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Optimization of alcohol insoluble polysaccharides (AIPS) extraction from the *Parkia speciosa* pod using response surface methodology (RSM)

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ABSTRACT

Central composite design (CCD) was employed to optimize the incubator temperature (X_1: 60–90 °C), extraction time (X_2: 1–3 h) and pH (X_3: 3–6) to obtain a high alcohol insoluble polysaccharide (AIPS) yield with high uronic acid content and antioxidant property from *Parkia speciosa* pod. Analysis of variance (ANOVA) showed that the contribution of quadratic model was significant for the responses. Optimization study using response surface methodology (RSM) was performed and 3-D response surfaces were plotted from the mathematical model. The optimal conditions were: extraction temperature of 90 °C, extraction time of 2.8–3.0 h, and pH of 3.0. These optimum conditions yielded AIPS of ~17–18%, uronic acid content of ~97–99 mg/g and %DPPHsc of ~48–50% and close agreement between experimental and predicted values was found.

1. Introduction

The food industry is experiencing a constantly growing demand for new ingredients from natural sources. This demand has therefore drawn researchers' attention to these ingredients obtained from agro-industrial waste (Guerrero, Torres, & Nuñez, 2008; Kasankala, Xue, Weilong, Hong, & He, 2007; Levigne, Ralet, & Thibault, 2002; Masmoudi et al., 2008; Renjie, 2008; Wu, Cui, Tang, & Gu, 2007). Biomass in the form of agro-industrial wastes containing these precious bio-materials is produced in large quantities generating environmental problems and caused loss of potentially valuable resources. Renewed awareness, in developing and developed countries alike, of the potentials of the agro-waste derived material has called for new research approaches to the exploitation of the agro-waste. In the developed countries, plant-derived materials are now regarded as either versatile functional ingredients or as biologically active components (McCleary & Prosky, 2001; Biliaderis & Izydorczyk, 2007). These plant materials found broad applications in the areas of foods (e.g. thickener, gelling agent, emulsifier, coating, fat substitute) and pharmaceuticals (e.g. radical scavenging agent, diet supplement) (Castro, Tirapegui, & Benedicto, 2003; Ebringerová et al., 2008). In addition, a diet rich in plant materials is believed to protect against a wide range of diseases (e.g. constipation, diverticular disease, colon-rectal diseases, diabetes, obesity, gall stones, colon cancer) (McCleary & Prosky, 2001; Biliaderis & Izydorczyk, 2007). This search for plant-derived bio-materials sources has therefore stimulated research interest in extracting and isolating these materials from under-utilized known agro-waste, such as empty bean pods from *Parkia speciosa*.

Parkia speciosa is known as stink bean or "petai". It bears long and flat beans pod with green seeds. These beans are popular in Southeastern Asia including Malaysia, and Northeastern India. They are sold in the pods or the seeds are sold in plastic bags. They are also pickled in brine and exported in jars. *Parkia speciosa* is believed by the locals that it has medicinal properties. Jamaluddin, Mohamed, and Lajis (1994, 1995) has reported the hypoglycaemic activity of the pod and seed using alloxan diabetic rats. Other researches showed that *Parkia speciosa* seeds contain antibacterial cyclic polysulfides (e.g. hexathionane, tetrathionane, trithiolane, pentathiepane, pentathiocane and tetrathiepane) which are also responsible for their strong pungent flavour. Thiazolidine-4-carboxylic acid which has anticancer activity is also present in *Parkia speciosa* (Pandeya, 1972; Susilo & Gmelin, 1982). These pods are regarded as waste during the processing in the industry and extraction of polysaccharide from this material has not been studied. Therefore, it is possible to extract alcohol insoluble polysaccharide (AIPS) from the pods as a novel functional carbohydrate. Preliminary study (Gan, Abdul Manaf, & Latiff, 2009) showed that different extraction pH conditions yielded different extracts which have different uronic content and antioxidant property.

The objectives of this study were to explore the potential of *Parkia speciosa* pod in producing AIPS and to optimize the conditions for the extraction of AIPS that obtain high extraction yield, uronic acid content and antioxidant property (i.e. %DPPHsc). As many factors can influence the extraction yield, response surface methodol-

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ogy (RSM) was applied to fit and to exploit a mathematical model representing the relationship between the responses (i.e. extraction yield, uronic acid content and %DPPHsc) and variables (i.e. temperature, extraction time and pH).

2. Materials and methods

2.1. Materials

One batch of 10 kg of *Parkia speciosa* was purchased from local market located in Penang, Malaysia. The pods were immediately separated from the seeds and the former was lyophilised and milled. The powder obtained was sieved (60-mesh size screen) and stored at 4 °C until use. Ethanol and hydrochloride acid (HCl) were purchased from Fisher Scientific (Malaysia) and Labscan (Thailand), respectively. All chemicals used in the experiment were of analytical grade.

