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Optimization of Total Phenolic from *Cleistocalyx nervosum* by Microwave-Assisted Extraction

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Abstract: The objective of this research was to extract phenolic compounds from Makiang (*Cleistocalyx nervosum*) seeds waste obtained from the elaboration of makiang beverages using the microwave assisted extraction method. The optimal conditions of microwave assisted extraction of total phenolics from makiang seeds were determined using response surface method. The variables of microwave power, extraction time and ethanol proportion on effect of total phenolic were designed the experiment by Box Behnken design. The estimation of the mathematical model indicated that the second-order polynomial model was appropriate to determine optimal conditions of microwave assisted extraction of total phenolics from makiang seeds. The highest yield of total phenolic from makiang seed and antioxidant activity were obtained when the extraction process was set at a microwave power, 450 W, an extraction time of 213 second and an ethanol proportion of 51%(v/v). Under these optimal conditions, the predicted and experimental values of total phenolic from makiang seeds were the 75.659 mgGAE/gDW and 75.132±0.576 mgGAE/gDW, respectively. The research showed that total phenolics from makiang seeds by microwave assisted extraction have the high efficiency in terms of high yield and antioxidant activity within short time extraction which can apply to use in cosmetic product, health food and pharmaceutical industry.

Keywords: *Cleistocalyx nervosum*, Total Phenolic, Microwave-Assisted Extraction, Antioxidant Activity

Introduction

Makiang (*Cleistocalyx nervosum*) seeds are commonly used for the elaboration of beverages because of its high content of carbohydrates, vitamin b1, vitamin b2, calcium and flavonoids (Taya *et al*., 2014; Khumtue and Naphrom *et al*., 2013). However, there is an abundant waste of makiang seeds rich in phenolic compounds during the process of elaboration of beverages (Patthamakanokporn *et al*., 2008; Sriwanthana *et al*., 2007). Many studies report that numerous plants contain phenolic compounds that help reduce the risk of cancers, heart and neurodegenerative diseases (Joseph *et al*., 2005; Nuengchamnong and Inkaninan, 2009).

A traditional method of extracting phenolic compounds was by using water bath; however, this extraction process requires long periods of time. At present, the extraction of phenols using microwave (-assisted) has gain interest, because it requires a shorter extraction period, uses less solvent, has a better extraction rate and operates under a lower cost. This technique has been widely used in the extraction of pheolic compounds from several plants and vegetables (Gallo *et al*., 2010; Garofulic *et al*., 2013; Song *et al*., 2011). However, extraction of Total Phenolic compounds from Making Seeds (TPMS) using microwave methods has yet to be attempted. Therefore, microwave-assisted extraction of TPMS was investigated by response surface methodology to determine the optimal condition of process. Moreover, antioxidant activities of TPMS were studied.

Box Behnken Design (BBD) is a statistical technique of response surface methodology that uses experimental data to simulate mathematical equation and then solve problem to determine the optimal condition. This method has been applied to optimize biochemical and physical process in many researches (Cansee *et al*., 2008; Omar *et al*., 2004; Zhu *et al*., 2010; Xu *et al*., 2008; Narkprasom *et al*., 2013) because, this excellent design spends minimal experiment to obtain the best outcome.

Therefore, microwave-assisted extraction of TPMS was investigated by response surface methodology to determine the optimal condition of process. Moreover, antioxidant activities of TPMS were studied.

Materials and Methods

Material

Samples of makiang seeds (*Cleistocalyx nervosum*, RIT) were obtained from the waste beverage process of pilot plant located at Maejo University. The seeds were separated manually from the rest of the waste and then were washed with fresh water. After washing, the seeds were dried at 60°C for 3 days and then were milled to powder at 40 mesh. The powder was then packed in aluminum foil bags and kept in desiccators.

Chemicals

Gallic acid, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, Na_2CO_3 and Folin-Ciocalteu's phenol were purchased from union science (Chiang Mai, Thailand).

