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MODELLING OF SIMULTANEOUS ENCAPSULATION OF BETACYANINS AND ANTIOXIDANT DURING SPRAY DRYING OF RED AMARANTH EXTRACT

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ABSTRACT

Amaranth betacyanin, responsible for red or violet color was extracted from *Amaranthus gangeticus by* using water extraction method and microencapsulated by spray drying. The physicochemical, and nutritional properties of microencapsulated betacyanins were assessed as influenced by inlet temperature and maltodextrin concentration. The process was conducted on a mini spray dryer and maltodextrin was used as encapsulating agent. Central composite design was used and thirteen experiments were carried out. The responses were betecyanin retention, moisture content, water activity, particle densities, particle size, colour values and antioxidant activity. Quadratic effect of inlet temperature was found positive on betacyanin retention whereas antioxidant was affected by linear change of maltodextrin. Moisture content and water activity are hardly affected by inlet temperature and maltodextrin concentration individually.Only the effect maltodextrin concentration was found significant on color value. Particle densities and sizes are slightly affected by the process conditions studied.

***Keywords*:** Natural colorant, amaranth, pitaya, color stability, spray drying modeling

INTRODUCTION

The awareness concerning the natural colorant in food use is widespread among the consumer groups as well as the manufacturers. Most of the cases, natural colorants are the extract of plant and tuber or synthesized microbiologically. It has been extensively studied that processing of colored fruits or vegetables into coloring foodstuffs rather than isolation of pure compounds are the best option to produce natural colorant. Providing nutritional values besides their coloring ability are the major benefits of natural colorant. Betacyanins is a water soluble natural colorant, which is red in color and has potential to use as natural red colorant in food [1]. There are many plant species which are the potential sources of it like, pitaya, red amaranth, red beet, red spinach and so on [2-4]. The major problem associated with natural colorant is the stability. Betalain stability is exponentially affected by water activity. From this perspective spray drying is used for producing powder with lower water activity having improved stability of colorant [4-6]. Spray drying is also a common method for encapsulation of sensitive ingredients into a coating material or “wall” to isolate them from outside environment and to protect against oxidation. In this work, betacyanins from red amaranth were microencapsulated by spray drying. Besides assessing microencapsulation yield, morphological characteristics, physicochemical and nutritional properties of produced powder as affected by operating parameters of spray drier were investigated.

MATERIALS AND METHODS

Raw materials

A 15kg of red amaranth (*Amaranthus gangeticus)* was collected from Pasar Borong, Seri Kembangan, Selangor Darul Ehsan, Malaysia. It was cleaned by removing the root, soil as well as rinsing by tap water. After that it was stored in the freezer at -20^{o} C.

Betacyanin extraction & spray drying

Betacyanin from amaranthus gangaticus has been extracted by water extraction following the method described by Cai et al. (1998b)[7]. Maltodextrin DE10 was used as coating agent for spray drying. Wall materials (MD) was dispersed in water (6-34%) and finally made volume 200mL with constant stirring. Wall material was previously swollen in distilled water for 12h. About 10% concentrated pigment extracts were added to the mixture and homogenized each preparation with a homogenizer (WiseMix^{TM} Homogenizer HG15A, Malaysia) at10000 rpm for 5min. The homogenized suspension then was ready for spray drying. The resulted solutions were fed to a laboratory spray drier (SD-05 England). The spray drier was operated at inlet temperature ranging from 132 to 188^{0}C. The air flow, rate of feeding, nozzle size were 10ml/min, 0.7mm, respectively and controlled the same for all the runs. The powders obtained were stored to exclude light and were kept at -20^{0}C until analyzed.

