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# Optimization of Extraction Conditions of Antioxidant Activity from *Zingiber zerumbet* Oleoresin

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**Abstract:** The health promoting capacity of natural antioxidant from phytochemicals has increase attention from researchers and public. However, processing is affecting the activity and the bioavailability of bioactive compounds. Therefore, the optimization of extraction condition of antioxidant activity from *Zingiber zerumbet* oleoresin was investigated. A Box-Behnken design technique was employed to study the effect of different range parameters of soxhlet extraction. Analysis of variance and response surface methodology were applied to identify the optimal processing parameter. Independent variables were extraction time (8, 10 and 12), type of solvent used (hexane, acetone, ethanol) and blanching treatment (steam treated, boil treated, untreated). The response and variables were fitted well to each other by multiple regressions. All the independent parameters affected oleoresin yield and antioxidant activity significantly. The optimal processing parameter that fulfilled the requirement for yield of oleoresin and antioxidant activity were found to be 12 h extraction time, ethanol as the solvent used and untreated sample. While, the optimal yield of oleoresin was 13.05% w/w and antioxidant activity was 16.01% w/w.

**Key words:** Antioxidant activity, *Zingiber zerumbet*, oleoresin, experimental design

## INTRODUCTION

Z. zerumbet rhizome is popularly used as an ingredient in the traditional health supplements known as "jamu" and is eaten as an appetizer or to cure stomachache. Recently, several products derived from Z. zerumbet have appeared in the market in the form of spray dried powder of the water extract or oleoresin (Ruslay et al., 2007). Oleoresins are standardized products in terms of active ingredients, color, flavor and physical properties hence facilitate consistency in end-usewhich is not always possible in raw spices (Hashim et al., 2002).

Antioxidant activity was selected as the initial biopotential assessment, since antioxidants have strongly associated with the defence mechanisms of living cells against oxidative damage (Miller, 1995). Plant materials with retained antioxidants are more functional towards improving shelf life of food products and providing health promotion compared to compare to materials whose antioxidants have been removed or destroyed during processing (Lindley, 1998; Nicoli et al., 1999). Processing is known to affect content, activity and bioavailability of bioactive compounds. Therefore, antioxidants preservation during processing has become increasingly important.

The traditional approach of optimization which is single dimensional search involve extensive experiments in obtaining value and interactive relationship among variables especially if several set of variables are involved (Stowe and Mayer, 1966). This system also is not applicable where several factors are simultaneously involved, time consuming and the interaction between the variables are difficult to relate. Hence, Response Surface Methodology (RSM) is selected due to its ability to link one or more responses to a set of variables when firm interaction is known (Zakaria and Amin, 2002).

The objective of this study was to evaluate the combined effects of blanching treatment, type of solvent used and extraction time on the yield of *Z. zerumbet* oleoresin and its antioxidant activity.

## MATERIALS AND METHODS

Materials and sample preparation: Fresh Z. zerumbet rhizomes were obtained from a local market in Skudai, Johor, Malaysia. The rhizomes were cleaned and sliced into small pieces of 2 mm cross-section each. The sliced rhizomes were undergoing through three different treatments of blanching process accordingly. The three treatments were: (1) control (untreated) (2) boiled for one

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h and (3) steamed for 15 min. The treated rhizomes were then dried at 50°C for three days in air forced convection oven and ground into powder. The obtained powder particles were 0.5 mm mesh size. The residual water content of the oven dried samples was less than 10%. All samples were refrigerated at 4°C prior to extraction.

Extraction of *Z. zerumbet* oleoresin: Eight gram sample was extracted in a soxhlet by different solvent (hexane, acetone and ethanol) and extraction time (8, 10 and 12 h). The samples were then evaporated in rotary evaporator (Laborota 4000, Heidolph, Germany). The oleoresins obtained were stored in air-tight amber bottles at 4°C until tested and analysed. The yield of oleoresin produced was calculated based on Eq. 1 as follows:

Yield of Oleoresin 
$$\left(\%\frac{w}{w}\right) = \frac{x(g) \text{ of oleoresin obtained}}{20 \text{ gof dried sample}} \times 100\%$$
 (1)

Evaluation of the antioxidant activity of Z. zerumbet oleoresin using  $\beta$ -carotene-linoleic acid: In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade), 25 µl linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL distilled water saturated with oxygen (30 min, 100 mL min<sup>-1</sup>) was added with a vigorous shaking. This reaction mixture (2500 μL) was dispersed to test tubes and 350 µL portions of the extracts prepared at 2 g L-1 concentrations were added and emulsion system was incubated up to 48 h at 27°C. Similar procedure was repeated with synthetic antioxidant, butylated hydroxytoluene (BHT) as positive control, as well as the blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHT and blank.

