of the sample extract (0.5 ml) was added to 2.5 ml of the 0.2 N FC reagent and allowed to react for 5 min. Then, 2 ml of 75 g/l sodium carbonate was added to the reaction mixture and diluted to 25 ml using distilled water. Finally, the reaction mixture was incubated for two hours at room temperature, and the absorbance was measured at 760 nm using a 4802 UV–Vis double-beam spectrophotometer. Methanol was used as the reference. Tannic acid (0–100 mg/l) was used to produce a standard calibration curve. The total phenolic content was expressed in mg of tannic acid equivalents (TAE/g of spray-dried powder).

2.7. Total flavonoid content

The total flavonoid content was determined using the Dowd method, as adopted by Arvouet-Grand et al., 1994. A total of 5 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.4 mg/ml). Absorption readings at 415 nm using a UV–Vis double-beam spectrophotometer were taken after 10 min against a blank sample consisting of a 5-ml extract solution with 5 ml of methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with catechin (0–100 mg/l) as the standard. The content is expressed as mg of catechin equivalents (CE/g of extract).

2.8. Experimental design

The response surface methodology (RSM) was applied using a commercial statistical package, Design-Expert version 8.0.2 (Statease Inc., Minneapolis, USA) to identify optimum levels of two variables of temperature (°C) and M_{core}/M_{wall} (no unit)

Table 1 Factors and levels tested for the experimental design.

Parameter	Temperature (°C)	M_{core}/M_{wall}
Factor	A	B
Max. parameter	140	1
High level	+1	
Average parameter	115	0.625
Medium level	0	
Min. parameter	90	0.25
Low level	−1	

regarding four responses: moisture content, DPPH scavenging activity, TPC, and TF of spray dried *M. citrifolia* L. fruit extract. The coded and uncoded independent variables used in the RSM design are listed in Table 1. The experiments were designed according to the D-optimal design, as shown in Table 2. The order of experiments has been fully randomised. The data were analysed by multiple regressions using the least-squares method. A second order polynomial equation was used to express the responses as a function of the independent variables.

A quadratic model was used to express the responses as a function of independent variables, where A and B are coded values of temperature and M_{core}/M_{wall}. The test of statistical significance was performed on the total error criteria, with a confidence level of 95%. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of the model was checked by calculating the R^2 and adjusted-R^2. The numerical and graphical optimisation techniques of the Design-Expert software were used for the simultaneous optimisation of the multiple responses. The desired goals for each variable and response were chosen. All of the independent variables were kept within range, while the responses were either maximised or minimised.

3. Results and discussion

3.1. Model fitting

The responses of MC, DPPH scavenging activity, TPC, and TF obtained from the experiments are listed in Table 2. The experimental data were used to calculate the coefficients of the quadratic equation, and Tables 3 and 4 summarise the ANOVA results for the significance of the coefficients of the models and regression coefficient, respectively. For any of the terms in the model, a large regression co-efficient and a small p-value would indicate a more significant effect on the respective response variables. ANOVA showed that the resulting quadratic model adequately represented the experimental data, with coefficients of multiple determinations (R^2) of 1.00, 0.99, 0.91, and 0.97 for the responses of MC, DPPH activity, TPC, and TF for maltodextrin as wall material.

The coefficient of determination, R^2, is the proportion of variation in the response attributed to the model rather than to random error. It has been suggested that a good-fitting model should have R^2 no less than 80%. When R^2 is close to unity, the empirical model is suitable for fitting the actual data. A lower value of R^2 indicates that the model is inappropriate for explaining the relation between variables (Little and Hills, 1978; Mendenhall, 1975).

It should be noted that adding a variable to the model will always increase R^2, regardless of whether or not the additional variable is statistically significant. Thus, a large value of R^2 does not always imply the adequacy of the model. For this reason, it is appropriate to use an adjusted-R^2 of over 90% to evaluate the model adequacy. Only for the TPC of maltodextrin were the adj-R^2 values found to be less than 0.90. Higher adj-R^2 indicated that non-significant terms have not been included in the model.