Experimental design

A rotatable central composite design (CCRD) constituted by 13 experiments was used. The design consist of three level full factorial design (+1, -1), superimposed by the centre point (0, 0), and star points (+α, -α). The central composite design is made rotatable by the choice of α and for two factors the value of α is 1.414 [8]. The independent variables considered were the temperature of drying (140 to 180 ^{0}C) and wall materials (10 to 30%; ratio of core materials to coating materials from 1:1 to 1:3) for maltodextrin. The response variables were the efficiency of betacyanins encapsulated and their total antioxidant activity. The variables studied and their effects on responses have been shown in Table 1. The behaviour of the system was displayed as regression equation which has been fitted to data. The equation of the fitted model is shown as below:

$$y = \lambda_0 + \lambda_1 T + \lambda_2 C + \lambda_{11} T^2 + \lambda_{22} C^2 + \lambda_{12} TC \qquad (1)$$

Where y is response, λ_0 is interception coefficient, λ_1 and λ_2 are linear terms, λ_{11} and λ_{22} are quadratic terms, λ_{12} is interaction term, T is inlet temperature and C is carrier concentration.

The analysis of variance, test for the lack of fit, determination of regression coefficients, plot of experimental vs predited, generation of three dimensional graph were carried out using Statgraphics Centurian VI, 2011.

Analysis of physicochemical properties of powder

The physicochemical properties of the powder were determined by the methods as described by [9]. The moisture content of the powder was determined by using oven drying. Water activity of powder was measured by using water activity meter (FA-ST/lab, GBX Instrumentation Scientifique, France). The colour of the powder was measured by using a colour reader (CR-10, Konica Minolta Sensing America's Ltd, Ramsey, USA). The absolute, tap and bulk densities of the powder were measured. A gas pycnometer (AccuPyc II 1340, **Micromeritics,** Norcross, USA) was used to determine the absolute density of the powder. The particle size of the powder was measured by using a particle size analyzer (Malvern Mastersizer 2000, Malvern Instrument Ltd, UK)**.** The betacyanin concentration of powder was determined by using a spectrophotometer (DR 2800 Spectophotometer, HACH, Colourado, USA) as described by Lim et al. (2011)[10]. Antioxidant activity was determined in accordance with 2,2-diphenyl-1-picryl hydrazyl (DPPH) method [11]. TROLOX was used as standard and the results were expressed as IC_{50}.

Table 1: Experimental design with different level of factors and their effects on responses

Inlet Temp. (° C)	MD conc. (%)	BR (%)	MC (%)	a_w	True density (kg/m^3)	Tapped density (kg/m^3)	Bulk density (kg/m^3)	Particle size (μm)	a^* value	IC_{50} (mg/ml)
160	20	81.72	5.06±0.12	0.29±0.01	1455±2	429±10	327±16	7.63±0.08	13.50	2.70
160	6	76.11	5.24±0.16	0.30±0.01	1467±2	417±0	295±18	5.85±0.03	17.42	0.70
160	20	82.38	5.24±0.06	0.34±0.02	1402±7	455±0	328±25	7.46±0.07	13.59	3.60
160	20	77.90	4.81±0.17	0.33±0.01	1393±1	438± 5	339±23	11.06± 0.09	15.11	2.70
180	30	55.57	4.39±0.21	0.29±0.02	1431±4	483±15	349±14	9.34±0.07	11.93	4.80
132	20	56.24	6.37±0.05	0.45±0.00	1476±10	406±18	358±26	7.88±0.03	13.10	2.90
140	10	70.85	5.00±0.15	0.29±0.01	1478±1	401±16	307±11	6.09±0.02	15.01	1.40
160	34	66.16	4.85±0.08	0.27±0.02	1429±6	442±12	349±14	10.26±0.31	12.36	7.60
160	20	77.90	5.77±0.19	0.33±0.02	1503±0	442±12	324±18	8.62±0.12	14.01	2.60
188	20	68.55	4.51±0.13	0.30±0.01	1384±6	417±0	278±0	9.21±0.02	14.32	2.80
180	10	60.98	5.51±0.12	0.33±0.01	1416±14	417±0	273±9	6.85±0.03	16.02	1.30
160	20	72.61	6.13±0.16	0.43±0.00	1448±3	417±0	326±6	7.85±0.04	13.82	2.9
140	30	58.97	6.01±0.29	0.42±0.02	1443±2	461±10	366±16	9.48±0.04	12.09	4.20

MD=Maltodextrin Concentration, BR=Betacyanin Retention, MC=Moisture Content, a_w=Water Activity

RESULTS AND DISCUSSION

Effect of spray drying on microencapsulation of betacyanins

The response, betacyanin retention, in the experiments has been presented in Table 1 along with overall design. The percent betacyanin retention was the percentage of the betacyanin content in the powder relative to the total betacyanin content in the feed.

