Optimization of ultrasound-assisted extraction of anthocyanins and bioactive compounds from butterfly pea petals using Taguchi method and Grey relational analysis

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Abstract Ultrasound-assisted extraction (UAE) was used to extract anthocyanins, antioxidants and phenolic compounds from butterfly pea petals, as an alternative to traditional methods. Taguchi method with three factors: extraction time (30, 45, 60 min), temperature (40, 60, 80 °C) and liquid–solid ratio (5, 7.5, 10 mL distilled water/mg butterfly pea) was used to obtain the high extraction yield. Grey relational analysis was employed to convert multi-response problem into single response optimization. The high extraction efficiency could be achieved at optimal parameter condition using 45 min of extraction time, 40 °C and 10 ml distilled water/mg butterfly pea. Liquid–solid ratio exhibited the highest contribution for anthocyanin and total phenolic content. A high temperature of ultrasonication resulted in a negative effect on antioxidant capacity

and total phenolic content. The findings from this study indicated that the UAE process optimization would be an efficient and sustainable method for the preparation of bioactive compounds from medical plants with saving of reaction time and cost in which extraction yields of antioxidant capacity and total phenolic content were also increased. The color response analysis results suggested that the gelatin film incorporated with butterfly pea extract can be potentially used as pH-indicator for detecting food spoilage for intelligent packaging.

Keywords Ultrasound-assisted extraction · Multiple response optimization · Taguchi method · Grey relational analysis · Butterfly pea · Anthocyanins

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Introduction

Clitoria ternatea L., well-known as butterfly pea in South East Asia, has become intensively attractive as it has promising potential applications both in modern medicine, agriculture and as a source of natural coloring agents and antioxidants in food applications (Mehmood et al. 2019; Oguis et al. 2019). This has occurred because of their beneficial properties such as food coloring effect, pH indicator, anti-inflammatory, inhibition of DNA damage in cancer cells and other degenerative diseases (Cortez et al. 2017; Khoo et al. 2017). Although numerous studies has attempted to study the biological activities and bioactive components of butterfly pea, there is currently no the large scale synthesis of anthocyanins from butterfly pea petal extract. The novel extraction technologies are continuously developed in order to improve the extraction efficiency and reduce cost. Among solvent extraction methods, ultrasound-assisted extraction (UAE) is considered to be an

effective method due to short time, low cost, low solvent and high productivity (Chotphruethipong et al. 2019; Mojerlou and Elhamirad 2018; Paraíso et al. 2019). The mechanism of UAE involves the mechanical effect that accelerates the release of organic compounds in the plant cells due to breakdown of cell wall, increases mass transfer across cell membrane, and enhances accessibility of solvent to cell (Malinowska et al. 2018). The UAE technique has been commonly used for extraction of anthocyanins and polyphenols from various plants such as rosemary leaves (Hosseini et al. 2018), mulberry (Espada-Bellido et al. 2017), blueberries, cherries and red pear peels (Wang et al. 2016). Previous studies have also observed that the efficiency of extraction depends on various parameters including extraction time, temperature and liquid–solid ratio. The use of high temperature usually increases the rates of diffusion and the solubility. However, the higher degradation rate of anthocyanins and phenolic compounds can be occurred at a very high extraction temperature ($> 80\text{ }^{\circ}\text{C}$) (Moldovan et al. 2016; Sarkis et al. 2019; Turturică et al. 2018). Therefore, it is necessary to investigate an optimal extraction temperature. The extraction efficiency of polyphenols from plant materials is also influenced by the extraction time, and long extraction time may increase the decomposition of extracted polyphenol compounds (Hosseini et al. 2018). Moreover, the effect of liquid–solid ratio on the extraction of bioactive compounds from plants has been extensively studied. The increase of liquid–solid ratio enhanced the mass transport driving force and the internal diffusion rate, improving the extraction yield (Liu et al. 2018). However, the solvent consumption is directly related to the cost of extraction process and the amount of solvent waste. A suitable liquid–solid ratio is an important parameter to improve the extraction yield and reduce the waste of solvent. Consequently, the optimization of UAE process is necessary to maximize extraction efficiency. Taguchi method is one the optimization techniques that can reduce the number of experiment, enhance quality of products and determine design solutions (Ravanfar et al. 2015; Subroto et al. 2017). Generally, Taguchi method has been used to determine the optimization of single response. For optimization of multi response problem, Taguchi method integrated with Grey relational analysis can convert the multi response results into a single grade relational (Kasemsiri et al. 2017). The extraction parameters of bioactive compounds have been successfully optimized by Taguchi method. Ravanfar et al. studied the extraction of red cabbage using UAE (Ravanfar et al. 2015). The parameters such as output power, time, temperature and pulse mode were optimized employing Taguchi method. The maximum yield of anthocyanin of 20.9 mg/L can be obtained from optimal condition. Kapadiya et al. also observed isolation of clove oil from

