

## Response surface optimisation of high antioxidant jelly from *Musa paradisiaca* and *Trigona* sp. honey using central composite design as a convenient functional food

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### Article history

Received: 30 August, 2018

Received in revised form:

23 January, 2019

Accepted: 17 April, 2019

### Keywords

Antioxidant capacity

Carbohydrate content

Acceptability

Response surface

methodology

### Abstract

The optimum combination of *Musa paradisiaca* (MP) and *Trigona* sp. Honey (TH) in formulating high antioxidant jelly was analysed for total carbohydrate content (CHO), antioxidant capacity (AC), and acceptability via the Response Surface Methodology. Central composite design was employed to optimise the combination effect of two independent variables; namely MP ( $X_1$ : 20-100%) and TH ( $X_2$ : 20-100%) on the recovery of three responses; total carbohydrate content ( $Y_1$ ), antioxidant capacity ( $Y_2$ ), and acceptability ( $Y_3$ ). A polynomial model generated a satisfactory fitting of the experimental data with regards to total carbohydrate content ( $R^2 = 0.8974$ ,  $p < 0.0024$ ), total antioxidant capacity ( $R^2 = 0.9702$ ,  $p < 0.0001$ ), and acceptability ( $R^2 = 0.9136$ ,  $p < 0.0001$ ). The optimum combination for maximum recovery of CHO, AC and acceptability were 20% of MP and 20% of TBH, with a predicted CHO of 33 Kcal/5 g, AC of 0.34 nm and acceptability score of 6.16 ( $< 5$ : not accepted;  $> 5$ : accepted).

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### Introduction

Nowadays, the incorporation of local natural resources in formulating functional foods has become a trend among researchers and food technologists worldwide. Fruits and natural-based products are favoured because they contain sufficient phytochemicals, macronutrients and micronutrients. Moreover, they are proven to be safe for routine consumption (Ibrahim *et al.*, 2010).

Banana (*Musa paradisiaca*) is extensively cultivated in Malaysia, and frequently used in traditional snacks, beverages, medicines and cosmetics. The supplementation of banana flour in a food product has been reported to enhance its nutritional properties by increasing the energy, polyphenols, dietary fibre and starch (Zuwariah and Noor Aziah, 2009). Several studies have demonstrated that banana flour contains approximately 95% total carbohydrates, phenols and flavonoids (Zuwariah and Noor Aziah, 2009).

Ahmad *et al.* (2015) reported that the different parts of banana namely tepal, skin and flesh exhibited high total phenolic content ranging from 2,150  $\mu\text{g}$  to 8,000  $\mu\text{g}$  gallic acid equivalent (GAE) in 1 g banana extracts. The antioxidant effect in banana was also studied in rats for hypercholesterolemic model, in which the concentrations of peroxide products such as malondialdehyde (MDA), hydroperoxides and conjugated dienes significantly decreased whereas the activities of catalase and superoxide dismutase significantly increased (Loganayaki *et al.*, 2010).

Apart from banana, honey is a globally documented natural food rich in nutritional value and valuable health-promoting properties. The utilisation of honey as medicines and nutritional foods since ancient times is due to the fact that it contains various functional compounds (Nayik *et al.*, 2014). Honey is a natural supersaturated sugar solution, mainly composed of approximately 65% to 80% of glucose and fructose, which can be used as a rapid energy source upon consumption (Chua and Adnan, 2014;

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Nayik and Nanda, 2016b). Honey is frequently associated with high antioxidant activity mainly due to the presence of major polyphenols in the form of phenolics acids (chlorogenic, ferulic, caffeic, ellagic, vanillic, benzoic, cinnamic, coumaric acids) and flavonoids (pinocembrin, apigenin, hesperitin, chrysin, quercetin, luteolin, myricetin, pinobanksin, galangin, kaempferol) (Nayik and Nanda, 2016a). Malaysian honey contains high phenolic (15.21 mg to 42.23 mg GAE in 1 kg honey) and flavonoid contents, ferric reducing/antioxidant power assay (FRAP) value, and high IC<sub>50</sub> of DPPH radical-scavenging activities (5.24 mg to 17.51 mg in 1 mL honey extract) (Khalil *et al.*, 2011).