Table 2 Experimental design conditions and results.

S. No.	Two factors				Responsive value							
	Factor 1	Factor 2	Factor 1	Factor 2	MC%		DPPH scavenging activity%		TPC mg of TAE/g of SDP		TF mg of CE/ g of SDP	
	Temperature (°C)	M_{core}/M_{wall} (no unit)	A	B								
	Uncoded values		Coded values		AV	PV	AV	PV	AV	PV	AV	PV
1	90.00	1.000	−1	1	11.60	11.39	18.58	18.92	33.00	34.50	35.00	36.23
2	140.00	0.625	1	0	6.20	5.81	11.90	11.87	27.00	28.51	30.00	29.91
3	90.00	0.625	−1	0	12.40	12.76	28.36	26.57	54.00	45.72	45.00	42.45
4	140.00	1.000	1	1	4.40	4.38	5.44	5.04	18.00	16.80	22.50	21.39
5	140.00	0.625	1	0	6.00	5.81	11.00	11.87	25.50	28.51	28.00	29.91
6	115.00	0.250	0	−1	9.00	8.60	13.80	13.05	27.00	24.83	32.50	32.51
7	140.00	0.250	1	−1	7.10	7.27	8.06	7.91	25.00	22.70	30.00	29.45
8	90.00	0.250	−1	−1	14.30	14.16	23.80	23.44	39.00	39.43	40.00	39.70
9	90.00	0.250	−1	−1	14.00	14.16	22.50	23.44	37.00	39.43	38.00	39.70
10	90.00	0.250	−1	−1	14.20	14.16	23.00	23.44	38.00	39.43	40.00	39.70
11	115.00	0.625	0	0	7.10	7.17	16.24	16.60	30.00	30.88	35.00	34.12
12	115.00	1.000	0	1	5.60	5.77	9.56	9.36	21.00	19.41	27.50	26.75
13	90.00	1.000	−1	1	11.50	11.39	18.50	18.92	32.00	34.50	36.00	36.23
14	115.00	0.625	0	0	7.00	7.17	16.00	16.60	28.00	30.88	32.50	34.12
15	140.00	1.000	1	1	4.20	4.38	5.20	5.04	18.00	16.80	21.00	21.39
16	140.00	0.250	1	−1	7.00	7.27	8.05	7.91	22.50	22.70	30.00	29.45

AV – actual values; PV – predicted values.

Table 3 Variance analysis for responses.

	Sum of Squares	Degrees of freedom	Mean of squares	*F*-value	*p*-Value prob > *F*
MC					
Regression	191.38	5	38.28	492.05	< 0.0001
Residual	0.78	10	0.08		
Total	192.16	15			
DPPH scavenging activity					
Regression	737.57	5	147.51	218.22	< 0.0001
Residual	6.76	10	0.68		
Total	744.33	15			
TPC					
Regression	1152.97	5	230.59	19.06	< 0.0001
Residual	120.97	10	12.10		
Total	1273.94	15			
TF					
Regression	598.71	5	119.74	57.78	< 0.0001
Residual	20.72	10	2.07		
Total	619.44	15			

Table 4 Estimated coefficients for the experimental design and their VIF.