buds of *Syzygium aromaticum* using microwave assisted extraction (Kapadiya et al. 2018). The optimization of extraction process can improve the overall quantity and quality of essential oil. Furthermore, the optimal condition would be able to reduce the amount of water usage, along with extraction time resulting in a lower energy consumption.

The objective of this study was to investigate the optimal extraction conditions of butterfly pea using UAE. Three factors, including extraction time, temperature and liquid–solid ratio, and three levels were optimized employing Taguchi method and Gray relational analysis. The anthocyanin content was characterized using UV–visible light, in addition to the evaluation of antioxidant activity and total phenolic content. Finally, the gelatin films incorporated with butterfly pea extract obtained from the UAE optimization were fabricated to use as prototype freshness indicator for evaluating the color change of films at different pH values and the application test on food spoilage. It is expected that the findings from this study would be beneficial to have a better understanding of effective bioactive compound production in the large scale application of intelligent food packaging films.

Materials and methods

Chemicals

The dried butterfly pea (*Clitoria ternatea*) was obtained from local market in Khon Kaen, Thailand. The dried petals of butterfly pea were ground by grinder, Philips HR2102. The particle size of powder was in the range of 0.17–0.25 mm. Potassium chloride and sodium acetate from Ajax Finechem were used as-received. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was supplied by RCI Labscan Limited. Folin-Ciocalteu reagent, sodium carbonate and garlic acid at analytical grade were obtained from Fluka and Sigma Chemical Co., respectively. Glycerol (analytical grade, QRec Chemical Co.) and gelatin (125 blooms, Gelita) were used in this experiment.

UAE of bioactive compounds and experimental design

The ground petal was mixed with distilled water and the mixture was placed in water bath. The UAE process was performed using DT 255 H Bandelin ultrasonicator at an output power of 160 W. The UAE conditions such as extraction time (factor A = 30, 45, 60 min), temperature of water bath (factor B = 40, 60, 80 $^{\circ}\text{C}$) and liquid-solid ratio (factor C = 5, 7.5, 10 mL distilled water/mg butterfly pea) and the extracts from butterfly pea petals are given in

Table 1 Results of experimental design using Taguchi method for L9 orthogonal array 3 factors 3 levels

Run	Independent variables			Responses		
	Time	Temperature	L/S ratio	Anthocyanin	Total phenolic	DPPH activity
	(A, min)	(B, °C)	(C, mL/g)	(mg cy-3-glu/g DW)	(mg gallic acid/g DW)	(%)
1	30	40	5.0	1.05 ± 0.06	3.30 ± 0.03	53.95 ± 3.91
2	30	60	7.5	1.80 ± 0.07	5.09 ± 0.10	53.20 ± 0.79
3	30	80	10.0	2.19 ± 0.14	4.94 ± 0.08	43.84 ± 2.04
4	45	40	7.5	1.59 ± 0.12	4.85 ± 0.01	58.63 ± 4.96
5	45	60	10.0	2.12 ± 0.28	6.67 ± 0.01	56.68 ± 1.63
6	45	80	5.0	1.43 ± 0.15	3.09 ± 0.02	41.90 ± 0.54
7	60	40	10.0	1.91 ± 0.18	6.59 ± 0.03	63.24 ± 1.17
8	60	60	5.0	1.56 ± 0.01	3.10 ± 0.01	45.20 ± 1.14
9	60	80	7.5	1.89 ± 0.08	4.14 ± 0.02	40.44 ± 3.22

Values are mean ± standard error (SE) ($n = 3$)

Table 1. Each experiment was performed with a minimum of three replicates. The average values and the standard deviation error bar were determined and used for all responses containing anthocyanin content, total phenolic content and antioxidant activity.