Response surface methodology (RSM) is a combination of mathematical and statistical methods, utilised in modelling and minimising problem in which the desired response is influenced by a number of variables and the intention is to optimise the response (Saniah and Hasimah, 2008; Dailey and Vuong, 2015; Nayik *et al.*, 2016). The conventional method of formulating food product is laborious and time-consuming (Quispe-Fuentes *et al.*, 2017). Therefore, RSM was applied in the present work as it is able to analyse the relationship between several independent variables and the preferable responses or dependent variables (Prasad *et al.*, 2011).

The health concerns together with the abundance of artificial functional foods in the market have driven people to look for functional foods from natural sources. The demand for high energy and antioxidant food products is steadily increasing (Adewoyin *et al.*, 2017), in line with the rising number of people suffering from many degenerative diseases caused by malnutrition. Globally, malnutrition and specific nutrient deficiencies are the leading underlying causes of immune deficiency, leading to infection and other diseases like cancer. Approximately 40% of cancer patients, despite the cancer type, experience malnutrition and the prevalence has remained unchanged for over 30 years (Righini *et al.*, 2013). The percentage of malnourished patients are particularly high among patients with gastrointestinal or head and neck cancers.

As such, the present work aimed to formulate a high-energy and antioxidant functional food to cater to the aforementioned diseases and illnesses. The formulated functional food was designed to contain dense carbohydrate to provide adequate energy supply. The high antioxidant property can boost immune deficiency in the general consumers as well as the target patients. In addition, the formulated functional food has also been tested for consumers' preference in order to provide an insight into its marketability (Ahmed *et al.*, 2015).

## Materials and methods

### Plant and honey materials

'Pisang awak' (*Musa paradisiaca*) was purchased from a local market at its green stage. Firstly, the banana was peeled using a stainless-steel knife to remove the skin and then soaked in water for 30 min. The banana was then sliced into pieces about 5 to 10 mm thickness and placed on a tray before drying in a universal oven drier (Memmert 100-800 Incubator, Germany) at 60°C for 18 h. After drying, the dried banana slices were ground using multi-purpose swing disintegrator (Z500 medicine crusher, China), and later stored in an airtight plastic container at room temperature. *Trigona* sp. honey was collected from a selected local controlled farm in Ketereh, Kelantan, Malaysia, and immediately transported to the Laboratory of Food Analysis, Kulliyah of Allied Health Sciences, International Islamic University Malaysia. The authentication of the honey sample was confirmed by phenolic compounds analysis as described by Soares *et al.* (2017).

### Chemicals and reagent

Chemicals used in the present work were of analytical grade. Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany). Ammonium molybdate, sodium phosphate, DPPH, gallic acid, and quercetin were obtained from Sigma-Aldrich (St. Louis, USA).

### Jelly preparation and extraction

The optimised formulation of the jelly was developed by manipulating two factors, *Musa paradisiaca* ( $X_1$ : 20-100%) and *Trigona* sp. honey ( $X_2$ : 20-100%), enrolled in central composite design (CCD) using five levels for each variable as shown in Table 1. Jelly (5 g) was extracted in 25 mL 80% aqueous methanol, with 1% concentrated HCl. The extraction was done using a rotary shaker at 150 rpm and 55°C for 30 min. Next, the extract was filtered using Smith filter papers (102 qualitative; diameter 125 mm, A0336), and the filtrate was used to measure the antioxidant capacity (AC), total phenolic content (TPC) and DPPH free radical-scavenging activities.

Table 1. Independent variables and their coded and actual values used for optimisation.

Independent variable	Unit	Symbol	Coded level				
			-1	0	1	Axial (- $\alpha$ )	Axial (+ $\alpha$ )
<i>Musa paradisiaca</i>	g	$X_1$	20	60	100	3.43	116.57
<i>Trigona</i> sp. honey	g	$X_2$	20	60	100	3.43	116.57

### Total carbohydrate content

The carbohydrate content was analysed using total carbohydrate assay kit from Cell Biolabs, Inc. (San Diego, USA). The absorbance was read using microplate reader at 490 nm. A standard curve was constructed using glucose standard solution (0, 0.0625, 0.125, 0.25, 0.5, 1, 2, and 4 mM,  $R^2 = 0.9909$ ). The result of total carbohydrate content was expressed as energy equivalent (Kcal) per 5 g jelly.