Co-efficient	Value	Standard error	Confidence interval at 95% confidence		VIF
			Low	High	
MC					
Intercept	7.17	0.16	6.81	7.52	
A-Temperature	−3.48	0.08	−3.66	−3.29	1.04
B-M_{core}/M_{wall}	−1.41	0.09	−1.60	−1.22	1.02
AB	−0.03	0.09	−0.24	0.18	1.02
A^2	2.11	0.17	1.74	2.48	1.06
B^2	0.02	0.16	−0.33	0.37	1.09
R^2	1.00				
Adj-R^2	0.99				
DPPH scavenging activity					
Intercept	16.60	0.47	15.54	17.65	
A-Temperature	−7.35	0.24	−7.89	−6.81	1.04
B-M_{core}/M_{wall}	−1.85	0.25	−2.41	−1.29	1.02
AB	0.41	0.28	−0.21	1.03	1.02
A^2	2.62	0.49	1.54	3.71	1.06
B^2	−5.39	0.46	−6.42	−4.36	1.09
R^2	0.99				
Adj-R^2	0.99				
TPC					
Intercept	30.88	2.00	26.44	35.33	
A-Temperature	−8.61	1.02	−10.89	−6.33	1.04
B-M_{core}/M_{wall}	−2.71	1.06	−5.07	−0.34	1.02
AB	−0.24	1.18	−2.86	2.38	1.02
A^2	6.23	2.07	1.62	10.85	1.06
B^2	−8.76	1.96	−13.12	−4.40	1.09
R^2	0.91				
Adj-R^2	0.86				
TF					
Intercept	34.12	0.83	32.28	35.96	
A-Temperature	−6.27	0.42	−7.22	−5.33	1.04
B-M_{core}/M_{wall}	−2.88	0.44	−3.86	−1.90	1.02
AB	−1.15	0.49	−2.23	−0.07	1.02
A^2	2.06	0.86	0.15	3.97	1.06
B^2	−4.49	0.81	−6.29	−2.68	1.09
R^2	0.97				
Adj-R^2	0.95				

Each of the experimental values was compared to the predicted value calculated from the model (Table 2). The results suggest that the models used in this study were able to identify the optimum operating condition of spray drying of *M. citrifolia* L. fruit extract.

3.2. Response surface analysis of moisture content

The response surface analysis (RSA) of the data in Table 2 demonstrates that the relationship between the moisture content and independent variables is quadratic, with a very good regression coefficient ($R^2 = 1.00$). Eq. (1) shows the relationship between moisture content and the independent variables (temperature (A) and M_{core}/M_{wall} (B)).

$$MC = 7.167 - 3.475A - 1.415B - 0.032AB + 2.114A^2 + 0.017B^2 \quad (1)$$

Figs. 1 and 2(A) are the response surface plot and isopleths showing the effects of temperature and M_{core}/M_{wall} on the moisture content. Temperature showed a negative linear effect and a positive quadratic effect on the moisture content ($p < 0.0001$; $p < 0.0001$). M_{core}/M_{wall} showed a negative linear effect on the response ($p < 0.0001$). The moisture content decreased when M_{core}/M_{wall} increased, which revealed that a higher ratio is favourable for obtaining a low moisture content at a lower temperature. At a higher temperature, the moisture content also decreased when M_{core}/M_{wall} increased, which revealed that a higher ratio of 1.00 is favourable for obtaining a low moisture content. As the temperature increases at the lower ratio of M_{core}/M_{wall} (more binding material), the moisture content decreases, which may be due to the temperature effect on the powder obtained. These results are consistent with other findings (Goula et al., 2004; Quek et al., 2007). However, in the experimental region, the lowest moisture