Taguchi method containing three factors and three levels was used to optimize the conditions of UAE process in terms of single response and multiple responses. In the case of single response optimization, the signal to noise (S/N) ratio was used to determine the influence of each parameter on response using ANOVA. Generally, three categories of S/N ratios: (1) nominal-the-better, (2) smaller-the-better, and (3) larger-the-better were applied for optimization (Roy 1990). In this study, all responses such as content of anthocyanin, total phenol content and antioxidant activity were targeted to be maximized. Therefore, the “larger is better” was selected to analyze S/N ratio, as explained in Eq. (1).

$$(S/N) = -10 \log \left[1/R \sum_{j=1}^R 1/y_j^2 \right] \quad (1)$$

where R is the number of all data points and y_i is the value of i th data point.

For multiple response optimization, S/N ratios of all responses can be converted to single response optimization using grey relational analysis. The obtained results from Taguchi method can then be calculated to determine the highest overall grey relational which represents the optimal parametric combination. Before grey relational analysis, data preprocessing is normally required, that is a process of transferring the original sequence to a comparable sequence that is normalized within the range of zero to one (Pervez et al. 2016). The reference sequence and comparable sequence can be denoted by $x_0(k)$ and $x_i(k)$ for $i = 1,$

2,..., m; $k = 1, 2, \dots, n$, respectively, where m is the total number of experiment to be considered, and n is the total number of observation data. The appropriate equation for the normalization also depends on the type of the quality characteristic. In this work, the-larger-the-better quality characteristic was applied for the normalization of all responses that as expressed in Eq. (2).

$$x_i(k) = \frac{y_i(k) - \min y_i(k)}{\max y_i(k) - \min y_i(k)} \quad (2)$$

where $x_i(k)$ is the value after grey relational generation, $\min y_i(k)$ is the smallest value of $y_i(k)$ for k th response, and $\max y_i(k)$ is the largest value of $y_i(k)$ for k th response.

Grey relational coefficient can be calculated using Eq. (3).

$$\gamma(x_0^*(k), x_i^*(k)) = \frac{\Delta_{\min} + \zeta \Delta_{\max}}{\Delta_{0i}(k) + \zeta \Delta_{\max}} \quad (3)$$

$0 < \gamma(x_0^*(k), x_i^*(k)) \leq 1$. Where

$$\Delta_{0i}(k) = |x_0^*(k) - x_i^*(k)|,$$

$$\Delta_{\max} = \max_j \max_k |x_0^*(k) - x_j^*(k)|,$$

$$\Delta_{\min} = \min_j \min_k |x_0^*(k) - x_j^*(k)|,$$

ζ is the distinguishing coefficient, $\zeta \in [0, 1]$.

If all process parameters have equal weighting, ζ is set to be 0.5. The grey relational grade is the average of all grey relational coefficients, which can be determined using Eq. (4).

$$\gamma(x_0^*, x_i^*) = \frac{1}{n} \sum_{k=1}^n \beta_k \gamma(x_0^*(k), x_i^*(k)) \quad (4)$$

Finally, the optimal condition of UAE process for butterfly pea extraction can be the level corresponding to the highest value of average gray relational grade of each factor.

