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Optimized microwave reflux extraction and antioxidant activities of piperine from black and white *piper nigrum*

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Abstract: In this study, the microwave reflux technique was employed to extract piperine from black and white pepper. This is due to its ability to combine the microwave and the conventional solvent extraction together with a high degree of selectivity and quality extract. The extraction process was optimized using theL_9-Taguchi experimental design. This investigated the effects of four independent factors (irradiation time (x_1), microwave power level (x_2), feed particle size (x_3) and molar ratio (x_4)) on piperine yield. Antioxidant activity of the oleoresin extracts were later evaluated using DPPH (1, 1-diphenyl-1-picrylhydrazyl) radical scavenging assay. The optimum extraction condition in black pepper refluxation was attained at 90 min irradiation time (x_1), 350 W power level (x_2), 0.105 mm feed particle size (x_3) and 10 mL/g molar ratio (x_4) with an extremum ranking in decreasing order of x_3> x_4> x_2> x_1.However,from the white pepper refluxation an optimum condition was achieved at 120 min irradiation time (x_1), 350 W power level (x_2), 0.300 mm feed particle size (x_3) and 6 mL/g molar ratio (x_4) with a corresponding decreasing extremum order of x_1> x_4> x_2> x_3.From the results obtained from concentration-dependent radicals scavenging activity it was concluded that white oleoresins extract were much higher in inhibitory activity than that of black oleoresin extract.

Keywords: *Microwave reflux extraction; Taguchi optimization DPPH radical scavenging activities; Piperine; Piper nigrum.*

Introduction

Black and white peppers are tropical crops of *piperaceae* family with over a thousand species usually produced using the seed of the plant from the freshly plucked spice berries[1]. The major difference between both species is that the fruit of white pepper is allowed to matured completely after which it is soaked in water for some time[2]. The outer layer is scratched off consequent to the mollification of fresh fruit to form a creamy white *piper nigrum*[3].Black *piper nigrum* on the other hand is produce by drying an unripe fruits to form a black reticulated seed as shown in Figure.1.

Figure 1: Green and matured *Piper nigrum* seeds.

Piperine is the major active alkaloids in black and white *piper nigrum*withcharacteristicanti-inflammatory, anticancer,antipyretic, analgesic, antitumor, anti-depressant, and antioxidant properties[4]. It is an important component responsible for the pungency in *piper nigrum*. It is slightly soluble in water and these increases at elevated temperatures. Many researches had investigated the nutraceutical and medicinal nature of white and black pepper due to the presence of bioactive-piperine[4,5,6].Abdurahman[7]opined that the variability in Piperine and capsaicicoids content in different cultivars of pepper is determined by variety, climate, geographical location, maturity, and the method of processing. However, the classical microwave refluxation is comparatively useful in the isolation of bioactive-alkaloids from different species of piper nigrum. It is such a method with an higher selectivity, lower solvent consumption, environmentally friendly and shorter irradiation time[8]. This is achieved by a selectively localized heating of the extracting solvent making it suitable for matrix with plant origin[9]. The ability of microwave reflux to isolate and extract targeted compounds from plant matrix is therefore due to the ionic conduction and dipole interaction of the electric and magnetic field[10]. The presence of antioxidants inside *piper nigrum* matrix offers remarkable advances in the treatment of some life-threatening free radical disorder such as cancer, cardiovascular diseases and neurological disorders. This has drawn the attention of researchers to focus on unveiling the therapeutic and free radical scavenging potentials of black and white *piper nigrum*.This research therefore investigated the

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optimal conditions for the extraction of essential bioactive piperine and compared the radical scavenging activities for both cultivars of *piper nigrum* under different extraction conditions.

Materials and Methods

Materials and reagents

A standard grade black and white *piper nigrum* were obtained from the Malaysian Pepper Board (MPB) located in Sarawak Malaysia at initial moisture of 3-4%.Moreover, an analytical-grade ethanol, acetone, and water were obtained from the Chemical Laboratory, Universiti Malaysia Pahang, Malaysia. The DPPH (1,1-diphenyl-2-picrylhydrazyl) reagent was supplied by Sigma Aldrich Chemical Co.

Sample and reagents preparation

The two pepper samples were pulverized into a finely defined powder using eppendorf grinder(200- model Hamburg, Germany) and then kept in an airtight container. The powdered samples were clarified into five different sizes of 0.105mm, 0.154mm, 0.300mm, 0.450mm and 0.900mm.Furthermore, a 0.0238g dark purple crystalline solid of DPPH was dissolved in 95% distilled ethanol to make up a final volume 100ml of 0.6mM DPPH stock solution.