2.2. Alcohol insoluble polysaccharide (AIPS) extraction

Extractions were carried out in a glass flask placed in an incubator as follows according to Masmoudi et al. (2008): 1.0 g of lyophilised *Parkia speciosa* powder was added to 50 ml of the distilled deionised water (solid–liquid ratio: 1:50, w/v) in each flask. The pH's of the mixtures were adjusted with 0.1 M HCl/NaOH and subsequently incubated in an incubator without agitation at different temperatures and incubation times (Table 1). The resulting slurries were allowed to cool to room temperature (25 °C) under running water after incubation and filtered through a muslin cloth. The filtrates were centrifuged at 20 °C for 30 min at 5000*g* to remove solid particles. Two volumes of 95% (v/v) ethanol were subsequently added to one volume of the extracts in order to precipitate AIPS. The obtained mixtures were kept for 5 h at 4 °C prior to filtration. The precipitates were washed three times with 50%, 75% and 100% ethanol and filtered in order to remove the mono and disaccharides. The obtained AIPSs were then dried at 50 °C to a constant weight. The extraction yields (Y), subject of this study, were calculated as follows:

$$Y(\%, \mathrm{w/w}) = \frac{W_E}{W_p} \times 100 \quad (1)$$

where W_E is defined as weight of extracted AIPS whereas W_p is defined as weight of dried pod used.

Table 1
Experimental domain of central composite design (CCD).

X_j	Factor levels				
	−1.68179	−1	0	1	1.68179
Temperature (°C)	49.77	60	75	90	100.23
Time (h)	0.32	1	2	3	3.68
pH	1.98	3	4.5	6	7.02

2.3. Determination of uronic acid

Uronic acid was determined by *m*-hydroxydiphenyl method (Blumenkrantz & Asboe-Hansen, 1973). Samples (0.5 ml) were mixed thoroughly with 3.0 ml of 0.0125 M sodium tetraborate solution (in concentrated sulphuric acid) in an ice bath. The mixtures were heated in a boiling bath for 5 min and subsequently cooled in an ice bath. The mixtures were added with 0.05 ml of 0.15% *m*-phenylphenol (in 0.5% sodium hydroxide solution). The absorbances at 520 nm were recorded after standing for 5 min. Standard curve was obtained using galacturonic acid (0–100 μg/ml).

2.4. Radical scavenging activity

The DPPH free radical scavenging activity of each sample was determined according to Liu et al. (2009). Samples were pre-diluted to concentration of 0.01 mg/ml prior to analysis. Aliquots of each sample (0.1 ml) were added to 3 ml of ethanolic DPPH solutions (0.1 mM). Discolorations were measured at 517 nm after incubation for 30 min at 30 °C in the dark. The %DPPH which was scavenged (%DPPHsc) was calculated using:

$$\%\mathrm{DPPHsc} = (A_{\mathrm{cont}} - A_{\mathrm{sample}}) \times 100/A_{\mathrm{cont}} \quad (2)$$

where A_{cont} was defined as absorbance of the control whereas A_{sample} was defined as absorbance of the sample (the extracts).

Table 2
CCD with the observed responses and predicted values for yield of pectin (%), uronic acid content (mg/g) and %DPPHsc (%).

Run	Coded variable levels			Observed (y_1)*			Predicted (y_0)		
	x_1 (temperature)	x_2 (time)	x_3 (pH)	Yield (%)	Uronic acid content (mg/g)	%DPPHsc (%)	Yield (%)	Uronic acid content (mg/g)	%DPPHsc (%)
1	−1	−1	−1	8.7	38.1	70.9	7.7	35.8	76.6
2	1	−1	−1	15.7	47.0	48.0	13.9	48.0	53.9
3	−1	1	−1	11.6	48.2	51.4	11.5	47.4	55.6
4	1	1	−1	18.6	97.0	42.0	17.9	99.1	48.0
5	−1	−1	1	10.9	81.3	61.8	10.7	83.3	60.2
6	1	−1	1	17.0	63.2	37.9	16.3	68.0	38.1
7	−1	1	1	13.5	87.1	41.1	14.5	90.45	39.6
8	1	1	1	20.3	108.0	33.9	20.5	114.6	32.6
9	−1.68179	0	0	10.6	53.4	53.4	10.4	53.8	51.6
10	1.68179	0	0	19.2	91.3	31.0	20.6	84.38	26.7
11	0	−1.68179	0	11.5	15.2	52.6	13.3	13.4	48.7
12	0	1.68179	0	20.6	67.0	28.8	20.0	62.3	26.5
13	0	0	−1.68179	5.8	82.0	98.5	7.5	83.9	87.7
14	0	0	1.68179	12.7	145.2	56.2	12.1	136.9	60.9
15	0	0	0	12.8	44.5	45.5	12.4	58.1	46.7
16	0	0	0	12.1	60.5	47.6	12.4	58.1	46.7
17	0	0	0	12.5	55.3	47.5	12.4	58.1	46.7
18	0	0	0	12.4	55.0	48.3	12.4	58.1	46.7
19	0	0	0	12.9	68.7	45.3	12.4	58.1	46.7
20	0	0	0	12.0	63.7	44.7	12.4	58.1	46.7