Total anthocyanin content determination

Anthocyanin content (ATC) was measured following the method carried out in the previous work (Cheok et al. 2013). The samples were diluted to a factor of 1:9 using two buffers: potassium chloride buffer (0.025 M, 1.0 pH) and sodium acetate buffer (0.4 M, pH 4.5). The absorbance of each dilution was measured at 520 and 700 nm using UV–visible spectrophotometer (Agilent Cary 60 UV–Vis Spectrophotometer). The total monomeric anthocyanin concentration was calculated following Eq. (5).

$$\text{Anthocyanin content} = \frac{A \times M_w \times DF \times 1000}{\varepsilon \times L} \quad (5)$$

where $A = (A_{520} - A_{700})_{\text{pH } 1.0} - (A_{520} - A_{700})_{\text{pH } 4.5}$; $M_w = 449.2 \text{ g/mol}$ for cyanidin-120 3-glucoside; DF = Dilution factor and $\varepsilon = 26,900 \text{ L/mol/cm}$ the molar extinction coefficient.

Total phenol content determination

The total phenol content (TPC) of samples was evaluated using a Foline Ciocalteu method and gallic acid as standard for external calibration according to Trongchuen et al. with minor modification (Trongchuen et al. 2018). The sample 0.2 mL was mixed with 1 mL of Foline-Ciocalteu reagent for 8 min. In the next step, 2 mL of 7.5% sodium and distilled water was added before the mixture was kept at room temperature in the dark for 2 h. The absorption was measured at 765 nm using UV–vis spectrophotometer. The calibration curve of gallic acid was used to determine the concentration of TPC. The results were expressed as mg gallic acid/g dry weight (DW).

Antioxidant activity determination

The antioxidant activity of samples was evaluated by DPPH scavenging assay according to Brand-Williams et al. (Brand-Williams et al. 1995). 1.5 mL of sample was mixed with 0.0025 mL of DPPH solution (0.1 mol/L in 95% methanol) using a vortex (Scientific Industries G560E 3200 rpm). After shaking the sample for 5 min, the mixture was incubated for 30 min at a temperature of 28 °C in the dark condition. The absorbance of mixture was measured at 517 nm using UV–vis spectrophotometer. The percentage of DPPH free radical scavenging activity of sample was expressed by the following Eq. (6).

$$\text{DPPH scavenging activity} = \frac{\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{DPPH}}} \times 100 \quad (6)$$

where Abs_{DPPH} and $\text{Abs}_{\text{Extract}}$ are the absorbances at 517 nm of the DPPH solution and extracted sample, respectively.

Color response analysis

The gelatin film containing 15 wt% butterfly pea extract was prepared by dissolving 3.5 g of gelatin in 20 mL of distilled water at 60 °C under magnetic stirring for 30 min, and then mixing with 1.05 g of glycerol and 0.525 mL of anthocyanin-rich extract from butterfly pea petals at room temperature. Then, the mixed solutions were cast on a Petri dish and dried in an oven with air flow circulation at 40 °C for 3 h.

To evaluate the color change of films at different pH values, the samples of gelatin-based film were cut into a $1 \times 1 \text{ cm}^2$ square and immersed in buffer solutions with different pH values ranging from 2 to 11 for approximately 10 min at room temperature. After removing the buffer solutions, film strip was placed on a Petri dish, and its color was then observed without drying. The images of the film were acquired by a digital camera.

To achieve the application test on food spoilage, the gelatin-based film used as prototype freshness indicator for fish spoilage. Mackerel was placed into a container and stored at room temperature, as presented in Fig. 3g. The film sample was faced with fish sample to observe the pH change after the storage for 24 h in ambient conditions. The images of the film color change were taken with a digital camera.

Results and discussion

Effect of extraction time on butterfly pea petal extraction

As can be seen in Fig. 1a, the extraction periods were varied to investigate the effect of time on extraction of anthocyanins, total phenolic and antioxidants from butterfly pea petals. The anthocyanin content slightly increased with increasing extraction time, and the highest yield was observed at 60 min. This could be due to the fact that the efficiency of extraction was influenced by the contact time between solute and solvent during UAE process. It was noticeable that the use of extraction time in all range gave similar tendency results of which TPC and DPPH antioxidant activity increased with the extraction time from 30 to 45 min. Subsequently, this trend slightly decreased with an