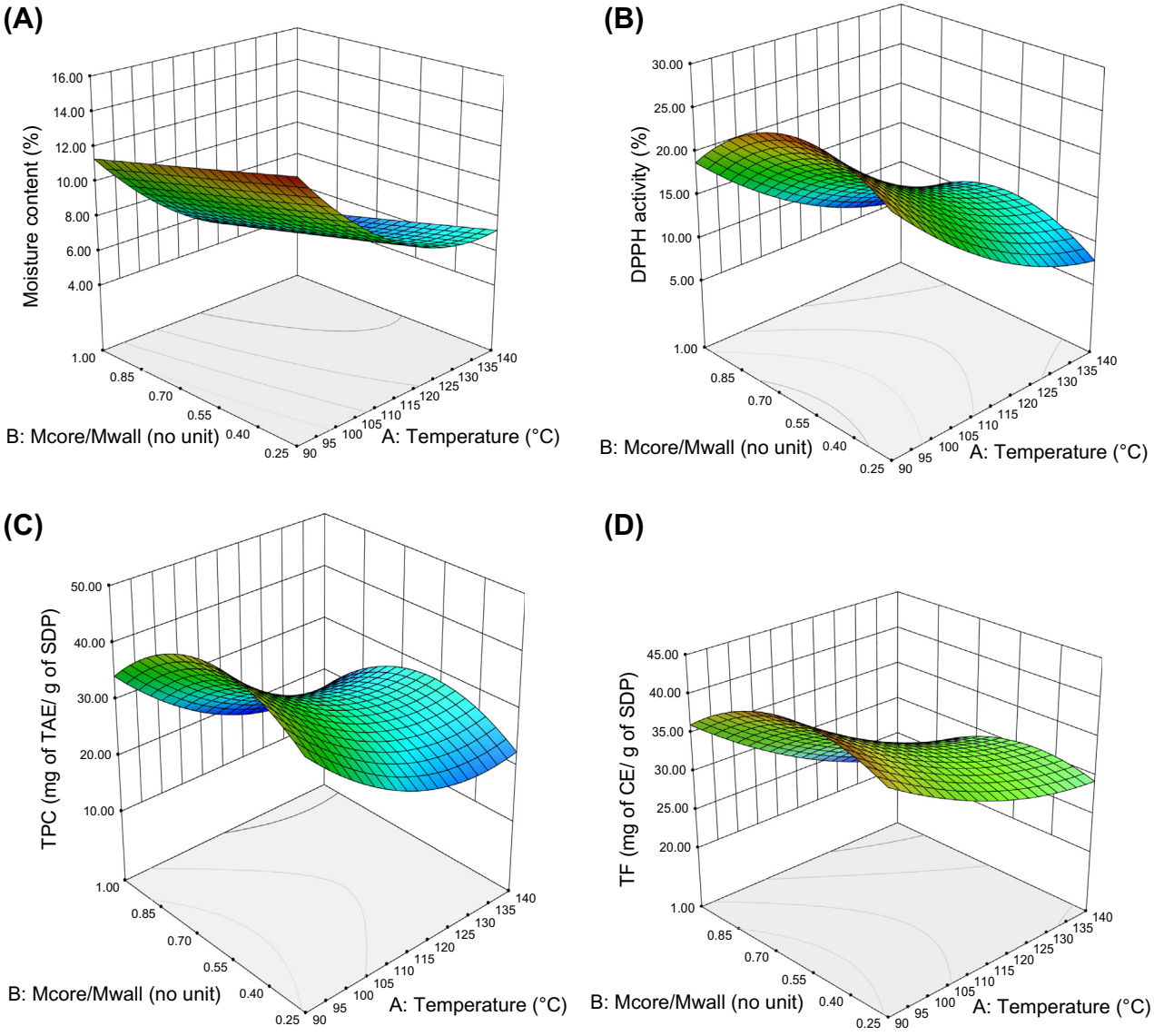

Figure 1 Variations in output experimental design with temperature and M_{core}/M_{wall} (maltodextrin, (A) moisture content, (B) DPPH scavenging activity, (C) TPC, and (D) TF).