### Antioxidant capacity

The antioxidant capacity was evaluated based on the method designed by Prieto *et al.* (1999). Briefly, 1 mL reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was freshly prepared and combined with 0.1 mL sample filtrate in a tube. The tube was wrapped with aluminium foil and kept in an incubator at 95°C for 90 min. Next, the mixture was brought to room temperature. Then, the absorbance was measured at 695 nm using a UV spectrophotometer. A blank solution containing 1 mL reagent solution and 0.1 mL solvent was used for the sample extraction. The antioxidant capacity was expressed as the absorbance value.

### Total phenolic content

The total phenolic content of jelly samples was estimated using a slightly modified spectrophotometric Folin-Ciocalteu method. Firstly, 200 µL jelly extract was mixed with 1 mL Folin-Ciocalteu's reagent. The mixture was retained at room temperature for 3 min. Then, 1 mL 10% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture and modified to 10 mL with distilled water. Next, the mixture was kept in the dark for 90 min. Then, the absorbance was read at 725 nm by a UV/VIS-spectrophotometer (Schott UVLine 9400, USA). A standard curve of gallic acid (0.0003, 0.0049, 0.0120, 0.0781, and 0.3125 of mg/mL,  $R^2 = 0.9994$ ) was constructed. The results were averaged with standard deviations, and expressed as milligrams of gallic acid equivalents (GAEs) per gram jelly.

### Free radical-scavenging activity

The DPPH free radical-scavenging activity was used to evaluate the antioxidant properties of the optimised jelly. The evaluation was established using the method described by Ferreira *et al.* (2009). Firstly, 1 mL jelly extract was dissolved in 2 mL 0.03 mM DPPH solution. The mixture was vigorously shaken before being allowed to react in the dark for 35 min. The reduction of the DPPH radical was evaluated by reading the absorbance at 517 nm. Quercetin was used as the standard reference (0.0003, 0.0006, 0.0013, 0.0025, and 0.005 mg/mL,  $R^2 = 0.9881$ ). The

radical scavenging activity (RSA) was evaluated using Equation 1:

$$\% \text{ RSA} = ([A_{\text{DPPH}} - A_{\text{S}}] / A_{\text{DPPH}}) \times 100 \quad (\text{Eq. 1})$$

where  $A_{\text{S}}$  = absorbance of the solution added with jelly extract, and  $A_{\text{DPPH}}$  = absorbance of DPPH solution. The  $\text{IC}_{50}$  was determined as the concentration of the tested jelly causing 50% reduction of the initial DPPH concentration, which was measured from the linear regression concentration curve of the test extract against the percentage of the radical scavenging inhibition.

### Sensory evaluation

The sensory evaluation was performed in a sensory room, Nutrition Laboratory, Department of Nutrition Sciences, Kulliyah of Allied Health Sciences, International Islamic University Malaysia, Kuantan, Pahang, with 39 untrained panellists. Panellists were properly introduced and explained to the attributes, the terminology to define each attribute, and the scale used to indicate the degree of liking towards the jelly. Approximately 5 g jelly was served and randomly coded with three digits. Sensory attributes evaluated included the degree of liking (DOL) for colour, appearance, softness, odour, flavour, after-taste and overall acceptability. The acceptability attribute was expressed as the mean of seven sensory attributes. A 9-point hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) was applied for the evaluation.

### Experimental design

The relationship between the process variables concerning response functions as well as the optimised formulation of the jelly, in terms of its antioxidant capacity, total carbohydrate content and acceptability, were identified by adopting a two-factor inscribed central composite design (CCD). The independent variables investigated were *Musa paradisiaca* concentration ( $X_1$ : 20-100%) and *Trigona* sp. honey ( $X_2$ : 20-100%). The desired response variables were total carbohydrate content ( $Y_1$ ), antioxidant capacity ( $Y_2$ ), and acceptability ( $Y_3$ ). A preliminary experimental data was considered in the selection of the range for those two-factor variables. The optimised independent variables were coded at 3 levels -1, 0, +1 (Table 1). Based on the CCD, 13 randomised experiments comprising five replicates of centre points were constructed. The values of independent variables constructed, and experimental and predicted values for response variables are shown in Table 2.