* Mean of triplicate determination.

2.5. Experimental design

The extraction parameters were optimized using RSM. A central composite design (CCD) was employed in this regard. Incubator temperature (X_1), extraction time (X_2) and pH (X_3) were chosen for independent variables. The range and center point values of three independent variables presented in Table 1 were based on the results of preliminary experiments (Gan et al., 2009). The experimental design consists of eight factorial points, six axial points at a distance of ± 1.68179 from the center and six replicates of the central point (Table 2). Yields of AIPS, uronic acid content and %DPPHsc were selected as the responses for the combination of the independent variables given in Table 2. Three experiments of each condition were carried out and the mean values were stated as observed responses. Experimental runs were randomized to minimize the effects of unexpected variability in the observed responses.

The variables were coded according to the equation:

$$x = (X_i - X_o)/\Delta X \quad (3)$$

where x is the coded value, X_i is the corresponding actual value, X_o is the actual value in the center of the domain, and ΔX is the increment of X_i corresponding to a variation of 1 unit of x.

The mathematical model corresponding to the composite design is:

$$Y = \beta_o + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=1+1}^{3} \beta_{ij} X_i X_j \quad (4)$$

where Y is the dependent variables (extraction yield, uronic content or %DPPHsc), β_o is the model constant, and β_i, β_{ii} and β_{ij} are the model coefficients. They represent the linear, quadratic and interaction effects of the variables. Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (version 6.0, USA). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

3. Result and Discussion

The effects of three process variables (i.e. temperature (X_1), time (X_2) and pH (X_3)) were studied during experimentation. Three responses of interest were extraction yield, uronic acid content and %DPPHsc. The results of 20 runs using CCD design are presented in Table 2 that include the design, observed responses and the predicted values. Results showed that there was a close agreement between experimental and predicted values. In addition, it was observed that the yield ranged from 8.7% to 20.6%. The maximum yield (20.6%) was found under the experimental conditions of X_1 = 75 °C, X_2 = 3.68 h and X_3 = pH 4.5. A wide range of uronic content was also found (15.2–145.2 mg/g) and the maximum point (145.2 mg/g) was found in conditions of X_1 = 75 °C, X_2 = 2 h and X_3 = pH 7.02. On the other hand, the antioxidant property (%DPPHsc) ranged from 31.0% to 98.5%. The highest %DPPHsc (98.5%) value was found in conditions of X_1 = 75 °C, X_2 = 2 h and X_3 = pH 1.98. These conditions seemed to be varied depending on the response required. Therefore, an optimum process should be investigated in order to obtain high yield, uronic acid content and antioxidant property.

3.1. Model fitting

Table 3 presents the results of fitting quadratic models to the data. The results of analysis of variance (ANOVA) indicate that the contribution of quadratic model was significant. The fitted quadratic model for extraction yield, uronic acid content and %DPPHsc in coded variables is given in Eq. (5, 6 and 7), respectively. The significance of each coefficient was determined using the F-test and p-value in Table 3. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller (Atkinson & Doney, 1992).

$$\text{Extraction yield} = 12.40 + 3.03x_1 + 1.98x_2 + 1.38x_3 + 1.10x_1^2 + 1.51x_2^2 - 0.90x_3^2 + 0.073x_1x_2 - 0.13x_1x_3 + 0.011x_2x_3 \quad (5)$$

$$\text{Uronic acid content} = 58.08 + 9.09x_1 + 14.53x_2 + 15.76x_3 + 3.89x_1^2 - 7.14x_2^2 + 18.49x_3^2 + 9.86x_1x_2 - 6.89x_1x_3 - 1.11x_2x_3 \quad (6)$$

$$\%\text{DPPHsc} = 46.67 - 7.41x_1 - 6.61x_2 - 7.96x_3 - 2.67x_1^2 - 3.20x_2^2 + 9.76x_3^2 \quad 3.78x_1x_2 + 0.14x_1x_3 + 0.094x_2x_3 \quad (7)$$

Table 3
ANOVA for response surface quadratic model: Estimated regression model of relationship between response variables (yield, uronic acid content and %DPPHsc) and independent variables (X_1,X_2,X_3).