Robust experimental design

This is an experimental design methodology which permits a higher level of consistent uniformity in the extraction parameters involving minimum number of experimental runs. This leads to a reduction in extraction time and cost of the sample used[11].Taguchi optimization design also allows for an independent analysis of each extraction factor with an admittance that not all the control variables with higher variation can be controlled in actual practice[12].

Table 1: Extraction Factors and operating levels.

Extraction factors	Black *piper nigrum*			White *piper nigrum*		
	-1	0	+1	-1	0	+1
Irradiation Time (x_1)	30	60	90	60	90	120
Microwave Power Level (x_2)	300	350	400	250	300	350
Feed Particle Size (x_3)	0.105	0.154	0.300	0.105	0.154	0.300
Molar Ratio (x_4)	30	60	90	6	8	10

The extraction factors considered for this were irradiation time (x_1), microwave power level (x_2), feed particle size (x_3), and molar ratio (x_4).The search for an optimal yield from the two samples were achieved using a three-level orthogonal design of these factors[13]. The factors and levels for the orthogonal design designated with x_1, x_2, x_3, and x_4 as presented in Table 1. This was analyzed using Minitab 17® experimental design software [14].

Microwave reflux extraction

A 5 g of *piper nigrum* powder sample was mixed and hydrated with distilled water. The purpose of hydrating the sample was to allow for homogeneity in the mixture. This was then loaded into the microwave reactor as illustrated in the schematic diagram (Fig.2) and irradiated in accordance with the operating conditions generated from the Taguchi experimental array.

Moreover, the microwave oven was operated in-step with the aid of 'easy-control' software, programmed for 3-level control of the microwave power, irradiation time, and temperature. The supernatant solution was thereafter filtered using the 0.45µm PTFE micro filter for subsequent spectroscopy quantification.

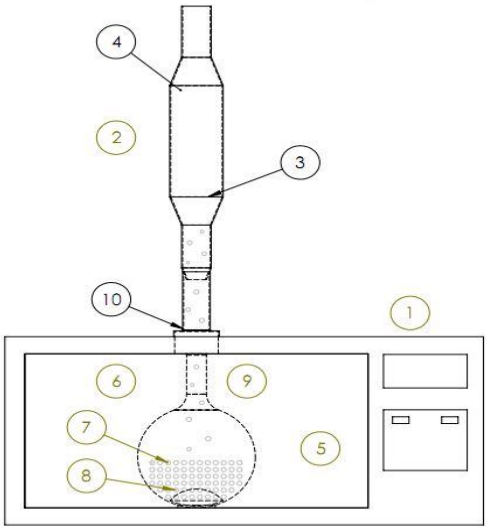

Figure 2: Schematic diagram of microwave experimental set-up. 1. Microwave oven, 2. Reflux system, 3. Ice water inlet, 4. Ice water outlet,

Spectrometry quantification

The components of the spice extract were obtained from peak area fragmentation fingerprints using a computer controlled GC-MS system, with the retention time indices and mass spectra matching as the basis for component identification[15]. An Agilent GC-MS(5973N-Agilent Technologies, Wilmington, DE, USA)) with 30 mm column diameter, 0.25 mm internal diameter, and 0.25 mm film thicknesses was employed

to detect and determine the quantity of the piperine present. The column oven was firstly set to 50°C for 5 min and then progressively increased to 125°C at 3°C per unit time, with a final 5min of heating at 300°C. The test was carried out by diluting a micro-filtered extract with an analytical standard grade acetone at ratio 1:10.1 µL. This was then injected into the GC-MS for components detection and quantification. The actual percentage of piperine was then calculated using the normalization techniques in relation to the quantity of the sample loading as shown in the equation below:

$$Piperine\ yield\ (w/w\ \%) = \frac{C_{content}}{W_{feed}} 100\%$$

where, $C_{content}$ and W_{feed} are the amount piperine (g) and weight of dried (g) samples respectively.

The component spectrometry identification was carried out in triplicates using the same GC-Mass spectrometric conditions. The validity of piperine yield was determined by the standard deviation errors (SD) given in the equation below. This accounted for the deviation inherent in the repeated use of the GC-MS.

$$Standard\ deviation = \sqrt{\frac{\sum (y_1 - y_0)^2}{N-1}}$$

DPPH free radical scavenging assay

The radical-scavenging assay is a biological activity test for evaluating the antioxidant potential of the extracts obtained from natural products. The choice of DPPH reagent for this study was due to its rapid and high sensitivity in the detection of a small variation in antioxidant activities in addition to the minimal use of test samples [18]. This assay was conducted to compare the antioxidant capacity of spice oleoresin extracts from black and white *piper nigrum*.