Fig. 1 The effects of main factor by using Taguchi method of **a** extraction time, **b** temperature and **c** liquid–solid ratio

increasing time to 60 min. The heat caused by ultrasonic waves during a longer extraction time might be the reason for the degradation and structural destruction of extracted phenolic compounds, thus resulting in the lower extraction efficiency. Accordingly, the higher amounts of anthocyanin were extracted when a longer extraction time was used, whereas the decomposition of extracted polyphenols can also be observed over a prolonged extraction time. From the economic perspective, shorter process time used, more economic process can be achieved, therefore, the optimization of extraction time and temperature can be considered to reach optimal extraction yield of all bioactive compounds. This finding was in good agreement with the gradually decreased yield of TPC and DPPH antioxidant activity of the chili extract with longer extraction time as

previous reported by Sricharoen et al. (Sricharoen et al. 2015). Makasana et al. suggested that the longer extraction time reduced the extraction efficiency by allowing the bubble grow inside the bubble; this resulted in the degradation of some antioxidant compounds (Makasana et al. 2016). Similar results were also reported by Altemimi et al. that increasing ultrasonication time led to the loss of antioxidant activity of spinach extracts (Altemimi et al. 2015).

Effect of temperature on butterfly pea petal extraction

The temperature has a critical effect on the extraction induced by ultrasonication process. The relationship between the temperature of UAE process and the yield of extracted compounds was illustrated in Fig. 1b. It was evident that the anthocyanin content increased as a function of temperature. Anthocyanin contents were in range of 1.518–1.837 mg cy-3-glu/g DW and the highest yield was at 80 °C. It is mainly due to the increase of solvent diffusivity and compound solubility during extraction by increasing temperature. Rajha et al. reported from the work on anthocyanin extract from grape that the increasing of temperature could reduce extraction time (Rajha et al. 2014). However, using high temperature might promote higher degradation rate of total phenolic and antioxidant compounds. As a result, DPPH antioxidant activity remarkably decreased with increasing temperature. The highest TPC was obtained at a temperature of 60 °C and decreased afterwards which is consistent with the findings of a previous research. The TPC consisted of various compounds: anthocyanin, delphinidin derivative, ternatin, quercetin derivative and rutin. Among these compounds, quercetin and rutin were easily degraded by time and temperature (Kim et al. 2018). Zhang et al. described that the ultrasonic extraction would produce cavitation bubbles that could explode and disrupt the structure to release component into solvent during extraction (Zhang et al. 2008). It is known that the vapor pressure is higher at high temperature than at lower temperature. The bubbles were produced at higher rate but less intensity and thus the intensity of mass transfer was reduced (Bonfigli et al. 2017). Another reason to explain this phenomenon is that low temperature operation permitted an easier dissipation for supersonic waves, whereas high dilatation and the expansion of solvent occurred as a result of the thermal effect, which reduced the efficiency of UAE (Altemimi et al. 2015).

Effect of liquid–solid ratio on butterfly pea petal extraction

Figure 1c shows the effect of liquid–solid ratio on the extraction using UAE. The similar trends of all responses were observed. The yields of extraction increased as the liquid–solid ratio increased from 5 to 10 mL distilled water/mg butterfly pea. This result is in good agreement with the previously reported result by Zhang et al. (2008). The use of a large amount of solvent could accelerate the diffusion process. It is a well-known fact that a larger volume of solvent accelerates the mass transfer by enhancing the contact area among solvent and plant materials. The reduction in concentration and viscosity of solvent could lead to higher dissolution of extracted in the solvent. More solvent could permeate into cell while the extracts could also dissolve into solvent. Such finding is consistent with the results reported by Ince et al. that the high yield of extracted phenolic compounds from nettle achieved by employing UAE at high solid to liquid ratio of 1:30 g/mL (Ince et al. 2014). Similar results were also observed for the extraction of various plants, i.e. flaxseed (Zhang et al. 2008) and corn silk (Prakash Maran et al. 2013).