Microwave-Assisted Extraction

A microwave oven (Samsung, ME711K) and soxhlet extractor set (Fig. 1) were used for the extraction of phenol compounds from the making seeds. The microwave oven was connected to a cooling system (Thermo Electron Haake WKL 25 Recirculator Chiller) that was set at 30°C to control temperature of circulated water. The leak of microwave-assisted extraction was checked with microwave leakage detector (RCME).

Quantification of Total Phenolic from Makiang Seeds

Makiang powder was mixed with ethanol at a ratio of 1:30 g mL^{-1}. The TPMS were extracted from the samples using different microwave power and extraction time. The samples were then centrifuged at 5000×g for 5 min to separate between the supernatant and solid portions of the dilution. One mL of the supernatant was diluted with 3 mL of distilled water. The 100 μL of mixed sample was added with 2 mL of 7%w/v Na_2CO_3 and 100 μL of Folin-Ciocalteu's phenol and then the samples were kept at room temperature for 30 min. TPMS was quantified using a spectrophotometer at 750 nm (Tookjit, 2011).

Assay of DPPH Radical Scavenging Activity

Antioxidant activity of TPMS was measured using Shimada *et al*. (1992) DPPH assay. About 100 μL of TPMS were mixed with 2900 μL of 0.1 mM DPPH in methanol. The samples were kept in the dark for 30 minutes and then, were measured DPPH radical scavenging activity by spectrophotometer at 515 nm. The antioxidant activity was calculated according to the following Equation 1:

$$\% \text{ Inhibition} = \left(\begin{array}{l} 1 - \text{absorbance of sample} / \\ \text{absorbance of control} \end{array} \right) \times 100 \quad (1)$$

Box Behnken Design and Statistical Analysis

The preliminary variables used in this study consisted of microwave power (x_1), extraction time (x_2) and ethanol proportion (x_3). The effects of these variables on the microwave-assisted extraction of the making seeds were investigated and analyzed using the ANOVA available at SPSS 17. The groups in homogenous subsets by Duncan method were displayed for designed level of BBD in next step. Based on ANOVA, the level of each factor was selected to design experiment which presented in Table 1.

Each level of the variables used in this study were coded -1, 0, 1. BBD was employed to determine the optimal condition. The whole design of 17 experimental points carried out and show on Table 2.

Fig. 1. Microwave-assisted extraction and microwave leakage detector for optimization of TPMS

Table 1. Factors and levels for designed experiment

Factor			Level -1	0	1
Microwave power	x_1	W	400	450	500
Extraction time	x_2	s	180	210	240
Ethanol proportion	x_3	%v/v	40	50	60

Table 2. Box Behnken design and response of TPMS

Runs	Microwave power (W) x_1	Extraction time (s) x_2	Ethanol proportion (%v/v) x_3	Yield of TPMS (mgGAE/gDW) Experimental	Predicted*
1	-1(400)	-1(180)	0(50)	60.13	60.552
2	1(500)	-1	0	61.23	61.972
3	-1	1(240)	0	60.29	59.547
4	1	1	0	66.14	65.717
5	-1	0(210)	-1(40)	57.39	58.355
6	1	0	-1	62.54	63.185
7	-1	0	1(60)	61.98	61.335
8	1	0	1	65.06	64.095
9	0(450)	-1	-1	68.25	66.862
10	0	1	-1	69.20	68.977
11	0	-1	1	69.33	69.552
12	0	1	1	68.79	70.177
13	0	0	0	74.88	75.560
14	0	0	0	76.23	75.560
15	0	0	0	75.57	75.560

*R^2 = 98.19%; Adjusted R^2 = 94.94%; Predicted R^2 = 99.09%; P-value<0.0001

The data obtained from the experiment was used to build a mathematical model (Equation 2). The model was modified to fit a quadratic equation in order to correlate the relationship between the independent variables and the dependent response for optimization:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 \quad (2)$$

Where:

Y = The predicted response
x_1, x_2 and x_3 = The independent variables
β_0 = The model constant
β_1, β_2 and β_3 = The linear coefficients
β_{12}, β_{13} and β_{23} = The interacted coefficients
β_{11}, β_{22} and β_{33} = The quadratic coefficients

Microsoft Excel 2007 was used to analyze the regression of quadratic model.