The antioxidants properties of the spice oleoresins is therefore a function of their free radical inhibition percents[19]. Radical-scavenging activity of spice extracts against stable the DPPH-solution was determined using spectrophotometer set at 517nm and the antioxidant activities was measured as the absorbance decreases. The method used by[20] for DPPH free radical scavenging was employed in this study. The negative control (A_0) was prepared by mixing 0.5ml of ethanol with 2.5ml of the DPPH solution and absorbance was measured after 30 min of keeping the mixture in the dark at 25°C.Five different concentrations of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL and 250 µg/mL at the optimal yield of the spice oleoresin extracts were prepared. Absorbance (A_1) was measured for a mixture of 0.5ml different concentration of spice oleoresin consecutively taken and mixed with 2.5ml of DPPH. However, in order to eliminate the colour effect of the spice extract, an absorbance denoted by A_2 was taken and this comprises a mixture of the spice extracts (different concentration) and 2.5ml ethanol. All analysis was performed in triplicates and the average value was used to estimate the inhibition percent. The percentage inhibition was calculated using the expression below:

$$Piperine\ yield\ (w/w\ \%) = \left(1 - \left[\frac{A1-A2}{A0}\right]\right) X\ 100$$

The percentage inhibition was plotted against the five concentrations (50-250 µg/mL) of the spice oleoresins extract to obtain a standard inhibition curve. The results were obtained by linear regression analysis, thereby generating a straight-line equation as shown in below:

$$I = mc + k$$

where *I*=Inhibition Percent, c=spice oleoresin concentration, m=slope and k=intercept. The IC_{50} value was thereafter estimated to determine the inhibition concentration of the spice oleoresin extract required to scavenge 50% of the DPPH radicals.

Results and Discussion

Determination of the optimized conditions

From design matrix in Table 2, the optimum extraction conditions for the black pepper (y_{black}) refluxation were attained at 90 min irradiation time (x_1), 350 W power level (x_2), 0.105 mm feed particle size (x_3) and 10 mL/g molar ratio (x_4). The main effects of the four extraction variables involved in black pepper microwave refluxation decreases in the order $x_3 > x_4 > x_2 > x_1$ in accordance with the extremum difference.

Moreover, optimum conditions for white pepper (y_{white}) microwave-refluxation were reached at 120 min irradiation time (x_1), 350 W power level (x_2), 0.300 mm feed particle size (x_3) and 6 mL/g molar ratio (x_4).The extraction yield of the bioactive-Piperine under the optimum conditions for black and white pepper refluxation were 2.0586 w/w% and 4.276 w/w% respectively. Hence the descending order of significance of the main effects in white pepper refluxation is given by $x_1 > x_4 > x_2 > x_3$ with respect to their extremum difference. The descending order of significance was arrived at from the delta values (Ϫ) generated by Minitab 17® software. In Taguchi designs, delta is the difference between the maximum and minimum mean response across levels of a factor. Triplicate parallel tests were conducted under the optimal response setting from the orthogonal parametric design.

Table 2: L_9 (3^4) Experimental design matrix and result.

Run	Coded Control Factors				Piperine Yield (w/w %)	
	x_1	x_2	x_3	x_4	(y_{black})	(y_{white})
1	-1	-1	-1	-1	1.3972 ±0.06	0.214± 0.02
2	-1	0	0	0	0.6984 ±0.30	0.166± 0.008
3	-1	+1	+1	+1	0.7160 ±0.62	0.258± 0.07
4	0	-1	0	+1	1.1944 ±0.38	0.494± 0.008
5	0	0	+1	-1	1.1118 ±0.23	1.827± 0.01
6	0	+1	-1	0	0.8678 ±0.02	0.616± 0.06
7	+1	-1	+1	0	0.5862 ±0.11	0.924± 0.04
8	+1	0	-1	+1	2.0586 ±0.04	1.87±0. 03
9	+1	+1	0	-1	0.7668 ±0.15	4.276± 0.02

Validity of the optimized conditions

The optimal extraction conditions obtained for a maximized Piperine extraction were experimentally validated. A close agreement between the predicted and experimental optimal Piperine yield was estimated using the X-goodness–of-fit-test, as shown in the equation below:

$$X = \sqrt{\frac{\sum (E - P)}{P}}$$

The X goodness-of-fit test was used to examine the validity of the optimum response setting [16]. According to Haldar[17] ,for an optimal condition to be valid the X-cut-off mark must not exceed 7.81 for a 3-degree of freedom at 95% confidence level.