Single response optimization of the UAE process

To study the effect of process variables on all single responses, the experimental results were transformed into S/N ratios. The main effects plots for S/N ratio of individual response are shown in Fig. 2. The optimal process condition for single performance characteristic was determined by analyzing the parameter values in each level having the highest value of S/N ratio. It was found that the highest anthocyanin content was recorded using $A_3B_2C_3$ condition with 60 min of extraction time (3rd level of factor A), 60 °C of temperature (2nd level of factor B) and 10 mL distilled water/mg butterfly pea (3rd level of factor C), while the highest amount of both TPC and antioxidant compounds was obtained at $A_2B_1C_3$ condition with 45 min of extraction time (2nd level of factor A), 40 °C of temperature (1st level of factor B) and 10 mL distilled water/mg butterfly pea (3rd level of factor C). The experimental data in Table 1 were conducted to predict the relationships between yields of bioactive compounds and factors using multi-linear regression as expressed in Eqs. (7–9).

$$\text{ATC} = -0.008 + 0.00367A + 0.00798B + 0.1456C \quad (7)$$

$$\text{DPPH} = 65.30 - 0.0235A - 0.4136B + 1.515C \quad (8)$$

$$\text{TPC} = 1.32 + 0.0055A - 0.0214B + 0.5816C \quad (9)$$

Fig. 2 The effect of three factors and their levels on S/N ratio and single response of **a** anthocyanin content, **b** total phenolic content and **c** DPPH activity

The analysis of variance (ANOVA) was also carried out at the 95% confidence level to identify the significant variables and to quantify their effects on the response characteristics, as depicted in Table 2. Considering the P values and percentage contributions of each factor, it revealed that liquid–solid ratio (factor C) had most influence on the extraction yield of anthocyanin and TPC among the process parameters due to its highest percentage contribution and low P value ($P < 0.05$). The liquid–solid ratio exhibited the highest percentage contribution for anthocyanin content and TPC of 77.72% and 84.00%, respectively. Whereas, the time and temperature of UAE process had very less significant on anthocyanin content and TPC

Table 2 The results of ANOVA for all responses and Gray relational grades

Factor	DF ^a	SS ^b	MS ^c	F value	P value	Contribution (%)	R ²	R ² (adj)
Anthocyanin content								
A	2	0.019	0.009	1.400	0.416	1.82		
B	2	0.198	0.099	14.790	0.063	19.17		
C	2	0.801	0.400	59.980	0.016*	77.72		
Error	2	0.013	0.007			1.30		
Total	8	1.031				100.00	93.73%	89.96%
Total phenolic content								
A	2	0.270	0.135	0.440	0.693	1.78		
B	2	1.541	0.771	2.530	0.283	10.20		
C	2	12.695	6.347	20.880	0.046*	84.00		
Error	2	0.608	0.304			4.02		
Total	8	15.113				100.00	91.47%	86.36%
DPPH activity								
A	2	12.510	6.256	0.630	0.613	2.35		
B	2	414.220	207.112	20.940	0.046*	77.78		
C	2	86.060	43.031	4.350	0.187	16.16		
Error	2	19.780	9.889			3.71		
Total	8	532.570				100.00	93.38%	89.41%
Gray relational grades								
A	2	0.006	0.003	0.67	0.599	2.02%		
B	2	0.031	0.016	3.65	0.215	11.03%		
C	2	0.239	0.120	27.77	0.035*	83.92%		
Error	2	0.009	0.004			3.02%		
Total	8	0.285				100.00%	93.09%	88.94%

A, B and C represent extraction time, temperature and liquid–solid ratio, respectively

^aDegree of freedom

^bSum of squares

^cMean square

*Significant at $P < 0.05$

($P > 0.05$). Furthermore, temperature played important role on the yield of DPPH antioxidant activity with the highest percentage contribution of 77.78% and low P value ($P < 0.05$). However, UAE process employing at high temperature during a long time had a negative effect on TPC and DPPH antioxidant activity owing to the degradation of bioactive compounds during this method.