Results

Effect of the Extraction Time on the Yield of TPMS

As observed in Fig. 2, the yield of TPMS increased at the beginning of the extraction time (120-210 s); however, yield of TPMS decreased after 210 s. The Maximum yield of TPMS (54.74±1.65 mgGAE/gDW was found at 210 s. Therefore, the level of extraction time for optimization was set at 180, 210 and 240 s.

Effect of Microwave Power on the Yield of TPMS

Results in Fig. 3 indicate that the yield of TPMS increased until the microwave was set to 450 W. The highest yield of TPMS as 63.03±2.62 mgGAE/gDW obtains at 450 W of microwave power. Voltages higher than 450 W reduced the yield of TPMS was deceased.

Effect of Different Ethanol Proportion on the Yield of TPMS

Different ethanol proportion was varied from 30-90%v/v to investigate the yield of crude extraction. Microwave power and extraction time were fixed at 450 W and 210 s, respectively. The results show that the highest yield of TPMS (58.11±0.51 mgGAE/gDW) was obtained when ethanol was diluted at a proportion of 50%v/v (Fig. 4).

The yield of TPMS increased when using an ethanol proportion ranging from 30 to 50%v/v. In contrast, when the solvent of ethanol and water was higher than 50%v/v, the yield of TPMS decreased.

Optimization of Microwave-Assisted Extraction for the Yield of TPMS

Table 2 indicates that the maximum yield of TPMS was found when microwave extraction process was set at a microwave power of 450 W, an extraction time of 210 s and ethanol proportion of 50%v/v. By applying regression analysis on the experimental data, the independent variables and dependent variable are related by the following second-order polynomial equation:

$$Y = 75.56 + 1.897x_1 + 0.685x_2 + 0.972x_3 + 1.187x_1x_2 - 0.517x_1x_3 - 0.372x_2x_3 - 10.381x_1^2 - 3.231x_2^2 - 3.436x_3^2 \quad (3)$$

where, Y was the yield of TPMS, whereas x_1, x_2 and x_3 are the variables of microwave power, extraction and ethanol proportion, respectively. Estimation of Equation 3 with OLS yielded a P-value of <0.0001 and high values of R^2 (0.98) and adjusted R^2 (0.94). This indicates that model (Equation 3) can be used to optimize of the extraction of TPMS using microwave-assisted extraction. The ANOVA was shown in Table 3.