Optimization of three variables by box- behnken design of response surface methodology: An experimental design with 17 experimental runs using three factorial variables to optimize the production of oleoresin and antioxidant activity was carried out. Design Expert software version 6.0.8 was utilized to carry out the regression analysis and to analyze the points of the datawhich was obtained from the preliminary experiment.

The Box-Behnken design technique was applied in order to select the optimum conditions of the three

Table 1: Independent variables and their levels for box-behnken design

		Levels			
Independent variables	Symbol	-1	0	1	
Extraction time (h)	A	8	10	12	
Extraction solvent	В	Hexane	Acetone	Ethanol	
Blanching treatment	C	Steamed	Boiled	Un-treated	

extraction factors in different experiments. The low, middle and high levels for all the independent variables were based on prior screening from literature review. The settings for the independent variables shown in Table 1 were as follows: extraction time of 8, 10 and 12 h; extraction solvent of hexane, acetone and ethanol; blanching treatment of steam treated, boil treated and untreated. Each variable to be optimized was coded at three levels: -1, 0 and +1. Five replicates at the centre (0, 0, 0) of the design were performed to allow the estimation of the pure error. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous matter (Shao *et al.*, 2008).

A quadratic polynomial regression model was assumed for predicting responses. The model proposed for each response of Y was:

$$Y = A + B + C + A^2 + B^2 + C^2 + AB + AC + BC$$
 (2)

where, Y is the predicted response variables (yield of oleoresin or antioxidant activity). The proposed model equation predicts the response as a function of the different levels of independent variables (A, B, C). The significance of each coefficient of the resulted model was determined by using the Student t-test and p-value (Shao *et al.*, 2008).

The adequacy of the quadratic polynomial model is expressed by the coefficient of multiple determinations, R2 and Analysis of Variance (ANOVA) was employed for the determination of the significance of the model. In addition, to visualize the relationship between response and experimental levels of each factor and to deduce the optimum condition, the fitted quadratic polynomial equation was also presented as the surface plot (Pericin *et al.*, 2009).

### RESULTS

**Model fitting:** Table 2 shows the value of independent process variables (A, B, C) for both responses (yield of oleoresin and antioxidant activity). The experimental values of yield of oleoresin and antioxidant activity were analysed by multiple regression to fit the quadratic polynomial equations (Eq. 1 and 2) shown in Table 3 and 4, respectively. The quality of fit to the equations was

Table 2: Responses of the dependent variables to extraction conditions

Experiment					Responses		
	Independent variables						
Std.	Run				Yield of oleoresin	AOX	
order	order	Α	В	С	% w/w	(%)	
1	12	-1	-1	0	3.08	0.67	
2	6	1	-1	0	3.00	3.33	
3	4	-1	1	0	73.00	2.67	
4	1	1	1	0	7.03	13.33	
5	5	-1	0	-1	12.36	2.70	
6	7	1	0	-1	3.30	13.50	
7	3	-1	0	1	5.06	3.81	
8	2	1	0	1	9.76	19.01	
9	10	0	-1	-1	2.93	2.67	
10	17	0	1	-1	3.76	10.50	
11	13	0	-1	1	3.35	2.70	
12	9	0	1	1	10.64	10.83	
13	15	0	0	0	10.64	14.24	
14	8	0	0	0	10.24	14.34	
15	11	0	0	0	10.11	14.30	
16	16	0	0	0	11.20	14.40	
17	14	0	0	0	10.33	14.37	

Table 3: Analysis of variance for the yield of oleoresin Effect đf MS F-value p-value  $\mathbb{R}^2$ SS Regression 202.29 22.5 <1E-4 0.984 Residual 3.35 0.48 Total

Table 4: Analysis of variance for the antioxidant activity						
Effect	SS	df	MS	F-value	p-value	$\mathbb{R}^2$
Regression	546.75	9	60.75	15.77	7E-4	0.953
Residual	26.97	7	3.85			
Total	573 73	16				

checked using the coefficient of determination (R²)which were 0.984 and 0.953 for yield of oleoresin and antioxidant activity, respectively. The coefficient for yield of oleoresin and antioxidant activity indicated a satisfactory agreement between predicted and actual responses.