The close agreement that existed between the predicted and experimental optimal yield were estimated using the X- goodness-of-fit test [16]. The test shows that there is no significant difference between the predicted and experimental optimum response settings with X^2 value of 0.043 and 0.059 for the Piperine yields in black and white pepper respectively as shown in Table 3. The X^2 –values are therefore negligible when compared with the 7.81 cut-off value for a 3-degree of freedom. This indicated valid optimal conditions at 95% confidence level.

Table 3: Validation for optimal condition.

Sample	Predicted (w/w)	Observed(w/w)			X Goodness of Fit
		Trial 1	Trial 2	Trial 3	
Black Pepper	2.06	2.06	1.99	2.07	0.043
White Pepper	4.27	4.16	4.30	4.26	0.059

Table 4 shows the scavenging activity dependence on spice oleoresin concentration with an IC_{50} value of 94.92µg/mL and 107.57µg/mL for both black and white oleoresin extracts respectively. This suggests that the DPPH radical scavenging activity of white spice oleoresin extracts is greater than that of the black extracts. This is attributable to the presence of higher concentration of Piperine in white pepper as compare to the black pepper [15]. This is consistent with the investigation carried out by Zhang and Xu[20], who concluded that the white ethanolic extracts of white spice oleoresin exhibits an higher scavenging potentials than that of black spice oleoresin.All experimental procedure were performed in triplicates and the one-way statistical analysis (ANOVA) was carried out to determine the coefficient of determination (R^2-value) and the significant differences between means ($p<0.05$) using Minitab17® software.

Table 4: DPPH radical scavenging assay.

Sample Name	IC_{50} value (µg/mL)	Regression Equations	R^2 - value	*p-value*
Black Spice Oleoresin	107.5667 ±0.04	I = 17.73c + 0.3000	0.85	0.01
White Spice Oleoresin	94.9219 ±0.01	I = 18.41c + 0.3328	0.87	0.01

Figure 3 shows the inhibitory plot which illustrated the comparison between the scavenging activities of black and white pepper oleoresin extracts. Deduction made from the graph indicated that the concentration-

dependent radicals scavenging activity of white oleoresin extract was 11.8% higher than that of black oleoresin extract. This is conformable with other results from previous investigation [2,20].

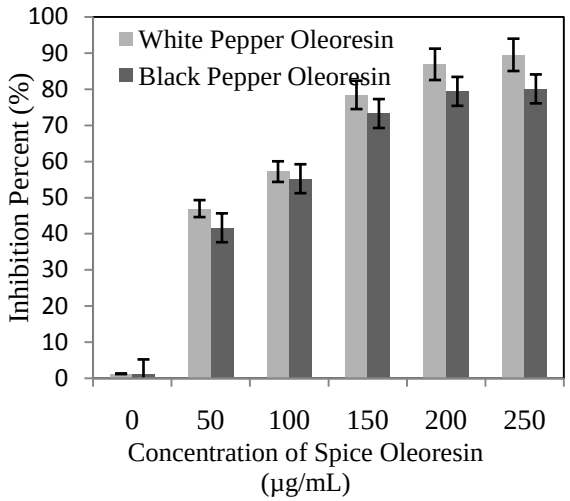

Figure 3: Comparison of inhibitory calibration plot for white and black free radical scavenging assay.

Conclusion

This study concluded that the white pepper contained a higher proportion of antioxidant than that of black pepper. Moreover, from the estimated IC_{50} value, it can be clearly seen that the white pepper oleoresin extracts offer a better antioxidant activity than the corresponding black pepper oleoresin extracts. This indicated that allowing an immature flowering plant of *piper nigrum* to mature increases the Piperine content and hence the radical scavenging ability of its oleoresin extracts. This gives substantial evidence that black and white oleoresin extract can be possibly used as dietary antioxidants for the protection of free radical related diseases.