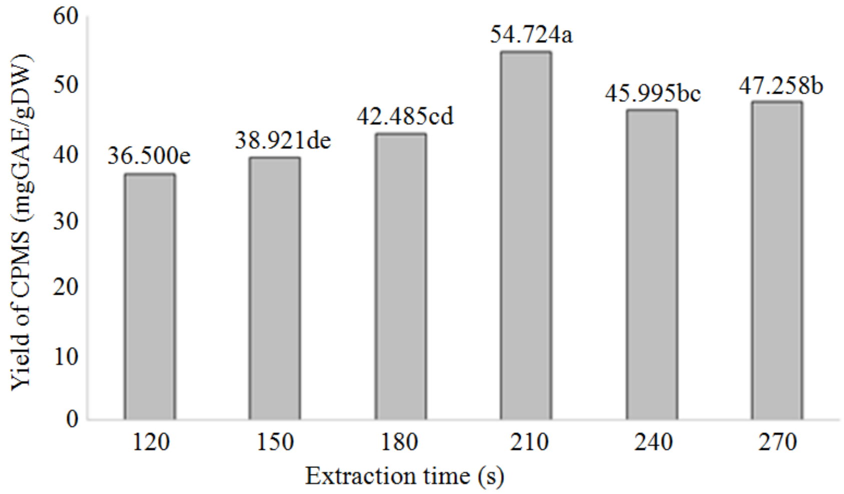

Fig. 2. Effect of different extraction time on extraction yield of TPMS

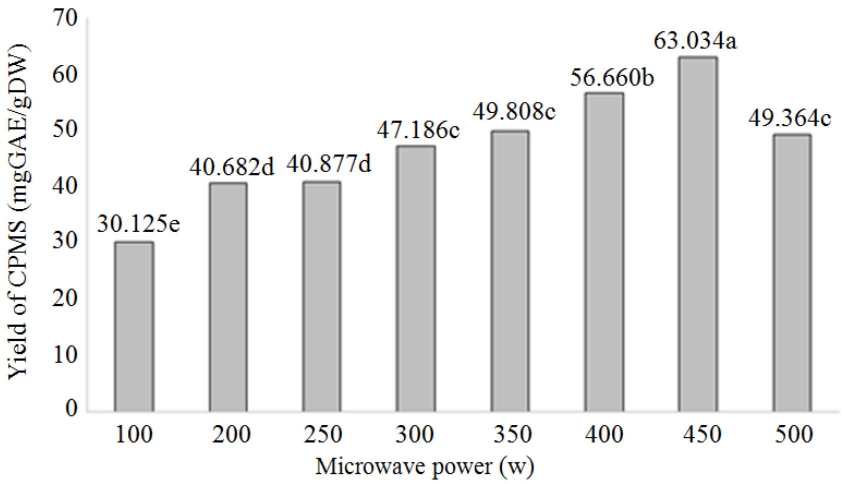

Fig. 3. Effect of different microwave power on extraction yield of TPMS