Source	Sum of Squares	DF	Mean Square	F-value	P-Value
Yield (%)[a]					
Model	270.07	9	30.01	18.29	<0.0001
Quadratic	64.96	3	21.65	13.2	0.0008
X_1	125.7	1	125.7	76.62	<0.0001
X_2	53.34	1	53.34	32.52	0.0002
X_3	25.9	1	25.9	15.79	0.0026
X_1^2	17.42	1	17.42	10.62	0.0086
X_2^2	32.75	1	32.75	19.96	0.0012
X_3^2	11.8	1	11.8	7.19	0.023
X_1X_2	0.043	1	0.043	0.026	0.8745
X_1X_3	0.13	1	0.13	0.079	0.7847
X_2X_3	9.61×10^{-4}	1	9.61×10^{-4}	5.86×10^{-4}	0.9812
Residual	16.40	10	1.64		
Total	286.47	19			
Uronic acid content (mg/g)[b]					
Model	14792.39	9	1643.60	28.14	<0.0001
Quadratic	6219.54	3	2073.18	35.49	<0.0001
X_1	1129.09	1	1129.09	19.33	0.0013
X_2	2885.20	1	2885.20	49.40	<0.0001
X_3	3391.22	1	3391.22	58.06	<0.0001
X_1^2	218.50	1	218.50	3.74	0.0819
X_2^2	734.32	1	734.32	12.57	0.0053
X_3^2	4926.63	1	4926.63	84.35	<0.0001
X_1X_2	777.62	1	777.62	13.31	0.0045
X_1X_3	379.87	1	379.87	6.50	0.0289
X_2X_3	9.84	1	9.84	0.17	0.6901
Residual	584.09	10	58.41		
Total	15376.48	19			
%DPPHsc (%)[c]					
Model	4107.99	9	456.44	14.29	0.0001
Quadratic	1780.79	3	593.60	18.58	0.0002
X_1	749.32	1	749.32	23.46	0.0007
X_2	597.60	1	597.60	18.71	0.0015
X_3	865.76	1	865.76	27.10	0.0004
X_1^2	102.86	1	102.86	3.22	0.1030
X_2^2	147.65	1	147.65	4.62	0.0571
X_3^2	1371.45	1	1371.45	42.93	<0.0001
X_1X_2	114.28	1	114.28	3.58	0.0879
X_1X_3	0.16	1	0.16	0.01	0.9445
X_2X_3	0.07	1	0.07	0.00	0.9635
Residual	319.46	10	31.95		
Total	4427.45	19			

[a] The coefficient of determination (r^2) of the model was 0.9427.
[b] The coefficient of determination (r^2) of the model was 0.9620.
[c] The coefficient of determination (r^2) of the model was 0.9278.

3.1.1. Extraction yield

It can be seen that the variable with the largest effect on extraction yield was linear term of incubator temperature (X_1 followed by linear term extraction time (X_2), quadratic term of extraction time (X_2^2), linear term of pH (X_3), quadratic term of temperature (X_1^2) and quadratic term of pH (X_3^2) (Table 3). However, the interaction terms (X_1X_2,X_1X_3 and X_2X_3) were found insignificant ($p > 0.005$). These results shown in Table 3 suggested that the change of extraction temperature, time and pH had significant effects ($p < 0.005$) on the yield of AIPS however there is no significant ($p > 0.005$) interaction effect between these parameters on the yield. The coefficient of determination (R^2) of the predicted models in this response was 0.9427. This would give a good fit to the mathematic model in Eq. (5).

3.1.2. Uronic acid content

Linear term extraction time (X_2), linear term of pH (X_3) and quadratic term of pH (X_3^2) showed the largest effect ($p < 0.0001$) on uronic acid content (Table 3). It was followed by linear term of incubator temperature (X_1) and interaction term of incubator temperature and extraction time (X_1X_2). Quadratic term of temperature (X_1^2), quadratic term of extraction time (X_2^2), and the other interaction terms of X_1X_3 and X_2X_3 were however not significant ($p > 0.005$). These results again showed significant ($p < 0.005$) effects of these parameters (extraction temperature, time and pH) on uronic acid content. The coefficient of determination (R^2) of the predicted models in this response was 0.9620, which suggesting a good fit to the mathematical model (Eq. (6)).