Table 2. Two-factor central composite design used for RSM with experimental and predicted values for the independent variables.

Standard order	Factor 1 ( $X_1$ ) <i>Musa paradisiaca</i>	Factor 2 ( $X_2$ ) <i>Trigona</i> sp. honey	Response 1 ( $Y_1$ ) CHO (Kcal)		Response 2 ( $Y_2$ ) AC (Abs in nm)		Response 3 ( $Y_3$ ) Acceptability (Score)	
			Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
1	-1	-1	35	33	0.340	0.340	6.070	6.160
2	+1	-1	17	17	0.328	0.340	5.280	5.200
3	-1	+1	28	23	0.239	0.254	5.890	6.160
4	+1	+1	30	27	0.267	0.254	5.120	5.200
5	-1.414	0	36	34	0.271	0.284	6.630	6.359
6	1.414	0	32	26	0.287	0.284	5.040	5.000
7	0	-1.414	22	21	0.364	0.371	5.740	5.680
8	0	1.414	19	21	0.234	0.249	5.650	5.680
9 <sup>a</sup>	0	0	31	30	0.291	0.310	5.790	5.680
10 <sup>a</sup>	0	0	31	30	0.317	0.310	5.620	5.680
11 <sup>a</sup>	0	0	32	30	0.313	0.310	5.590	5.680
12 <sup>a</sup>	0	0	28	30	0.312	0.310	5.740	5.680
13 <sup>a</sup>	0	0	28	30	0.308	0.310	5.650	5.680

Abs = absorbance; Exp. = experimental; Pred. = predicted. <sup>a</sup>Centre point.

### Statistical analysis

The statistical analysis was performed using the Design-Expert Version 6.0.10 (Minneapolis, MN) software. The outcome for total antioxidant capacity, total carbohydrate content and acceptability were expressed as mean values with standard deviations. The response surface analysis was utilised to verify the regression coefficients and statistical significance of the model terms. It was also used to fit the mathematical models of the experimental data that was intended to optimise the response variables. A second order polynomial model was used to fit the data (Equation 2):

$$Y = b_0 + b_1X_1 + b_2X_2 + b^2_1X^2_1 + b^2_2X^2_2 + b_1b_2X_1X_2 \quad (\text{Eq. 2})$$

where  $Y$  = predicted response variable;  $b_0$  = constant;  $b_1$  and  $b_2$  = regression coefficients for the linear effect terms;  $b^2_1$  and  $b^2_2$  = quadratic effect terms; and  $b_1b_2$  = interaction effect terms. The adequacy of the model was predicted through the regression analysis ( $r^2$ ) and the ANOVA analysis ( $p < 0.05$ ). From the ANOVA analysis, only the significant coefficients were included, while the non-significant coefficients were omitted from the initial model. A three-dimensional model graph was used to explain the relationship between the independent variables ( $X_1$  and  $X_2$ ) and the response variables ( $Y_1$ ,  $Y_2$  and  $Y_3$ ). The desired goal was set in numerical optimisation to generate the optimal condition, and point prediction was performed to discover the predicted values of the response.

### Model verification

The experimental data for total carbohydrate content, antioxidant capacity and acceptability were determined based on the optimum conditions suggested by the software. The verification of the response surface model was performed by comparing the experimental value obtained from the independent samples in contrast to the predicted value obtained from the optimised model.

## Results and discussion

### Identification of the range of independent variables

A preliminary study was performed to identify the minimum, centre point and maximum level of the two independent variables, *Musa paradisiaca* ( $X_1$ ) and *Trigona* sp. honey ( $X_2$ ). Based on the values of the response variables in Table 2, an optimum set of combinations was predicted for the development of jelly with a high total carbohydrate content, antioxidant capacity as well as acceptability among panellists. The total phenolic content and DPPH radical scavenging assay were also carried out for the optimised combination of independent variables.