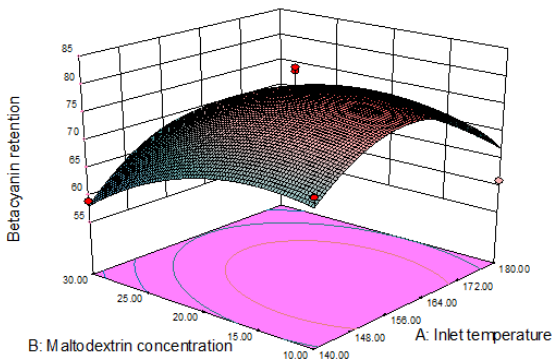


Fig. 1: Response surface plot showing relation between the betacyanin retention as affected by inlet temperature and maltodextrin concentration.

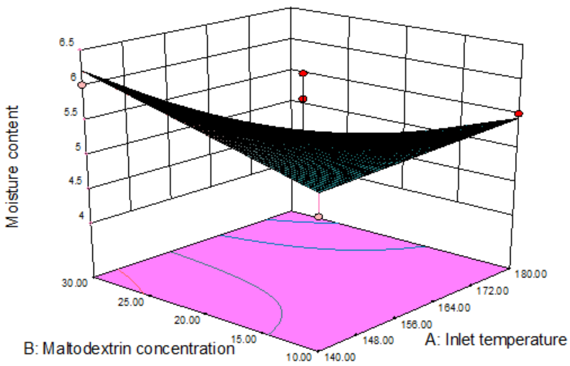
Figure 1 represents the graphical presentation of regression equation termed as response surface plot obtained using the Design expert. The relation between the betacyanin retention (response) as affected by factors (inlet temperature and maltodextrin concentration) are clearly demonstrated by this presentation. Table 2 represents the coefficient values and R^2 of proposed models for all responses. From Figure 1, it could be observed that the change of individual factors have insignificant effect on the betacyanin retention. According to ANOVA table, the effects of linear terms and their interaction are not significant. However, there is a significant effect of quadratic effect for inlet temperature. It indicated that the change of betacyanin retention was curvilinear as affected by the inlet temperature. Initially the betacyanin retention was slightly increased with increasing inlet temperature as well as the ratio of coating agents and extract up to center point (0 level) of both of the factors and then again decreased. Between the two factors, change of inlet temperature is more sensitive than carrier concentration for the variation of betacyanin retention. Contour plot of response surface for betacyanin retention is integrated with the 3D response plot.

As shown in Table 1, high retention of betacyanin was achieved at inlet temperature of 160 ^{o}C with carrier concentration of 20%, which means that during spray drying the betacyanins are well encapsulated in this formulation. Lower betacyanin retention was obtained at higher inlet temperature beyond 160 ^{o}C. As mentioned earlier, betacyanin is a heat sensitive pigment and thus higher inlet temperature during spray drying would decrease the recovery of betacyanin pigments. A related work was conducted by Cai and Corke (2000)[12]. They examined the production and properties of spray-dried *Amaranthus* betacyanin pigments. They found that compared to freeze drying process, spray drying process degraded betacyanin pigments 2.77% at 150 ^{o}C, 3.85% at 165 ^{o}C, 4.14% at 180 ^{o}C, 6.08% at 195 ^{o}C and 7.66% at 210 ^{o}C. Hence for spray drying process, the inlet temperature more than 180 ^{o}C was not desirable.