3.1.3. Antioxidant property (%DPPHsc)

In term of antioxidant property, it can be observed that quadratic term of pH (X_3^2) gave the largest effect followed by linear term of pH (X_3), linear term of incubator temperature (X_1) and linear term extraction time (X_2). Quadratic term of temperature (X_1^2), quadratic term of extraction time (X_2^2), and the interaction terms (X_1X_2,X_1X_3 and X_2X_3) were not significant ($p > 0.005$). The coefficient of deter-

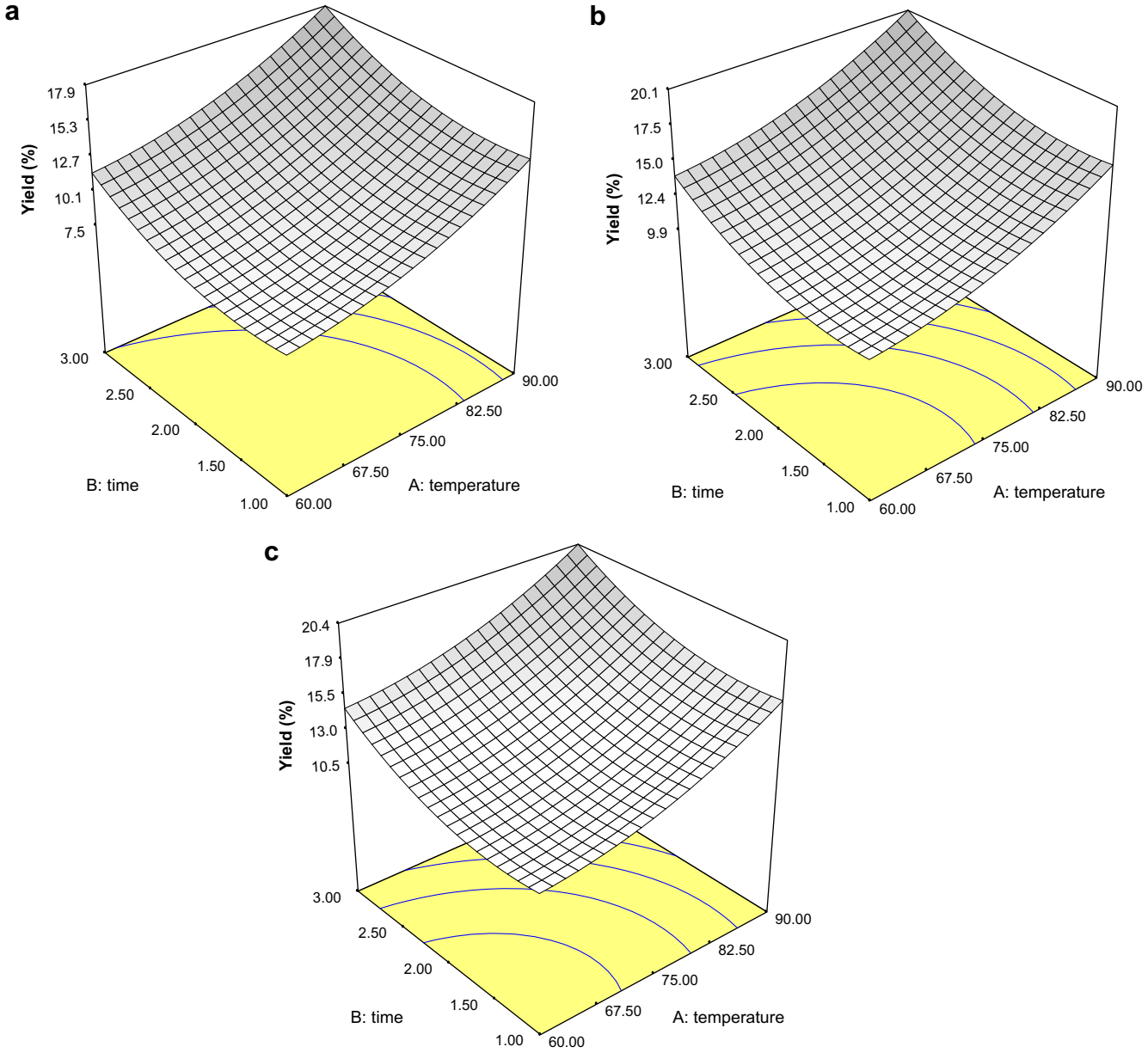

Fig. 1. Three-dimensional plot for extraction yield as a function of temperature and time at different pH: (a) pH 3; (b) pH 4.5; (c) pH 6.

mination (R^2) of the predicted models in %DPPHsc was 0.9278, which also suggesting a good fit to the mathematical model (Eq. (7)). The predicted models seemed can reasonably represent the observed values. Thus, the responses were sufficiently explained by the models.