In addition, p values determined also implied that equation found for yield of oleoresin and antioxidant activity can adequately predict the experimental results, as the fit was statistically significant (p<0.050). Furthermore, the yield of *Z. zerumbet* oleoresin and its antioxidant activity obtained for each of experiments versus the predicted values are plotted in Fig. 1 and 2, respectively, are also shows an acceptable level of agreement. All these results imply a satisfactory mathematical description of the extraction process by the fitted models (Eq. 1 and 2):

Yield of Oleoresin = 
$$+10.50 + 1.40 * A + 2.59 * B + 1.88 * C -1.82 * A^2 -2.13 * B^2 -3.20 * C^2 + 1.17 * A * B + 1.05 * A * C + 1.62 * B * C$$
(3)

Antioxidant activity = 
$$+10.50 + 1.40* A + 2.59 * B + 1.88* C -1.82 * A^2 -2.13* B^2 -3.20* C^2 + 1.17* A* B + 1.05 * A * C +1.62 * B* C (4)$$

**Effect of processing parameter:** The three-dimensional response surface graphs were drawn to illustrate the main

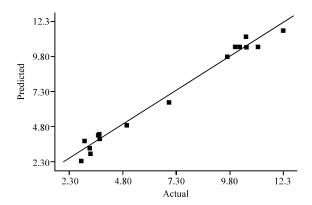


Fig. 1: Relationship between the predicted and actual value of the yield of oleoresin

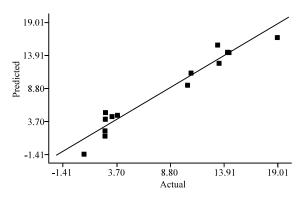


Fig. 2: Relationship between the predicted and actual value of the antioxidant activity

and interactive effects of the independent variables on the yield of oleoresin and antioxidant activity. Figure 3 shows the effect of extraction time and solvent on yield of oleoresin; quadratic effect for both variables was observed, though solvent had greater influence on the yield of oleoresin.

Yield of *Z. zerumbet* oleoresin was increased by time, but after 10 h of extraction a stationary area of yield of oleoresin could be noticed. After 11.5 h the yield started to decrease. Also higher values of yield could be noticed when the polarity of solvent took place between acetone and ethanol. Figure 4 shows the effect of extraction time and blanching treatment on the yield of *Z. zerumbet* oleoresin. The yield of oleoresin in fresh sample was increased by time. The optimal processing parameter to produce the highest amount yield of oleoresin (13.05% w/w) was obtained from fresh sample, ethanol as a solvent and 11.94 h extraction time.

The response surface on the antioxidant activity is shown in Fig. 5 and 6. Figure 5 shows the effect of extraction time and solvent. Quadratic effect for both variables through extraction time with great influence on the antioxidant activity was observed.

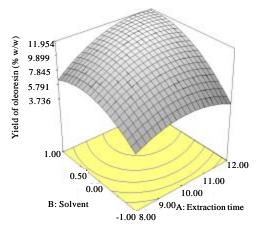


Fig. 3: Response surface plot showing the combine effect of extraction time and solvent on the yield of oleoresin

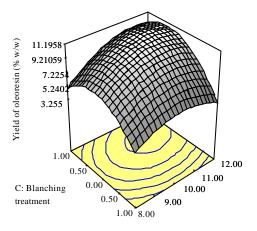


Fig. 4: Response surface plot showing the combine effect of extraction time and blanching treatment on the yield of oleoresin

Antioxidant activity increased in extraction time but after 11.5 h a stationary area of antioxidant activity could be noticed. While the higher value of antioxidant activity could also be noticed when the polarity of solvent took place between acetone and ethanol. Figure 6 shows the effect of extraction time and blanching treatment; quadratic effect of extraction time and a linear effect of blanching treatment on the antioxidant activity can be noticed.