Cai and Corke (2000)[12] pointed out that with increasing carrier concentration the betacyanin retention was also increased during spray drying. For our case as mentioned earlier, 20% of carrier concentration with inlet temperature of 160 ^{o}C exhibited highest betacyanin retention (82.38%) as shown in Table 1. Carrier concentration beyond 20% either increase or decrease resulted in low betacyanin retention. However, there were exceptions for run(180 ^{o}C, 30%), (160 ^{o}C, 34%) and (140 ^{o}C, 30%). For run (180 ^{o}C, 30%), the betacyanin retention was only 55.57% with 30% of carrier concentration. This was due to the high inlet temperature (180 ^{o}C) that caused the loss of betacyanin during spray drying. For run (160 ^{o}C, 34%), the betacyanin retention was 66.16%. High carrier concentration would increase the feed viscosity and this resulted the feed became in the form of paste rather than solids in drying chamber during spray drying [12]. For the case of run (140 ^{o}C, 30%) gave betacyanin retention of 58.97%. This could be explained by low inlet temperature. Low inlet temperature during spray drying might cause incomplete encapsulation of betacyanin in drying chamber as it was lost before transforming from droplet into powder.

Table 2: Coefficient values and R^2 of proposed models

Factors	λ_0	λ_1	λ_2	λ_{11}	λ_{22}	λ_{12}	R^2
Betacyanin retention	-510.0071	7.3414	0.3046	-0.0234	-0.0498	0.0081	0.7833
IC_{50}	-0.4777	0.0036	0.1378	-	-	-	0.9173
***a* value**	14.9877	0.0161	-0.1771	-	-	-	0.8635
Moisture content	0.6851	0.0299	0.4177	-	-	-0.0027	0.6314
a_w	-0.0536	0.0024	0.0346	-	-	-0.0002	0.4472
True density	1662.9273	-1.2757	-0.9218	-	-	-	0.3848
Tapped density	338.7971	0.3347	2.0169	-	-	-	0.5355
Bulk density	435.7567	-1.0259	2.6421	-	-	-	0.8743
Particle size	2.7453	0.0156	0.1515	-	-	-	0.6513



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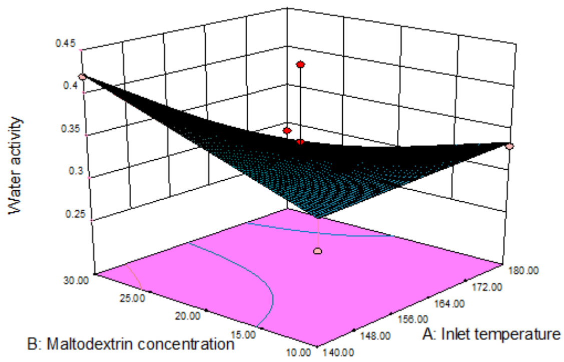

Fig. 2: Response surface plot showing relation between the moisture content (up) and water activity (down) as affected by inlet temperature and maltodextrin concentration.

Moisture content & water activity

The moisture content of the amaranth powder that produced at different inlet temperatures and maltodextrin concentrations are shown in Table 1 and Figure 2. The powder produced by spray drying obtained moisture content between 4.39 to 6.37%. This water content is sufficient to make food powder microbiologically safe. The range of moisture content is similar to spray dried pitaya fruit powder obtained by Ng et al. (2012)[9]. Higher inlet temperature reduces moisture content, shown in Figure 2. Moisture evaporated in the drying chamber during spray drying can be explained by droplet drying. In the first stage of drying, the majority of moisture was removed from the surface of liquid droplets. Second stage of drying took place when there was no more moisture from the surface and a crust was formed. At the moment the moisture removed from core of crust to the surface by diffusion and during this stage the moisture lost was limited. Hence, moisture content for all formulations was no much difference.

As shown in Table 1, temperature was the variable that showed the greatest influence on the powder moisture content. According to Quek et al. (2007)[13] at higher inlet temperature the rate of heat transfer to the particle is greater, which provides a great driving force for moisture evaporation. Finally the powders with reduced moisture content are formed. Therefore, results concerning inlet temperature effects at 20% maltodextrin concentration were consistent with this statement. Moisture content of spray dried powder was also affected by the content of maltodextrin. Due to the increase of the total soluble solids with the increasing of maltodextrin concentration, moisture content of produced powder decreased [14].