3.2. Interpretation of response surface model

3.2.1. Extraction yield

Three-dimensional (3-D) plots for extraction yield as a function of extraction temperature and time at different pH conditions are given in Fig. 1. It can be seen that the extraction time demonstrated an exponential increase on the response in Fig. 1a. The effect of incubator temperature also displayed an exponential increase at a range of 60–90 °C. With regard to the effect of pH, similar pattern of 3-D plot at condition of pH 4.5 was shown in Fig. 1b. However, the range of the extraction yield was slightly higher (from 9.9% to 20.1%) compared to that of pH 3.0 (from 7.5% to 17.9%). A much higher yield range (from 10.5% to 20.4%) could be observed using condition of pH 6.0 (Fig. 1c).

It was reported that an extended extraction time favours the production of AIPS (Cho & Hwang, 2000; Hou & Chen, 2008). This might be due to the time requirement of the exposure of the AIPS to the release medium (distilled deionised water) where the liquid penetrated into the dried powdered pod, dissolved the AIPS and subsequently diffused out from the pod. In addition, increasing extraction temperature would increase the solubility of the extracted AIPS, giving a higher rate of extraction. Also, the diffusion coefficient will increase and thus improves the rate of diffusion (Masmoudi et al., 2008). Higher pH condition would also induce cell wall disruption, protopectin hydrolysis and solubilization which disrupt the ester linkages and hydrogen bonds between AIPS and cell wall (Renard, Crépeau, & Thibault, 1995; Wang, Pagán, & Shi, 2002). Therefore, it is reasonable to anticipate that an extended period and higher temperature would increase the release of AIPS from the pod whereas different pH conditions gave different yield of the extracts.