### Fitting the response surface model

The experimental value of total carbohydrate content ( $Y_1$ ), antioxidant capacity ( $Y_2$ ), and acceptability ( $Y_3$ ) were employed in multiple linear regression analysis performed using response surface analysis to fit the polynomial equation. A satisfactory model was obtained as indicated by a minute difference

between the response values obtained experimentally and predicted values (Table 2). Coefficients of determination ( $R^2$ ), adjusted  $R^2$  values, probability values ( $p$ ), and lack-of-fit values for response variables are tabulated in Table 3. The coefficient of determination ( $R^2$ ) obtained were 0.8974, 0.9702 and 0.9136 for total carbohydrate content ( $Y_1$ ), antioxidant capacity ( $Y_2$ ), and acceptability ( $Y_3$ ) respectively, thus indicating that approximately (89 to 97%) of the variations was described by the model. The fitness of the model in predicting the variation was evaluated by performing the lack-of-fit test.

#### Effect of independent variables on total carbohydrate content

Second-order polynomial regression equation explained the effect of two independent variables in relation to the total carbohydrate content by means of the significant ( $p < 0.05$ ) coefficient. The combination of *Musa paradisiaca* and *Trigona* sp. honey showed significant ( $p < 0.05$ ) effect with regard to first-order linear effect ( $X_1$ ), second-order quadratic effect ( $X_2^2$ ), and interaction effect ( $X_1X_2$ ) towards CHO ( $Y_1$ ). The predicted model observed for CHO ( $Y_1$ ) is tabulated in Table 3.

As shown in Figure 1A, the energy for the 13 formulations varied from 17 Kcal to 36 Kcal, with the highest energy measured at the optimum (35 Kcal) when the *Musa paradisiaca* and *Trigona* sp. honey were set at the lowest (20%). Meanwhile, the energy load was measured at the highest (36 Kcal) when the formulation was set at ( $X_1$ :3.43%;  $X_2$ :60%) and the least (17 Kcal) when *Musa paradisiaca* was set at the highest (100%) and the *Trigona* sp. honey at the lowest (20%).

*Trigona* sp. honey exerted a significant effect on carbohydrate content as it has been laboratory-tested to contain approximately more than 80% of carbohydrate in the proximate analysis. A study by Chua and Adnan (2014) reported that carbohydrate content displayed a strong positive correlation (0.9994) towards energy. Additionally, *Musa paradisiaca* showed a significant effect towards carbohydrate content as it has been reported to contain approximately 95% total carbohydrate content. Unripe *Musa paradisiaca* is an excellent source of carbohydrate to be incorporated in a functional

processed food like jelly (Zuwariah and Noor Aziah, 2009; Wang *et al.*, 2012). From a nutritional point of view, the optimised formulated jelly is a good energy supplement as the carbohydrate load is derived not only from simple sugar like glucose and fructose from *Trigona* sp. honey, which is able to immediately metabolise in producing energy but also from dietary fibre and resistant starch from *Musa paradisiaca*, that can benefit many health physiological aspects such as bowel digestion, micronutrient absorption, blood lipid profile as well as glycaemic and insulinaemic responses (Nugent, 2005; Chong and Noor Aziah, 2008; Zuwariah and Noor Aziah, 2009).

#### Effect of independent variables on antioxidant capacity

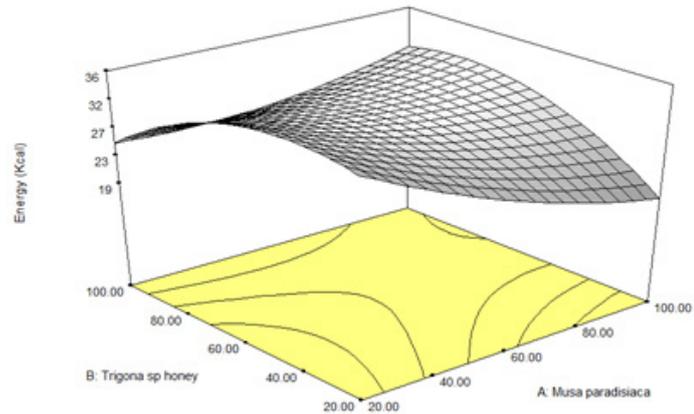
Second-order polynomial regression equation explained the effect of two independent variables in relation to antioxidant capacity by means of the significant ( $p < 0.05$ ) coefficient. The combination of *Musa paradisiaca* and *Trigona* sp. honey showed significant ( $p < 0.05$ ) effect with regard to first-order linear effect ( $X_2$ ) and second-order quadratic effect ( $X_2^2$ ) towards AC ( $Y_2$ ). Nevertheless, there was no interaction effect observed between the independent variables. The predicted model observed for AC ( $Y_2$ ) is tabulated in Table 3.