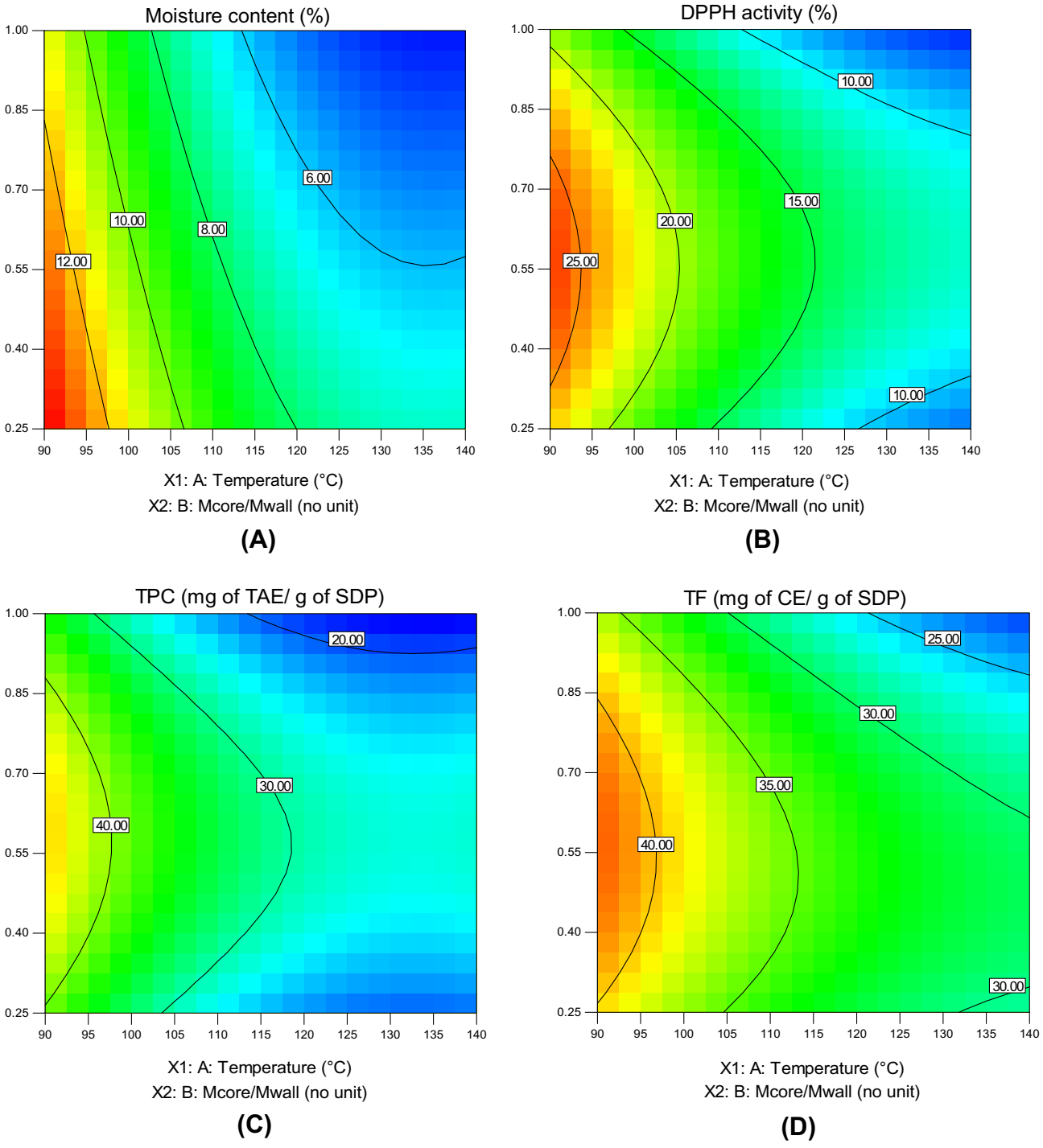

Figure 2 Isopleths of output experimental design with temperature and M_{core}/M_{wall} (maltodextrin, (A) moisture content, (B) DPPH scavenging activity, (C) TPC, and (D) TF).

content was observed for 1:1 at 140 °C. It was also clear that the experimental results of moisture content and the predicted values obtained using Eq. (1) are not significantly different (Fig. 3).