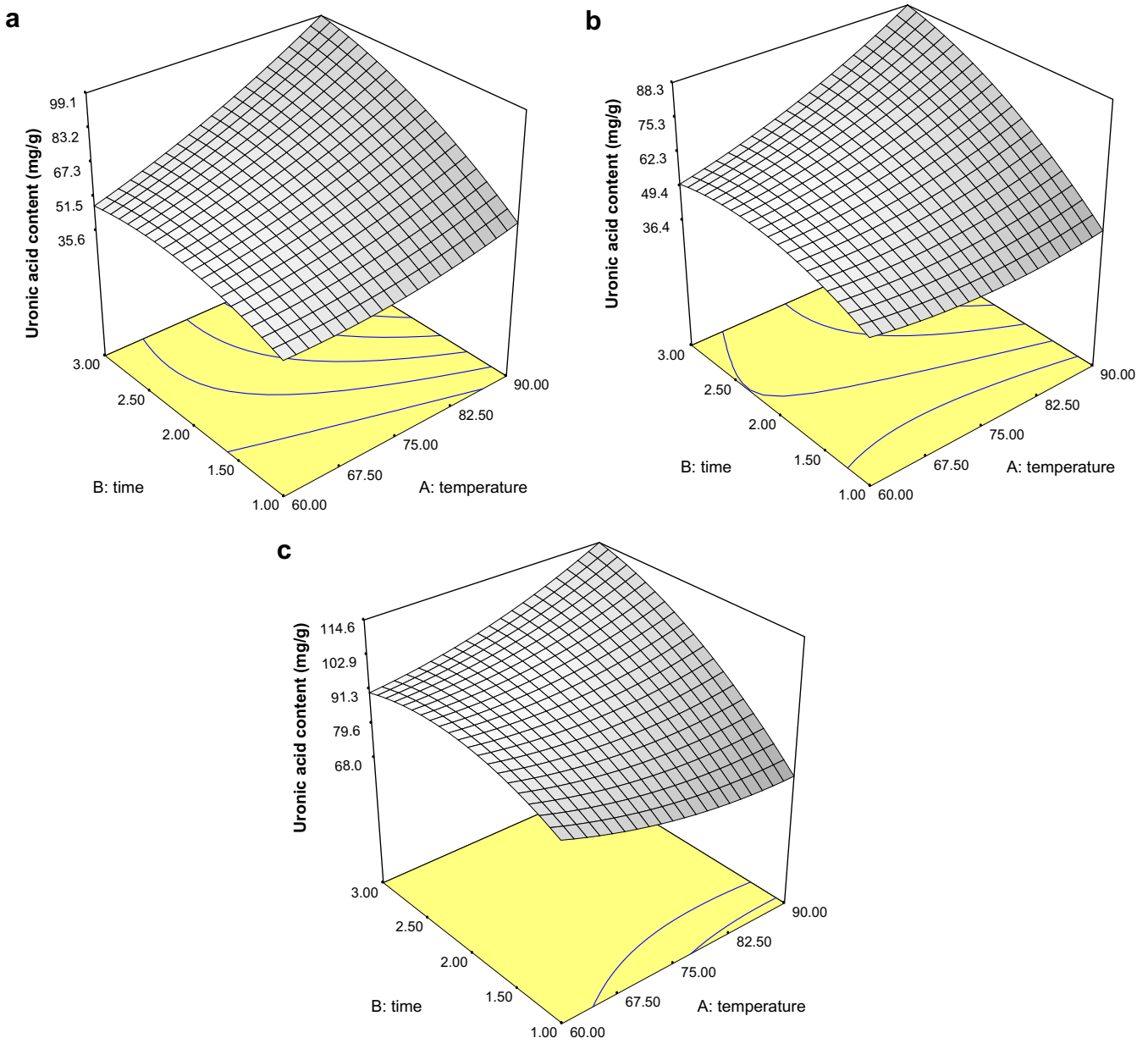

Fig. 2. Three-dimensional plot for uronic acid content as a function of temperature and time at different pH: (a) pH 3; (b) pH 4.5; (c) pH 6.

3.2.2. Uronic acid content

Different extraction conditions also gave effect to the uronic acid content of the extracts (Fig. 2). Extraction temperature gave a slight increase in uronic acid content whereas extraction time gave an exponential increase to uronic acid content within the first 2 h and gradually became constant at the following hour. The uronic acid contents of the extract were ranged from 36 to 99 mg/g at pH 3.0 (Fig. 2a). However, the range of uronic acid content decreased slightly to 36–88 mg/g at pH 4.5 (Fig. 2b). An apparent increase in uronic acid content was observed at subsequent increase of pH (pH 6.0) up to 114 mg/g, shown in Fig. 2c. The yield of the extraction was relatively correlated with the uronic acid content. Similarity in the response surface pattern between these responses was found. Therefore, this could be explained by the increase of temperature, time and pH aforementioned in Section 3.2.1. that favour the release of uronic acid by increasing the diffusion coefficient or the rate of diffusion (Cho & Hwang, 2000; Hou & Chen, 2008; Masmoudi et al., 2008) as well as by disrupting the linkages of pectin and cell wall (Renard et al., 1995; Wang et al., 2002).

3.2.3. Antioxidant property (%DPPHsc)

The 3-D response surfaces for %DPPHsc is given in Fig. 3. It was evident that the extracts consisted of different antioxidant property by scavenging free radicals. It was also observed that increase of extraction temperature and time decreased the %DPPHsc values. Extract from pH 3.0 seems to obtain a higher antioxidant property whereas extracts from pH 4.5 and 6.0 obtained a lower %DPPHsc values. This could be due to exposure of samples in high temperature, extended period and high pH during extraction that favours the oxidation. Reactive free radicals such as superoxide anion, hydroxyl radical and peroxy radical are the products of oxidation (Williams & Jeffrey, 2000) which may cause the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxida-