Based on the equation in Table 3, both *Musa paradisiaca* and *Trigona* sp. honey had an effect on antioxidant capacity, with *Musa paradisiaca* affecting antioxidant capacity more than *Trigona* sp. honey did. To date, honey has been identified as a novel antioxidant as it is rich in various antioxidants namely phenolic acid, flavonoid, ascorbic acid, catalase, peroxidase, carotenoid and products of Maillard reaction (Khalil *et al.*, 2011; Erejuwa *et al.*, 2012). *Musa paradisiaca* is commonly reported to contain a significant amount of polyphenols including flavonoids and tannins. The phenolic compound present in *Musa paradisiaca* is capable of absorbing and neutralising free radicals, quenching singlet oxygen or decomposing peroxides (Loganayaki *et al.*, 2010). Various parts of *Musa paradisiaca* contain high phenolic compound ranging from 2,150 to 8,000  $\mu\text{g/g}$  GAE that may explain the double effect of *Musa paradisiaca* on the antioxidant capacity analysed (Ahmad *et al.*, 2015).

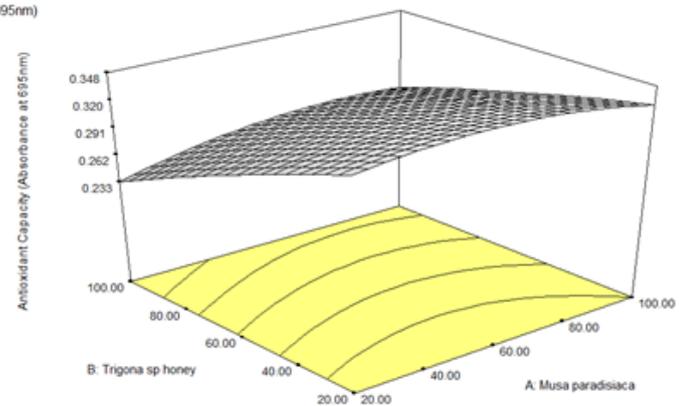
Table 3. Polynomial equations and statistical parameters calculated after implementation of two-factor central composite experimental design.

Regression coefficient	Polynomial equation	$R^2$	$R^2$ (adjusted)	Regression ( $p$ value)	Lack-of-fit
CHO, ( $Y_1$ )	$29.9-2.78X_1-4.69X_2^2+5.06X_1X_2$	0.8974	0.8241	0.0024	0.2978
AC, ( $Y_2$ )	$0.31-0.043X_2-0.013X_2^2$	0.9702	0.9489	< 0.0001	0.7971
Acceptability, ( $Y_3$ )	$5.68-0.48X_1$	0.9136	0.8963	< 0.0001	0.1260

DESIGN-EXPERT Plot

Energy (Kcal)  
X = A: Musa paradisiaca  
Y = B: Trigona sp honey

DESIGN-EXPERT Plot

Antioxidant Capacity (Absorbance at 695nm)  
X = A: Musa paradisiaca  
Y = B: Trigona sp honey

DESIGN-EXPERT Plot

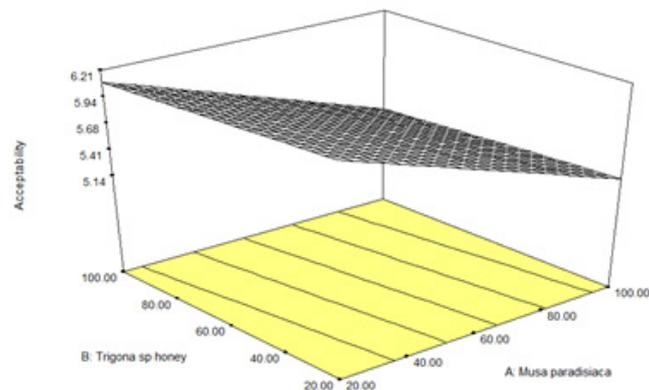
Acceptability  
X = A: Musa paradisiaca  
Y = B: Trigona sp honey

Figure 1. Effect of the combination of *Musa paradisiaca* and *Trigona* sp. honey on (A) carbohydrate content, (B) antioxidant capacity, and (C) acceptability.