3.3. Response surface analysis of DPPH activity

The RSA in Table 2 demonstrates that the relationship between the DPPH scavenging activity and independent variables is quadratic, with a good regression coefficient ($R^2 = 0.99$). Eq. (2) shows the relationship between DPPH scavenging activity and independent variables (temperature and M_{core}/M_{wall}).

$$\mathrm{DPPH} = 16.595 - 7.352\mathrm{A} - 1.849\mathrm{B} + 0.413\mathrm{AB} + 2.625\mathrm{A}^2 - 5.391\mathrm{B}^2 \quad (2)$$

Figs. 1 and 2(B) are the response surface plot and isopleths showing the effect of temperature and M_{core}/M_{wall} on the

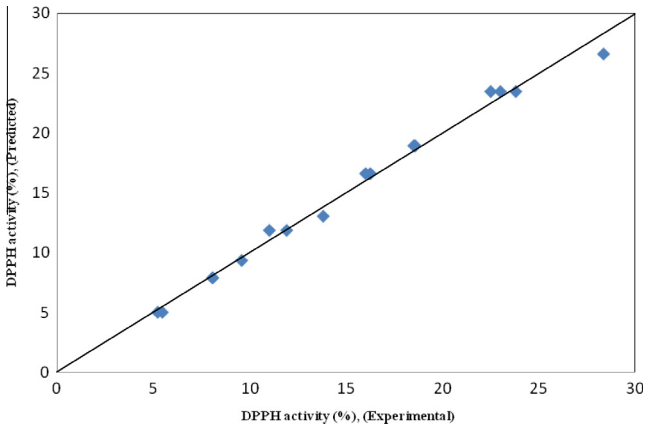

Figure 3 Comparison of the experimental results of dpph scavenging activity with those calculated via D-optimal design resulted equation.

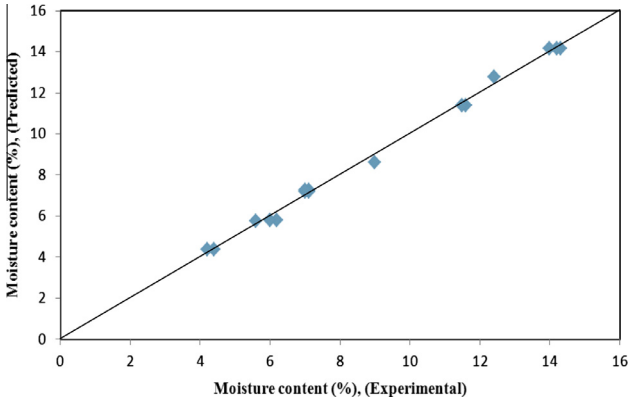

Figure 4 Comparison of the experimental results of moisture content with those calculated via D-optimal design resulted equation.

DPPH scavenging activity. Temperature showed a negative linear effect and a positive quadratic effect on the DPPH scavenging activity ($p < 0.0001$; $p < 0.001$). M_{core}/M_{wall} showed a negative linear effect and a negative quadratic effect on the DPPH scavenging activity ($p < 0.0001$; $p < 0.0001$). The DPPH scavenging activity first increased and then decreased when M_{core}/M_{wall} increased, which revealed that a ratio of 0.85 is favourable for obtaining high DPPH scavenging activity at a lower temperature. At a higher temperature, the DPPH scavenging activity increased and then decreased when M_{core}/M_{wall} increased, which revealed that a ratio of 0.55 is favourable for obtaining high DPPH scavenging activity. As the temperature increases at the lower ratio of M_{core}/M_{wall} (more binding material), the DPPH scavenging activity decreases. From the results it was clear that the activity is higher at a medium ratio and lower temperature, since the ingredients bind effectively to the wall material at these points. That is there is no leakage of active ingredients through the wall material and undesired materials are kept out (Gibbs et al., 1999; Mozafari et al., 2008). However, in the experimental region, the highest DPPH scavenging activity was observed as 1:1 at 90 °C. It was also clear that the experimental results of DPPH and the predicted values obtained using Eq. (2) are not significantly different (Fig. 4).