Multiple response optimization of the UAE process

Taguchi method with grey relational analysis was performed to optimize the UAE process condition. Initially, data preprocessing for the larger-the-better quality characteristic was applied for the normalization of obtained responses from Taguchi method using Eq. (2). Grey relational coefficients and grey relational grade were determined using Eq. (3) and (4), respectively. The values of normalized S/N ratio and grey relational analysis are tabulated in Table 3. The mean of grey relational grade of

process parameter for each level was then determined by employing the response table of Taguchi method. The average grey relational grade represents the level of correlation among the sequence. The greater value of grey relational grade suggested the comparability sequence that presents a stronger correlation with the reference sequence. Typically, the optimal level of the factors is the level with the highest value of average grey relational grade. By comparing the mean values of grey relational grade for all responses listed in Table 4, the optimal condition for butterfly pea extraction using UAE process was obtained as $A_2B_1C_3$ condition with 45 min of extraction time (2nd level of factor A), 40 °C of temperature (1st level of factor B) and 10 mL distilled water/mg butterfly pea (3rd level of factor C). ANOVA analysis was performed at the 95% confidence level for the average grey relational grade of all responses to identify the significant factors. The results revealed that liquid–solid ratio (factor C) was a statistically significant process parameter that influenced most on the

Table 3 The S/N ratio and Grey relational analysis for nine comparability sequences

Run	S/N ratio			Normalized S/N ratio			GRC			GRG	GRO
	ATC	TPC	DPPH	ATC	TPC	DPPH	ATC	TPC	DPPH		
1	0.38	10.36	34.64	0.00	0.09	0.65	0.33	0.35	0.59	0.42	7
2	5.12	14.14	34.52	0.74	0.65	0.61	0.66	0.59	0.56	0.60	4
3	6.79	13.88	32.84	1.00	0.61	0.18	1.00	0.56	0.38	0.65	3
4	4.05	13.71	35.21	0.57	0.59	0.79	0.54	0.55	0.71	0.60	5
5	6.53	16.48	35.07	0.96	1.00	0.76	0.93	1.00	0.67	0.87	2
6	3.13	9.79	32.44	0.43	0.00	0.08	0.47	0.33	0.35	0.38	9
7	5.64	16.38	36.02	0.82	0.99	1.00	0.74	0.97	1.00	0.90	1
8	3.85	9.82	33.10	0.54	0.01	0.25	0.52	0.34	0.40	0.42	8
9	5.54	12.33	32.14	0.81	0.38	0.00	0.72	0.45	0.33	0.50	6

Table 4 The response table for Gray relational grades and result of confirmation experiment

Levels	Factors		
	A = Time	B = Temperature	C = L/S ratio
1	0.558	0.641	0.409
2	0.616	0.629	0.567
3	0.607	0.510	0.805
Delta	0.058	0.131	0.397
Rank	3	2	1
Grey relation grade of A ₂ B ₁ C ₃	Predicted 0.875	Experiment 0.891	

multi-response characteristics because of its highest percentage contribution of 84% and low P value ($P < 0.05$).

Basically, Grey relation analysis is used to convert the multi responses to single response, and the larger grey relational grade indicates the better quality characteristic of multi responses. In this work, the verification experiment was carried out under A₂B₁C₃ condition. It was noticeable that the yields of anthocyanin content, TPC and DPPH activity were 1.77 ± 0.22 mg cy-3-glu/g DW, 7.35 ± 0.04 mg gallic acid/g DW and $63.80 \pm 0.01\%$, respectively. Although the anthocyanin content decreased, the higher amounts of TPC and DPPH activity was achieved in comparison with the results obtained from Taguchi method; thus the multiple responses improved under grey relational grade. From the result of the confirmation tests, the grey relational grade improved from 0.875 to 0.891, after validation. The percentage deviation between the predicted and experiment results was less than 10%. It implied that Taguchi method integrated with Grey relational analysis was effectively carried out to investigate the optimal extraction conditions of butterfly pea using UAE with high yields of extraction. Based on previous research reported by Kumar et al. who attempted to obtain the optimal parameters for the fabrication of polymer composites incorporated with fly ash using Taguchi–Grey relational analysis to achieve the hardness, flexural strength and moisture absorption, it was found that the highest grey

relational attributes could improve multiple performance characteristics (Kumar et al. 2018). The value of the grey relational grade was improved by 4.4% from 0.701 to 0.732, although the values of hardness and flexural strength were lower than those obtained from Taguchi experimental design.