Figure 1B reveals that there was a linear increase and a quadratic increase in antioxidant capacity (0.234 to 0.340 nm) with the reduction of *Trigona* sp. honey and *Musa paradisiaca* from 100% to 20%, respectively. This inconsistency between the concentrations of *Trigona* sp. honey and *Musa paradisiaca* with regards to antioxidant capacity might be due to the pro-oxidant activity which might have occurred due to various factors.

For *Musa paradisiaca*, our finding contradicted that of the earlier finding by Ahmad *et al.* (2015), in

which it was reported that the scavenging activity analysed via DPPH (2,2-diphenyl-1-picrylhydrazyl) assay increased with increasing concentration of *Musa paradisiaca*. *Musa paradisiaca* may be prone to oxidation and may become pro-oxidant if the harvesting, handling, storage and transportation are not carefully managed. According to Yang *et al.* (2008), storage of postharvest banana is associated with the production of reactive oxygen species (ROS), in which malondialdehyde (MDA), protein carbonyl, lipofuscin, hydroxyl radical and hydrogen peroxide

markedly increase after four days of harvesting. Therefore, the presence of ROS upon *Musa paradisiaca* harvesting and storage could possibly lead to the discrepancy between the concentration of *Musa paradisiaca* and the antioxidant capacity of the formulated jelly.

Our results seem to be consistent with Aissat *et al.* (2015), whereby honey was reported as a complex mixture of compounds, which may act as an oxidant or antioxidant when utilised in different concentrations. The *Trigona* sp. honey used is also a multiflora tropical honey that may act as peroxide-producing honey that could lead to the formation of free radical (Erejuwa *et al.*, 2014). Honey, which is rich in polyphenol, may exert pro-oxidant activities under certain experimental conditions. Polyphenols are capable of both scavenging activities and generating radicals. The benefits of polyphenols thus might be optimised by a combination of both mechanisms (Ahmed *et al.*, 2012). As the *Trigona* sp. honey used in the present work was harvested from a local farm in Kelantan, the handling and transportation factor might have exposed the honey to the air and heat which could have triggered hydrogen peroxide ( $H_2O_2$ ) production (Aoshima and Ayabe, 2007). In addition, the formulation of jelly by diluting the honey with water instead of other ingredients might have enhanced the production of hydrogen peroxide generated by the action of glucose oxidase, thus subsequently influencing the reduction of antioxidant capacity in the formulated jelly (Henriques *et al.*, 2006).

#### *Effect of independent variables on acceptability*

Second-order polynomial regression equation explained the effect of two independent variables in relation to antioxidant capacity by means of the significant ( $p < 0.05$ ) coefficient. The combination of *Musa paradisiaca* and *Trigona* sp. honey showed significant ( $p < 0.05$ ) effect with regard to first-order linear effect ( $X_1$ ) towards acceptability ( $Y_3$ ). Nonetheless, independent variables did not show any second-order quadratic effect as well as interaction effect. The predicted model observed for acceptability ( $Y_3$ ) is tabulated in Table 3. As detailed in the equation in Table 3, only *Musa paradisiaca* significantly affected the overall acceptability of the jelly among the panellists.

Figure 1C shows that the acceptability score for the formulated jelly increased (5.04 to 6.63) with the reduction of *Musa paradisiaca* concentration from 100% to 20%. This rather expected finding might be explained by the total soluble solid and slightly astringent taste of *Musa paradisiaca* towards

softness and after-taste, respectively. The reduction of *Musa paradisiaca* concentration from 100% to 20% would have reduced the total soluble solid and astringent taste, consequently lowering the softness and improving the after-taste attributes of the jelly. In addition, panel score for jelly acceptability decreased with the increase of *Musa paradisiaca* concentration from 20% to 100%. Another possible explanation for this relationship might be due to the fact that *Musa paradisiaca* flour is rich in starch granule but low in gluten content, resulting in the production of a jelly with harder and more compact structure (Chong and Noor Aziah, 2008). Zuwariah and Noor Aziah (2009), Loganayaki *et al.* (2010) and Ahmad *et al.* (2015) reported that *Musa paradisiaca* flour contains approximately 1,200 mg tannic acid per 100 g. The reduction of *Musa paradisiaca* from 100% to 20% might have reduced the tannic acid in the jelly thus improving the after taste of the jelly. Similarly, a study conducted by Villamor *et al.* (2013) reported that a high tannic acid concentration tended to intensify bitter or astringent taste, banana fruit aroma as well as the flavour of the jelly.