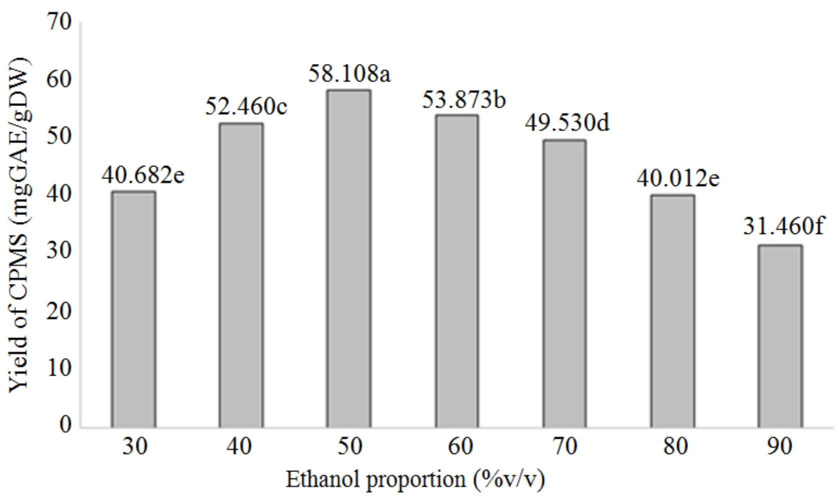

Fig. 4. Effect of different ethanol proportion on extraction yield of TPMS

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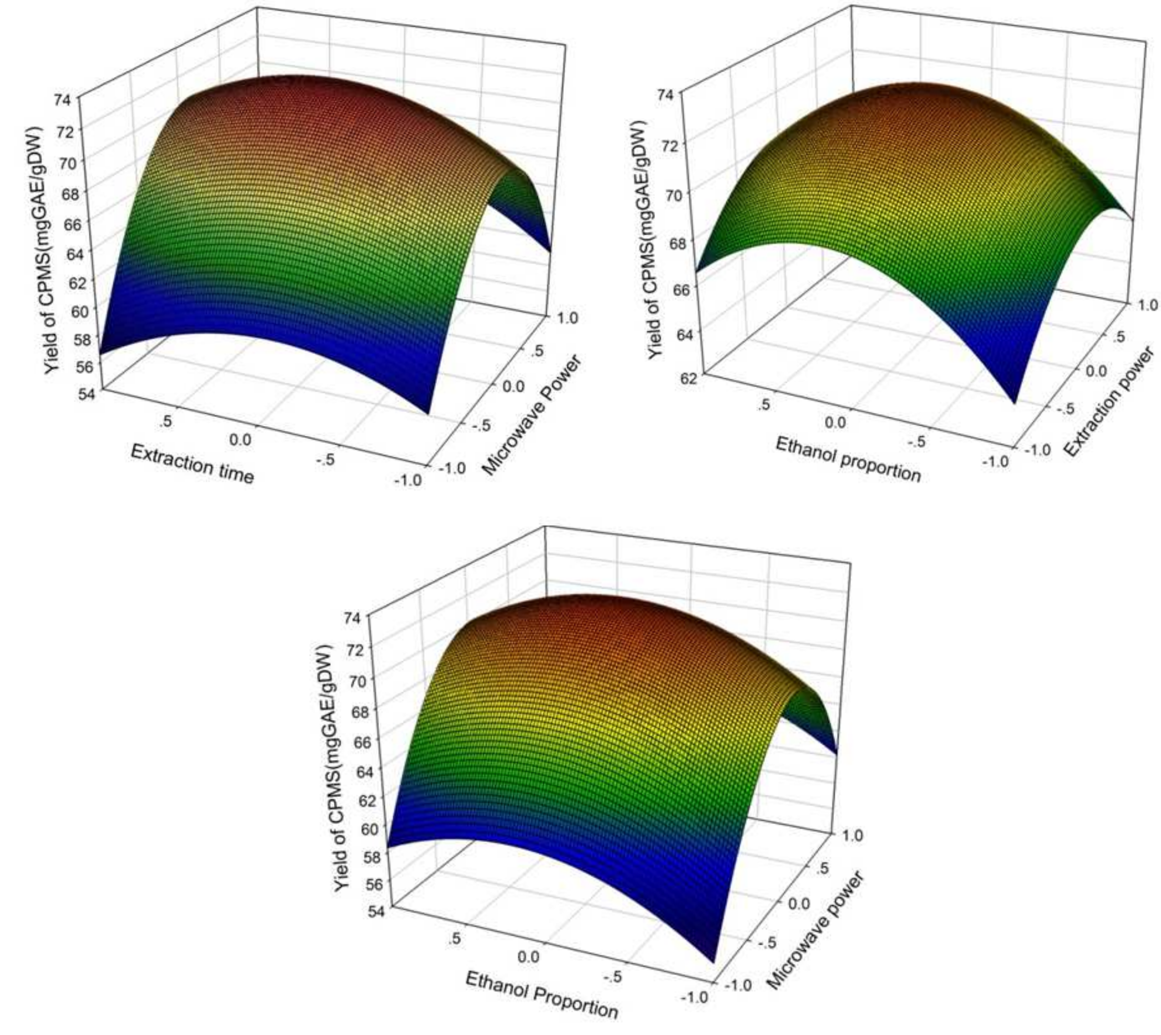
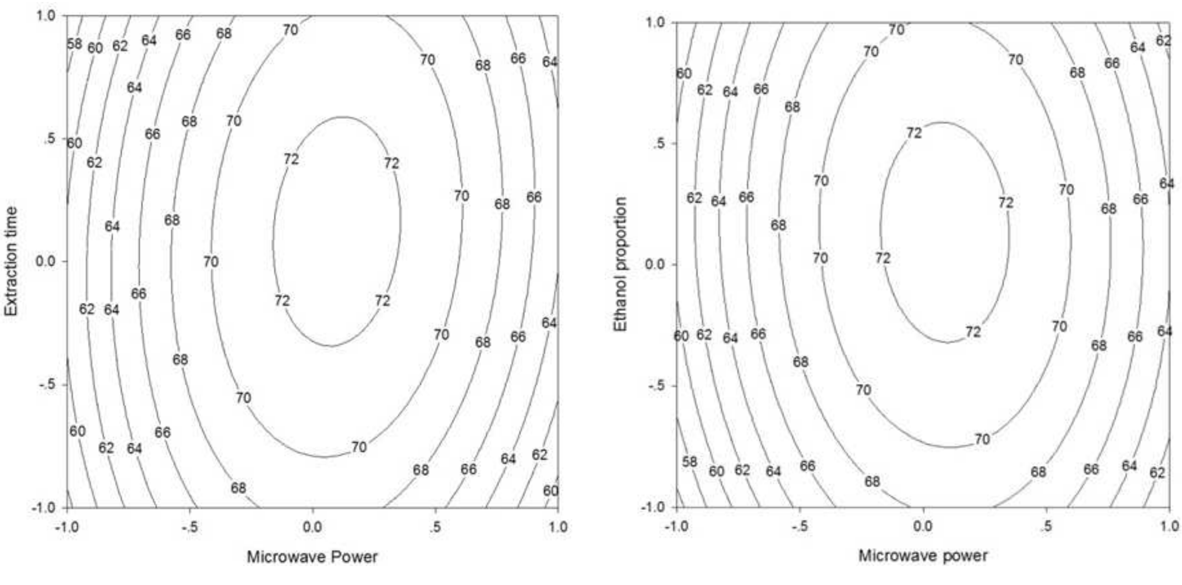