Color response analysis of pH-sensing films

It was noticeable that gelatin-based films containing butterfly pea extract could be used for further development of pH indicator. The color of gelatin-based film enriched with anthocyanins could change with pH. The visual appearance of films before and after immersion in buffer solutions at different pH values is illustrated Fig. 3. Initially, the color of films were dark bluish purple. When the film was immersed into the buffer solution at pH 2.0, the film presented a pinkish purple color, whereas at pH 5.0–7.0 it showed a light blue. At pH 9.0–11.0, a bluish green color became obvious and intensified with increasing pH. The color change is mainly due to the change of molecular structure of anthocyanins at different pH values (Mekar Saptarini et al. 2015). As in acidic pH condition, the red xanthine salt cations were an important part in the solution thus that the film exhibited red color. As the pH increased, the anthocyanin lost its cation to blue quinone; this led to the simultaneous decrease in the original xanthine salt ion

Fig. 3 Response of gelatin films containing anthocyanin-rich butterfly pea extract at different pH values: **a** control film, **b** pH 2, **c** pH 5, **d** pH 7, **e** pH 9 and **f** pH 11, and **g** application test on fish spoilage after storage for 24 h at room temperature

concentration and color intensity. Therefore the film gradually appeared blue. This was in agreement with the results of previous studies by Yong et al. reported that films containing chitosan and anthocyanin-rich extract from purple-fleshed sweet potato exhibited the color variations, changing from pink-red at pH 3.0–6.0 to greenish-green at pH 9.0–10.0 (Yang et al. 2019). Prietto et al. also reported a similar trend, in which the pH-sensitive films made from starch incorporated with glycerol and anthocyanins extracted from black bean seed coat and red cabbage showed the color changing from pink to purple and blue under different pH conditions and the color spectra difference depending on the source of anthocyanins that were used (Prietto et al. 2017). These observations are in accordance with the results obtained for other anthocyanin-rich films (Zhang et al. 2019).

In general, by microbial degradation during storage, various volatile nitrogen compounds, such as trimethylamine, ammonia, and dimethylamine, are produced in foods, leading to a pH change in the enclosed food package (Zhang et al. 2019) and causing foods to be undesirable for human consumption owing to changes in sensory characteristics (Morsy et al. 2016). In this work, the pH-sensing film was tested with fish sample in order to test its efficiency in practical applications. It can be seen that the film sample showed a dark bluish purple color at the beginning, and then changed to a bluish green color after being in contact with the fish which was stored for 24 h at room temperature, as depicted in Fig. 3g. Previous study reported that the changes in the color could indicate the

accumulation of volatile amines, such as trimethylamine, produced from the protein decomposition, in the existence of microorganisms causing *P. fragi* spoilage, leading to the increase of pH level in the headspace (Chun et al. 2014). This suggests that the film could indicate the freshness of fish via an obvious color change. Since pH value probably represents the freshness of food, and is correlated with bacterial growth as spoilage proceeded. The results are also consistent with the study of pork and fish spoilage estimation in visual pH sensing films based on chitosan and natural dyes extracted from the flower of *Bauhinia blakeana* Dunn (Zhang et al. 2014). Therefore, the simple and cheap pH indicator film fabricated in this work can be possibly used as a visible label for monitoring fish freshness during spoilage for intelligent packaging.

Conclusion

An optimization of ultrasound-assisted extraction of butterfly pea petals using Taguchi method and grey relational analysis was successfully carried out in this study. The extraction assisted by ultrasound of butterfly pea petal under the optimized parameter condition with 45 min of extraction time, 40 °C of process temperature and 10 mL distilled water/mg butterfly pea achieved the high extraction yield of bioactive compounds, especially total phenolic content. The results suggest that UAE is an effective and indeed feasible method for using in the industry compared to conventional method. Moreover, the prototype gelatin films containing anthocyanin-rich butterfly pea extract showed color changes at different pH values and could be used to indicate the fish freshness. Therefore, pH indicator film fabricated in this work can be potentially applied as a visible label for monitoring fish freshness during spoilage in our further study.

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