The score ranging from 5.04 to 6.63 shows that the 13 formulations received scores from “neither like nor dislike” to “like slightly”. Formulations ( $X_1:X_2$ ; 20:20) and ( $X_1:X_2$ ; 3.43:60) received scores of 6.63 and 6.07 respectively, which are considered as “like slightly” among the panellists. The other formulations received scores considered as “neither like nor dislike”. In a sensory evaluation, a product with a score value of more than 5 for overall acceptability can be considered as a good quality product (Chong and Noor Aziah, 2008)

#### *Optimisation of responses and verification of the model*

In order to produce a jelly with a high total carbohydrates content, antioxidant capacity and acceptability, the optimal level of total desirability from all three responses was determined based on the combination of *Musa paradisiaca* and *Trigona* sp. honey. The numerical optimisation was carried out to discover the optimal condition of independent variables from several solutions generated. The optimum combination was attained for all responses, which was 20% of *Musa paradisiaca* and 20% of *Trigona* sp. honey, with the predicted response values for total carbohydrate content, antioxidant capacity and acceptability were 33 Kcal, 0.340 nm and 6.16, respectively. The experiment yielding optimised condition was repeated to assess the power of the response surface models to predict the optimum response values. The observed value of the total

carbohydrates content, antioxidant capacity and acceptability were  $32 \pm 2$  Kcal,  $0.328 \pm 0.004$  nm, and  $6.08 \pm 0.91$ , respectively. The values of experimental and predicted were compared to verify the response surface model. The experimental response values were incongruent with predicted values as there was no significant difference ( $p > 0.05$ ) observed between the experimental and the predicted values for total carbohydrates content ( $E = 3.13\%$ ), antioxidant capacity ( $E = 3.66\%$ ), and acceptability ( $E = 1.33\%$ ).

#### Total phenolic content and DPPH free radical-scavenging activity

The total phenolic content for optimised formulated jelly was  $18.3 \pm 2.67$  mg GAE/100 g. The total content of polyphenol for the recommended optimum combination was investigated using the modified Folin-Ciocalteu assay. The Folin-Ciocalteu reagent reacted with polyphenol from the jelly to form a blue complex (chromophore), which could be detected spectrophotometrically. In terms of antioxidant activities, DPPH radical-scavenging activity assay is one of the rapid tests available to investigate overall hydrogen/electron-donating activity of a single antioxidant and health-promoting dietary antioxidant supplements. DPPH implied radical scavenging capability using DPPH radical, which will be reduced by antioxidants (Jauhari *et al.*, 2013). In DPPH radical-scavenging activity assay, the result was expressed as a percentage of inhibition, with the optimised formulated jelly showing  $51.24 \pm 5.27\%$ .

#### Conclusion

The optimisation using RSM was successfully adopted to optimise the total carbohydrate content, antioxidant capacity as well as acceptability responses. The model equation was found to be adequate to predict the effects of the variables and the optimum combination of the formulated jelly. The high antioxidant properties of the jelly were also successfully verified using TPC and DPPH radical-scavenging assays. The formulation for optimised high antioxidant jelly was determined as 20% of *Musa paradisiaca* and 20% *Trigona* sp. honey. Further in vivo assessments and clinical trials, however, are necessary before proceeding with industrial applications and commercialisation.

#### Acknowledgement

The present work was financed by the Malaysian Ministry of Higher Education (MOHE) under the

Prototype Research Grant Scheme (PRGS15-015-0025). The authors would like to thank Kulliyah of Allied Health Sciences, International Islamic University Malaysia (IIUM) for the laboratory facilities provided for the completion of the present work.

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