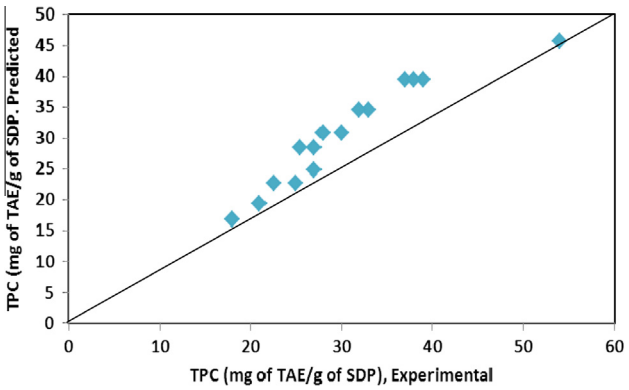

Figure 5 Comparison of the experimental results of total phenolic content with those calculated via D-optimal design resulted equation.

3.4. Response surface analysis of total phenolic content

The RSA of the data in Table 2 demonstrated a good regression value ($R^2 = 0.91$), and Eq. (3) shows the relationship between TPC and independent variables (temperature and M_{core}/M_{wall}).

$$TPC = 30.881 - 8.608A - 2.707B - 0.241AB + 6.235A^2 - 8.761B^2 \quad (3)$$

Figs. 1 and 2(C) are the response surface plot and isopleths showing the effect of temperature and M_{core}/M_{wall} on the total phenolic content. Temperature showed a negative linear effect and a positive quadratic effect on the TPC ($p < 0.0001$; $p < 0.05$). M_{core}/M_{wall} showed a negative linear effect and a negative quadratic effect on the response ($p < 0.05$; $p < 0.01$). The TPC increased gradually and then slightly decreased when M_{core}/M_{wall} increased, which revealed that a higher ratio of 0.85 is favourable for obtaining a high total phenolic content at a lower temperature. At a higher temperature, the total phenolic content first increased and then decreased when M_{core}/M_{wall} increased, which revealed that the ratio of 0.55 is favourable for obtaining a high phenolic content. As the temperature increases at the lower ratio of M_{core}/M_{wall} (more binding material), TPC decreases. From the results, it was clear that as with the DPPH scavenging activity, a higher ratio of 0.85 at a lower temperature resulted in maximum TPC. This is due to phenolic compounds also having diverse biological effects as those of antioxidants (Chung et al., 1998). However, in the experimental region, the highest total phenolic content was chosen as 1:1 at 90 °C. It was also clear that the experimental results of the total phenolic content are slightly lower than the predicted values (Fig. 5).

3.5. Response surface analysis of total flavonoids

The RSA in Table 2 demonstrated a good regression value ($R^2 = 0.97$) and Eq. (4) shows the relationship between TF and independent variables (temperature and M_{core}/M_{wall}).

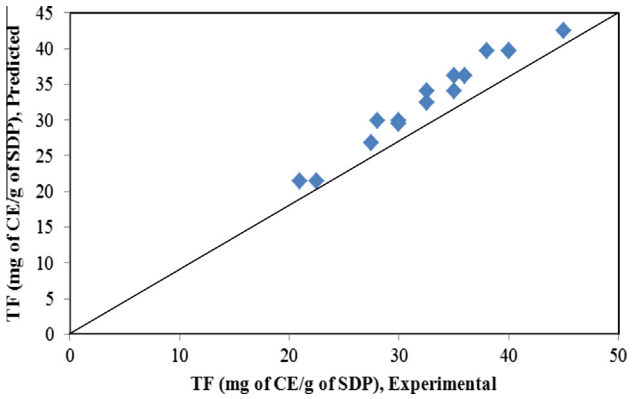

Figure 6 Comparison of the experimental results of total flavonoids with those calculated via D-optimal design resulted equation.

Table 5 Experimental verification of predicted independent variables.