Fig. 5. The 3D plots showing the effect of the microwave power, extraction time and ethanol proportion on the response of extraction yield of TPMS



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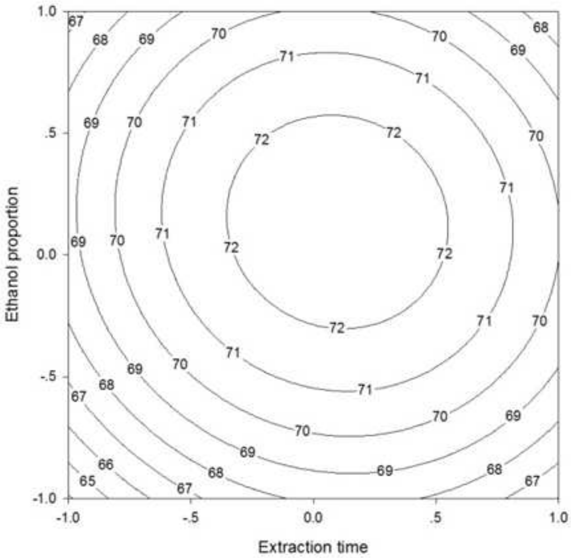

Fig. 6. The contour plots showing the effect of the microwave power, extraction time and ethanol proportion on the response of extraction yield of TPMS

Table 3. Estimated regression model of relationship between response variable and independent variables

Coefficients		Standard error	t Stat	P-value
β_0	75.560	0.775	97.467	0.000
β_1	1.898	0.475	3.997	0.010
β_2	0.685	0.475	1.443	0.209
β_3	0.973	0.475	2.049	0.096
β_{12}	1.188	0.671	1.769	0.137
β_{13}	-0.517	0.671	-0.771	0.476
β_{23}	-0.372	0.671	-0.555	0.603
β_{11}	-10.381	0.699	-14.856	0.000
β_{22}	-3.231	0.699	-4.624	0.006
β_{33}	-3.436	0.699	-4.917	0.004

The calculated significant of each coefficient was check by P-value. The calculated significant of each coefficient was check by P-value. The small P-value is more significant in corresponding coefficient which can found from the linear coefficient of β_1 and quadratic coefficients of β_{11} and β_{33}.

Estimates of Equation 3 were then made into 3D and contour plots (Fig. 5 and 6. respectively) to account for the interaction between the independent variables and the responses of the dependent variable.

The effects of microwave power, extraction time and ethanol proportion on the yield of TPMS were exhibited in Fig. 5 for 3D plots and Fig. 6 for contour plots, whereas the optimal conditions of model (Equation 3) was solved to calculate the maximum yield of TPMS under experimental condition.