The antioxidant activity was increased with time and the maximum value is in the untreated sample. The optimal processing parameter to produce the highest amount antioxidant (16.65% w/w) was obtained from fresh sample, acetone as a solvent and 11.92 h extraction time.

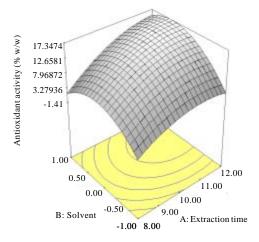


Fig. 5: Response surface plot showing the combine effect of extraction time and solvent on the antioxidant activity

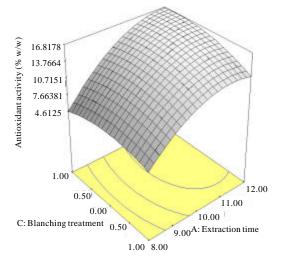


Fig. 6: Response surface plot showing the combine effect of extraction time and blanching treatment on the antioxidant activity

These results have shown that the response surface had a maximum point within the experimental range of the independent variables.

**Optimization of the process:** After the optimization process using design expert software version 6.0.8, five solutions were obtained. The first parameter was selected as optimal processing parameter as the desirability is equal to 0.96 which is the highest value achievable in this research. Table 5 shows the prediction solution from the first parameter chosen.

Table 5: The Predicted solution of the optimization of processing parameter Z. zerumbet oleoresin

Exn	Exn	Blanching	Yield	AOX	
time (h)	solvent	treatment	(% w/w)	(% w/w)	Db
12	Ethanol	Untreated	13.05	16.01	0.96

\*Exn: Extraction, AOX: Antioxidant, Db: Desirability

## DISCUSSION

Influential processing parameters on the yield of oleoresin: The optimum yield of oleoresin for the blanching treatment was obtained from fresh sample, while solvent polarity was between the polarity of acetone and ethanol. This suggests blanching treatments did not have any effect on the extraction of *Z. zerumbet* oleoresin. These findings contradict to the facts that blanching had originally been carried out to facilitate the extraction process by breaking up the cells walls (Gaikar and Dandekar, 2001) and cleanse the tissue as well as increase tissue temperature (Sharma et al., 2000). Concurrently, this study indicated that using solvents with polarity between acetone and ethanol is desired. This could be achieved by adjusting the concentration of the pure solvent or by using aqueous hydrotope solution where it allows water insoluble.

Organic compounds to be diluted in the aqueous solution (Gaikar and Dandekar, 2001). The extraction time was set based on the minimal time that gives the maximum response. In this study, extraction time was set at about 12 h.

Influential processing parameters on the antioxidant activity: In this study, the optimal processing parameter to produce the highest amount antioxidant was the combinations of the high polarity solvent and fresh sample. This is in agreement with the previous studies which reported that the most efficient solvents for extraction of antioxidants are those of high polarity such as methanol and ethanol (Hashim *et al.*, 2002). Phenolic compounds normally act as primary antioxidant via the mechanism of increased resonance stabilization of the free radical by donating a hydrogen atom to the radical (Gordon, 1990).

In term of extraction efficiency, longer extraction time means lower extraction rate. Higher yield suggests higher proportion of antioxidant properties. The result suggests a better response with longer extraction time. However, this is contrary to the fact that longer extraction time causes longer exposure to various influential environmental and processing factors towards the antioxidant properties such as heat, light, oxygen, temperature, type of substrate and physical state of the system (Yanishlieva-Maslarova, 2001).

#### CONCLUSION

Response surface methodology was successfully applied for optimization of *Z. zerumbet* oleoresin by Soxhlet extractor. The high regression coefficients of quadratic polynomial of the response showed that model fitted the data well. After the optimization process using Box-Behnken design technique of design expert software version 6.0.8, the optimal processing parameter that fulfilled the requirement for yield of oleoresin and antioxidant activity were found to be 12 h extraction time, ethanol as the solvent used and untreated sample. While, the optimal yield of oleoresin was 13.05% w/w and antioxidant activity was 16.01% w/w.

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