Concerning the water activity, it was found affected by both the inlet temperature and the maltodextrin addition, similarly to the water activity of spray-dried pitaya powder [9]. The water activities of the powders produced in the current work was between 0.27 to 0.45. This range indicated that powder produced by many of the conditions were microbiologically safe. Generally, food with water activity around 0.3 are stable both from microbiologically and chemically [15].

As shown in Figure 2, moisture content and water activity are hardly affected by inlet temperature and maltodextrin concentration individually. Their interactions have positive effect. With increasing both the factors, moisture content and water activity decreases.

Densities

Different types of density were analyzed and tabulated in Table 1 and the response plot is shown in Figure 3. As shown in Figure 3, bulk density changes linearly with changing maltodextrin concentration. Inlet temperature is found almost non-influencing factors on densities. The true density among the formulations ranged from 1384 kg/m^3 to 1503 kg/m^3, 400.67 kg/m^3 to 482.67 kg/m^3 for tapped density, and 273 kg/m^3 to 366.33 kg/m^3 for bulk density. The bulk density is related to spray drying inlet temperature as well as maltodextrin concentration. The relationship between bulk density and inlet temperature could be seen at 160 ^{o}C and 20% MD concentration, which bulk density ranged from 323.67 kg/m^3 to 338.33 kg/m^3, compared to at (132 ^{o}C, 20%) with 358.33 kg/m^3 and at (188 ^{o}C, 20%) with 278 kg/m^3. True density excludes open pores and closed pores of the powder, having the highest value compared to tapped density which only excludes open pores, followed by bulk density which includes both pores.

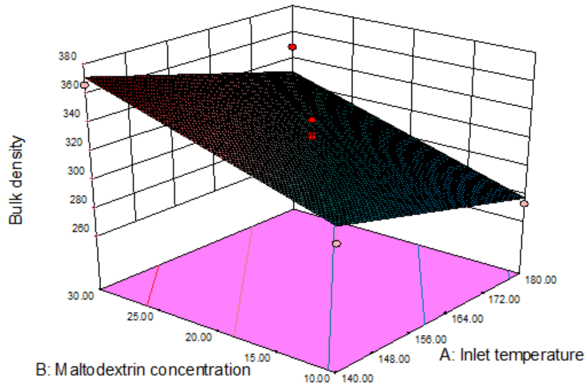

Fig. 3: Response surface plot showing relation between bulk density as affected by inlet temperature and maltodextrin concentration.

The inlet temperature is inversely proportional to bulk density. Similar trends were observed between run at (180 °C, 30%), (140 °C, 30%), as well as run at (140 °C, 10%) and at (180 °C, 10%). This was due to low inlet temperature caused low drying rate and the ratio of surface-volume for the spray-dried powder was also decreased, so high bulk density would be obtained. Additionally, the concentration of maltodextrin could also affect the bulk density. When the inlet temperature was fixed to 160 °C, increased maltodextrin concentration would increase solid content, resulting higher bulk density [12].

Particle size

As shown in Figure 4, an increase of inlet temperature resulted increase in particle size. However increase of maltodextrin concentration caused increased in particle size. Although inlet temperature insignificantly affected particle size, from Table 1 it could be noted for run (140 °C, 10%) and run (180 °C, 10%) with a fix maltodextrin concentration of 10%, as well as run (180 °C, 30%) compared to run (188 °C, 20%), with a fix maltodextrin concentration of 20%. This might be due to high inlet temperature caused high swelling. Another reason was that high inlet temperature exhibited fast drying rate which produced the structure early, the shrinkage of particles were limited and resulted in bigger particle size [16]. The particle size was greatly affected by maltodextrin concentration and this might be related to feed viscosity. The mean liquid droplet size would be larger with high maltodextrin concentration that causing high feed viscosity during atomization, thus produced larger particles after spray drying [17]. In our research, the evidence could be found in Table 1 when run (140 °C, 10%) compared to run (140 °C, 30%), with a fix inlet temperature of 140 °C, run (180 °C, 30%) compared to run (180 °C, 10%) with inlet temperature of 180 °C, as well as 4 runs of (160 °C and 20%) compared to run (160 °C, 6%) and run (160 °C, 34%).