According to the results from the solver in Microsoft Excel 2007, the highest yield of TPMS (75.659 mgGAE/gDW) was obtained when the extraction process was completed with a microwave power of 450 W; an extraction time of 213 sec and a dilution with ethanol at a proportion of 51%v/v. These results were confirmed by replicating the experiment with the aforementioned conditions. The mean yield of TPMS was 75.132±0.576 mgGAE/gDW., while the antioxidant activity was found to be 85% of inhibition.

Discussion

The parameters of microwave power, extraction time and ethanol proportion for microwave assisted extraction of TPMS were studies. The results of optimal condition according to Song *et al*. (2011) that long period of extraction time and higher power of microwave degrade phenolic compound from plants, which would explain the results reported in Fig. 2 and 3. The kind of solvent is also important for extraction process. From literature reviews found that ethanol is the best solvent for antioxidant compound and it is safe for human consumption (Dai and Mumper, 2010). According to Pavlović *et al*. (2013), the highest antioxidant activity in *in-vitro* was found when ethanol was used as a solvent in microwave-assisted extraction The TPMS from microwave assisted extraction was found antioxidant activity. According to many studies indicated that bioactive phenolic compound is the utilization of agricultural waste as natural food ingredients because, there are safe and healthy (Dorta *et al.*, 2014; Ribas-Agustي *et al.*, 2014).

Conclusion

The maximum yield of TPMS (75.659 mgGAE/gDW) was found when the microwave-assisted extraction was done using a microwave power of 450 W, an extraction time of 213 s and ethanol proportion of 51%v/v. The optimal conditions were replicated, the yield of TPMS (75.132±0.576 mgGAE/gDW) was closely with predicted yield. TPMS of this research may uses in food and pharma-ceutical industries to enhance nutritional values and also to add in healthy and cosmetic products.

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Author's Contribution

Nukrob Narkprasom: Contributed in all experiments, research plan, data-analysis and writing of manuscript.

Kanjana Narkprasom: Contributed in experiments, prepared the raw material and analysis of total phenolic and antioxidant activity.

Umaporn Upara: Coordinated all process of makiang production in pilot plant of Maejo University, Thailand.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manucript and no ethical issues involved.

References

Cansee, S., J. Uriyapongson, C. Watyotha, T. Thivavarnvongs and J. Varith, 2008. Amphoteric starch in simultaneous process preparation with boxbehnken design for optimal conditions. Am. J. Applied Sci., 5: 1535-1542. DOI: 10.3844/ajassp.2008.1535.1542

Dai, J. and R.J. Mumper, 2010. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15: 7313-7352. DOI: 10.3390/molecules15107313

Dorta, E., M. GonzДlez, M.G. Lobo, C. SДnchez-Moreno and B. de Ancos, 2014. Screening of phenolic compounds in by-product extracts from mangoes (*Mangifera Indica* L.) by HPLC-ESI-QTOF-MS and multivariate analysis for use as a food ingredient. Food Res. Int., 57: 51-60. DOI: 10.1016/j.foodres.2014.01.012

Gallo, M., R. Ferracane, G. Graziani, A. Ritieni and V. Fogliano, 2010. Microwave assisted extraction of phenolic compounds from four different spices. Molecules, 15: 6365-6374. DOI: 10.3390/molecules15096365

Garofulic, I.E., V. Dragovic-Uzelac, A.R. Jambrak and M. Jukic, 2013. The effect of microwave assisted extraction on the isolation of anthocyanins and phenolic acids from sour cherry marasca (*Prunus cerasus* var. Marasca). J. Food Eng., 117: 437-442. DOI: 10.1016/j.jfoodeng. 2012.12.043

Joseph, J.A., D.R. Fisher and D. Bielinski, 2005. Blueberry extract alters oxidative stress mediated signaling in COS-7 cells transfected with selectively vulnerable muscarinic receptor subtypes. J. Alzheimers Dis., 9: 35-42.