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References

1. A. Gupta, M. Gupta, and S. Gupta, "Isolation of Piperine and Few Sesquiterpenes from the Cold Petroleum Ether Extract of Piper nigrum (Black Pepper) and its Antibacterial Activity," International Journal of Pharmacognosy and Phytochemical Research,vol. 5, no. 2, pp. 3–7, 2013.
2. S. Singh, I. P. S. K. Gurdip, and S. Carola, "Chemistry , Antioxidant and Antimicrobial Potentials of White Pepper (Piper nigrum L .) Essential Oil and Oleoresins,"*The National Academy of Sciences, India* vol. 83, no. 3, pp. 357–366, 2013.
3. M. Meghwal and G. Tk, "Nutritional Constituent of Black Pepper as Medicinal Molecules : A Review," Open access report, vol. 1, no. 1, pp. 1–7, 2012.
4. C. Khushbu, R. Article, C. Khushbu, S. Roshni, P. Anar, M. Carol, and P. Mayuree, "Phytochemical and Therapeutic Potential of Piper longum linn: A review,"*International Journal of Research in Ayurveda & Pharmacy*, vol. 2, no. 1, pp. 157–161, 2011.
5. Z.Maitreyi, A. Khandhar, S. Patel, A. Patel, "Chemistry and Pharmacological *piper nigrum*," *International Journal of Pharmaceutical Sciences Review and Research*,vol.5,no.1,pp. 67-76,2010.
6. W. Epstein, "Isolation of Piperine from Black Pepper."*Journal Chemical Education*, vol.70, 598-599,1993.
7. N. H. Abdurahman, "A Comparative Review Of Conventional And Microwave Assisted Extraction In Capsaicin Isolation From Chili Pepper," *Australian Journal of Basic and Applied Science*, vol. 10, no. 6, pp. 263–275, 2016.
8. E. Durmaz, G. Sumnu, and S. Sahin, "Microwave-Assisted Extraction of Phenolic Compounds from Caper," *Separation Science. Technology*,vol.6395,no.8,2015.
9. M. Mohan, S. Khanam, and B. G. Shivananda, "Optimization of Microwave Assisted Extraction of Andrographolide from Andrographis paniculata and its Comparison with Refluxation Extraction Method,"*Journal of Pharmacognosy and Phytochemistry Optimization*, vol. 2, no. 1, pp. 342–348, 2013.
10. L. Sanchez-prado, C. Garcia-jares, and T. Dagnac, "Microwave-assisted extraction of emerging pollutants in environmental and biological samples before chromatographic determination," *Trends in Analytical Chemistry*, 71, pp. 119-143, 2015.
11. V. Mandal, Y. Mohan, and S. Hemalatha, "Microwave assisted extraction of curcumin by sample-solvent dual heating mechanism using Taguchi L9 orthogonal design," *Journal of Pharmaceutical and Biomedical Analysis*, 46, no. 2, pp. 322–327, 2008.
12. D. M. Steinberg and D. Bursztyn, "Noise Factors, Dispersion Effects, and Robust Design," *Statistical. Sinica.*, vol. 8, pp. 67–85, 1998.

13. H.Abdurahman,O.Olalere, "Taguchi-Based Optimization Technique in reflux microwave extraction of piperine from black pepper," Australian Journal of Basic and Applied Sciences, vol. 10, no.5. pp. 293-299, 2016.
14. Minitab, *Getting Started with Minitab 17*. 2014.
15. S. Singh, I. P. S. Kapoor, G. Singh, C. Schuff, M. P. De Lampasona, and C. A. N. Catalan, "Chemistry, antioxidant and antimicrobial potentials of white pepper (Piper nigrum L.) essential oil and oleoresins," *Proceedings of. Nationa. Academy Science India Section B - Biological. Sci.ence*, vol. 83,2013.
16. E. Subroto, R. Manurung, H. J. Heeres, and A. A. Broekhuis, "Optimization of mechanical oil extraction from Jatropha curcas L. kernel using response surface method," *Industrial Crops Product*, vol. 63, pp. 294–302, 2015.
17. S. Haldar, G. C. Majumdar, and H. N. Mishra, "Modeling the kinetics of extracting oleoresin from dried turmeric (Curcuma longa L.) rhizome using acetone as solvent," *Journal of Food Engineering.*, vol. 146, no.11, pp. 116–121, 2015.
18. L. S. Badwaik, P. K. Borah, and S. C. Deka, "Optimization of Microwave Assisted Extraction of Antioxidant Extract from *Garcinia pedunculata* Robx.," *Separation Science. Technology.*, vol. 50, no. 12, pp. 1814–1822, 2015.
19. G. Nahak and R. K. Sahu, "Phytochemical Evaluation and Antioxidant activity of Piper cubeba and Piper nigrum," *Journal of Applied Pharmaceutical Science*vol. 1, no. 8, pp. 153–157, 2011.
20. L.L Zhang and J.G. Xu, "Comparative Study on Antioxidant Activity of Essential Oil,"*European Journal of Food Science and Technology* vol. 3, no. 3, pp. 10–16, 2015.

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