Response	Maltodextrin	
	PV	AV[a]
Moisture content (%)	11.67	11.17 ± 0.33
DPPH scavenging activity (%)	24.78	24.22 ± 0.43
TPC (mg of TAE/ g of SDP)	42.81	42.02 ± 0.41
TF (mg of CE/ g of SDP)	40.97	40.50 ± 0.60

[a] All the experiments were repeated three times.

$$TF = 34.118 - 6.271A - 2.881B - 1.149AB + 2.061A^2 - 4.486B^2 \quad (4)$$

Figs. 1 and 2 (D) are the response surface plot and isopleths showing the effect of temperature and M_{core}/M_{wall} on the total flavonoid. Temperature showed a negative linear effect and a positive quadratic effect on the TF ($p < 0.0001$; $p < 0.05$). M_{core}/M_{wall} showed a negative linear effect and a negative quadratic effect on the TF ($p < 0.0001$; $p < 0.001$). Both independent variables showed a negative interaction effect on the response ($p < 0.05$). The TF increased gradually and then decreased when M_{core}/M_{wall} increased, which revealed that a medium ratio is favourable for obtaining a high total flavonoid at a lower temperature. At a higher temperature, when M_{core}/M_{wall} increased, the total flavonoid increased and then decreased, which revealed that a ratio of 0.70 is favourable for obtaining a high flavonoid content. As the temperature increases at the lower ratio of M_{core}/M_{wall} (more binding material), TF decreases. From the results, it was clear that a medium ratio of 0.70 at a lower temperature results in maximum TF. This shows the positive correlation between DPPH and TF (Krishnaiah et al., 2011b). However, in the experimental region, the highest total flavonoid was chosen as 1:2 at 90 °C. It was also clear that the experimental results of total flavonoid are slightly lower than the predicted values (Fig. 6).

3.6. Optimisation of spray drying operating condition

The spray drying condition would be optimum if the DPPH scavenging activity, TPC, and TF reached maximum values and moisture content reached minimum values. The values of all the responses at operating conditions were converted to a desirability function. The desirability values of the minimum and maximum were configured as 0 and 1, respectively. The maximum desirability function obtained was taken as the optimum operating condition (Bono et al., 2004). The optimal spray drying condition for maltodextrin as the binding material was found to be 1:1.5 (M_{core}/M_{wall}) at 95 °C.

3.7. Verification of predicted independent variables

The suitability of the model equation for predicting the optimum response values was verified using the optimal condition. The experimental values (Table 5) for maltodextrin are 11.17%, 24.22%, 42.02 mg of TAE/g of SDP and 40.50 mg of CE/g of SDP for the four responses of moisture content, DPPH scavenging activity, TPC, and TF, respectively.

4. Conclusions

With the advent of statistical techniques such as RSM and the availability of software packages for problem-solving, it is taking least ever time to optimise processes, products, and the design not only because of increased computer speed but also because the techniques reduce the amount of experimental data required for optimisation analysis. This has led to a better understanding of the process through the results represented in the form of graphs such as 3D surfaces and contours.

Many experiments have been conducted on spray drying of *M. citrifolia* L. fruit extract for different operating conditions: temperature (90–140 °C) and M_{core}/M_{wall} (1:1 to 1:4). An experimental design has been constructed as per the RSM D-optimal design conditions in order to study the influence of the above parameters on moisture content, DPPH scavenging activity, TPC, and TF. RSM and the conventional graphical and desirability function methods have been effective at determining the optimum zone within the experimental region. From the response surface quadratic model, it was found that the spray drying conditions were significantly affected by temperature and M_{core}/M_{wall}. At optimum spray drying condition of maltodextrin, moisture content, DPPH scavenging activity, total phenolic content, and total flavonoids were found to be 11.67%, 24.78%, 42.81 mg of TAE/g of SDP and 40.97 mg of CE/g of SDP respectively. This study also reveals that *M. citrifolia* L. fruit extract is a good source of antioxidants, phenolic compounds, and flavonoids.

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