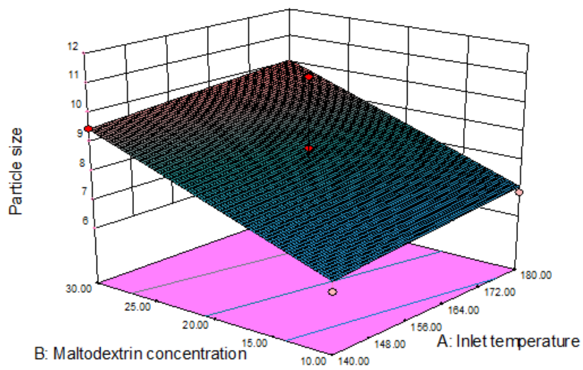

Fig. 4: Response surface plot showing relation between particle size as affected by inlet temperature and maltodextrin concentration.

Color

Table 3 represents the colour parameters of spray-dried powder with different formulations and Figure 4 shows the response plot. As shown in Figure 5, redness was greatly influenced maltodextrin concentration.

With increasing maltodextrin content, redness decreases, indicating higher amount of betacyanin compounds being encapsulated. Before spray drying, the colour parameters for concentrated red amaranth extracts *L**, *a**, *b**, h^o and *C** are 25.08, 4.16, 0.46, 0.11 and 4.18, respectively. After spray drying, an obvious increment of values could be found in *L**, *a** and *C**. Higher *L** value indicated higher lightness. The lightness after spray drying was contributed by the addition of whitish maltodextrin. The highest *L** was 84.28 which obtained in run (180 ^{o}C, 34%) as the highest amount of maltodextrin was added. *a** value was also influenced by maltodextrin concentration.

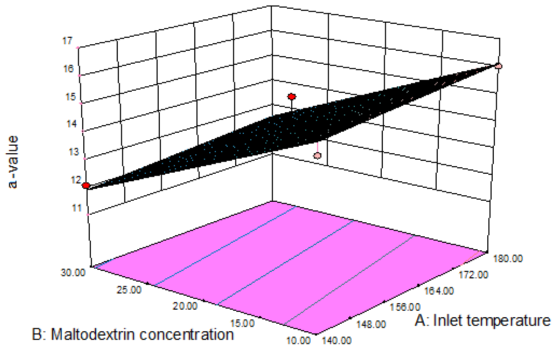

Fig. 5: The graphical presentation of regression equation showing the relation between the *a*-value as affected by inlet temperature and maltodextrin concentration.

Table 3. Colour parameters for formulated spray-dried powder

Run	Inlet temperature (° C)	Maltodextrin concentration (%)	*L**	*a**	*b**	*H°*	*C**
1	160	20	81.98	13.5	-0.9	356.19	13.53
2	160	6	76.08	17.42	-1.24	355.93	17.46
3	160	20	81.65	13.59	-1.01	355.75	13.63
4	160	20	80.88	15.11	-1.57	354.07	15.19
5	180	30	84.07	11.93	-1.27	353.93	12.00
6	132	20	81.9	13.1	-1.24	354.59	13.16
7	140	10	79.08	15.01	-1.26	355.2	15.06
8	160	34	84.28	12.36	-0.92	355.74	12.4
9	160	20	81.89	14.01	-1.77	352.80	14.12
10	188	20	81.19	14.32	-1.23	355.09	14.37
11	180	10	77.96	16.02	-0.96	356.57	16.05
12	160	20	81.54	13.82	-0.60	357.51	13.83
13	140	30	83.92	12.09	-1.38	353.49	12.17

The highest *a** value found was 17.42. Low maltodextrin concentration gave higher a* value as less powder produced and betacyanins get concentrated, thus increased the redness. For hue angle h^o, a pure redness is characterized by 0^o or 360^o. After spray drying, all the h^o values were slightly apart from 360^o, ranging from 352.80^o to 357.51^o. However, the redness of the powders produced were ensured as the h^o values were still near to 360^o. Chroma C* is related to colour purity. The C* value was improved from 4.18 to a range of 12.00 to 17.46 after spray drying.