Khumtue, S. and D. Naphrom, 2013. Effects of plant growth regulators on quality of makiang fruit (*Cleistocalyx nervosum* var. *paniala*). Proceedings of the International Graduate Research Conference, Dec. 20, iGRC2013, Chiang Mai University, Thailand, pp: 200-204.

Narkprasom, N., J.H. Guo, T.C. Huang and Y.K. Guu, 2013. Combination of statistical techniques for submerged fermentation for extracellular polysaccharide and biomass of *Ganoderma tsugae*. Am. J. Biostat., 3: 38-46. DOI: 10.3844/ajbssp.2013.38.46

Nuengchamnong, N. and K. Inkaninan, 2009. On-line characterization of phenolic antioxidants in fruit wines from family myrtaceae by liquid chromatography combined with electrospray ionization tandem mass spectrometry and radical scavenging detection. LWT-Food Sci. Technol., 42: 297-302. DOI: 10.1016/j.lwt.2008.04.012

Omar, R., M.A. Abdullah, M.A. Hasan and M. Marziah, 2004. Development of growth medium for *Centella Asiatica* cell culture via response surface methodology. Am. J. Applied Sci., 1: 215-219. DOI: 10.3844/ajassp.2004.215.219

Patthamakanokporn, O., P. Puwastien, A. Nitithamyong and P.P. Sirichakwal, 2008. Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. J. Food Composit. Anal., 21: 241-248. DOI:10.1016/j.jfca.2007.10.002

Pavlović, M.D., A.V. Buntić, S.S. Šiler-Marinković and S.I. Dimitrijević-Branković, 2013. Ethanol influenced fast microwave-assisted extraction for natural antioxidants obtaining from spent filter coffee. Separat. Purificat. Technol., 118: 503-510. DOI: 10.1016/j.seppur.2013.07.035

Ribas-Agustي, A., M. Gratacόs-Cubarsي, C. Sلrraga, M.D. Guàrdia and J. Garcيa-Regueiro *et al*., 2014. Stability of phenolic compounds in dry fermented sausages added with cocoa and grape seed extracts. LWT-Food Sci. Technol., 57: 329-336. DOI: 10.1016/j.lwt.2013.12.046

Shimada, K., K. Fujikawa, K. Yahara and T. Nakamura, 1992. Antioxidative properties of xanthans on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40: 945-948. DOI: 10.1021/jf00018a005

Song, J., D. Li, C. Liu and Y. Zhang, 2011. Optimization microwave-assisted extraction of Total Phenolics (TP) from *Ipomoea batatas* leaves and its antioxidant activity. Innovative Food Sci. Emerg. Technol., 12: 282-287. DOI:10.1016/j.ifset. 2011.03.001

Sriwanthana, B., W. Treesangsri, B. Boriboontrakul, S. Niumsakul and P. Chavalittumrong, 2007. *In vitro* effects of Thai medical plants on human lymphocyte activity. Songklanakarin J. Sci. Technol., 29: 17-28.

Taya, S., C. Punvittayagul, W. Inboot, S. Fukushima and R. Wongpoomchai, 2014. *Cleistocalyx nervosum* extract ameliorates chemical-induced oxidative stress in early stages of rat hepatocarcinogenesis. Asian Pacific J. Cancer Prevent., 15: 285-2830. DOI: 10.7314/APJCP.2014.15.6.2825

Tookjit, J., 2011. Production of ready to drink juice concentrate produced from makiang and mulbery using vacuum evaporation. MSc Thesis, Chiang Mai University.

Xu, H., L.P. Sun, Y.Z. Shi, Y.H. Wu and B. Zhang *et al*., 2008. Optimization of cultivation conditions for extracellular polysaccharide and mycelium biomass by *Morchella esculenta* As51620. Biochem. Eng. J., 39: 66-73. DOI:10.1016/j.bej.2007.08.013

Zhu, T., H.J. Heo and K.H. Row, 2010. Optimization of crude polysaccharides extraction from *Hizikia fusiformis* using response surface methodology. Carbohydrate Polymers, 82: 106-110. DOI: 10.1016/j.carbpol.2010.04.029