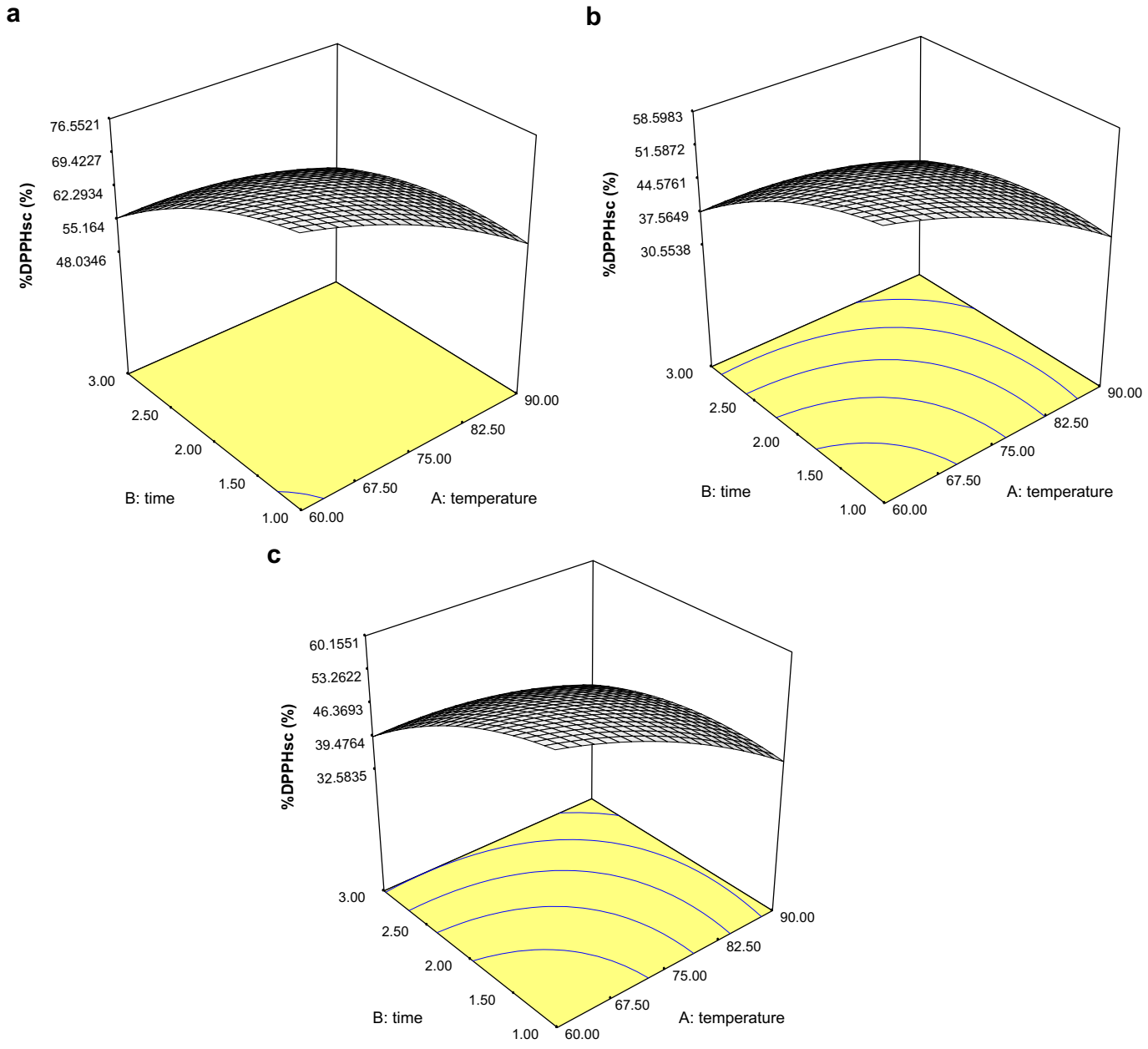

Fig. 3. Figure 1 Three-dimensional plot for %DPPHsc as a function of temperature and time at different pH: (a) pH 3; (b) pH 4.5; (c) pH 6.

Table 4
Predicted and experimental yield of pectin at optimum conditions.

Run	Process variables			Predicted yield (%)			Experimental yield* (%)		
	Incubator temperature (°C)	Extraction time (h)	pH	Yield (%)	Uronic acid content (mg/g)	%DPPHsc (%)	Yield (%)	Uronic acid content (mg/g)	%DPPHsc (%)
1	90.00	2.80	3.00	17.0	96.5	49.8	16.7	97.1	50.4
2	90.00	3.00	3.00	17.9	99.1	48.0	17.2	98.6	49.6

* Mean of triplicate determination.

tive DNA and alteration of platelet functions in human body (Fridovich, 1978; Kinsella, Frankel, German, & Kanner, 1993). These occurrences may result in many chronic health problems such as cancers, inflammation, aging and atherosclerosis. Therefore, the search for plant-derived bio-materials has therefore stimulated research interest in extracting natural products from under-utilized bulk agro-waste. In this study, the AIPS from *Parkia speciosa* pod gave an alternative to the synthetically produced food antioxidant.

3.3. Verification of predictive models

Based on the above findings, an optimization study was performed to evaluate the optimal operating conditions for the extraction with high yield of AIPS, uronic acid content and antioxidant property (%DPPHsc). Several combinations were found to maximize these responses taking into consideration of the efficiency, the energy conservation and the feasibility of the experiment. These are given in Table 4. The optimal conditions for desired extraction yield, uronic acid content and antioxidant property (%DPPHsc) corresponded to extraction temperature of 90 °C, extraction time of 2.8–3.0 h, and pH of 3.0. These optimum conditions yielded AIPS of ~17–18%, uronic acid content of ~97–99 mg/g and %DPPHsc of ~48–50%. Only small deviations were found between the actual values and predicted values. Thus, the model can be used to optimize the process of AIPS extraction from *Parkia speciosa* pod.

4. Conclusion

RSM was used to determine the optimum process parameters that gave a high extraction yield, uronic acid content and antioxidant property. ANOVA showed that the effects of all variables (i.e. incubator temperature, extraction time and pH) were significant and quadratic models were obtained for predicting the responses. The optimal conditions were: extraction temperature of 90 °C, extraction time of 2.8–3.0 h, and pH of 3.0. These optimum conditions yielded AIPS of ~17–18%, uronic acid content of ~97–99 mg/g and %DPPHsc of ~48–50%.

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