Antioxidant

Figure 6 illustrates graphical presentation of the response surface plot for the antioxidant activity. For the changes of independent variable inlet temperature, there was almost no effect on antioxidant concentration. On the other hand, remarkable increase of antioxidant concentration was observed with the increase of carrier concentration or core-coating ratio indicating carrier concentration as the major factor affecting the antioxidant of the encapsulated betacyanin

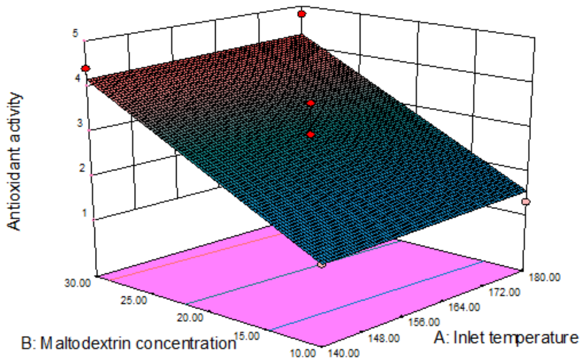

Fig. 6: The graphical presentation of regression equation showing the relation between the antioxidant activity as affected by inlet temperature and maltodextrin concentration.

The values of antioxidant concentration with different formulations are shown in Table 1. The relationship between inlet temperature and antioxidant concentration could be identified although there were no significant differences as discussed above. When 10% and 20% of carrier concentration were fixed, the relationship was unclear. However, a positive relationship was found when the carrier concentration was increased to 30%. During microencapsulation of bioactive compounds of cactus pear pulp using maltodextrin, the effect of inlet temperature was found insignificant at $P<0.05$ [4]. They found significant effect of types and ratio of core-coating materials on the yield of betacyanin and phenolic compound encapsulation. With maltodextrin concentration of 30%, at inlet temperature of 140 ^{o}C, the antioxidant concentration was lower (4.2 mg/ml) than that of at 180 ^{o}C, which was 4.8 mg/ml. This is similar to a research had been conducted by Couto and others in 2012 [18]. They examined the physicochemical and antioxidant properties of spray-dried rosemary extracts using 3^3 Box-Behnken design. A total of 15 experiments were conducted with 3 independent variables, which included inlet temperature (80 ^{o}C, 110 ^{o}C and 140 ^{o}C). According to their results, the antioxidant activity at higher inlet temperature is lower (18..8 ug/ml) than that of at higher inlet temperature (24.4 ug/ml). The response surface also explained that antioxidant activity was proportional to inlet temperature even ANOVA test showed no significant differences in linear and quadratic term of inlet temperature.

The antioxidant concentration was greatly affected by independent variable, the carrier concentration. As we observed from Table 1, the carrier concentration was proportional to antioxidant concentration at the same temperature among all formulations. This was because lower carrier concentration produced less powder after spray drying, made the antioxidant concentrated. Thus, lower antioxidant concentration would be expected and to be able to scavenge 50% of free radicals.

CONCLUSIONS

A response surface model and regression analysis were used to assess the effect of inlet temperature and maltodextrin concentration on the betacyanin retention and the antioxidant activity of encapsulated betacyanin. All models were significant except for water activity and true density. The quadratic effect was only found in betacyanin retention**.**

NOMENCLATURE

*L**	Lightness	
h^o	*Hue angle*	
*a**	Redness	m^2m^{-3}
*b**	Yellowness	
c*	Hue angle	
IT	Inlet temperature	^{0}C
MD	Maltodextrin concentration	%

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