Research Journal of Applied Sciences, Engineering and Technology 7(24): 5098-5105, 2014
DOI:10.19026/rjaset.7.904
ISSN: 2040-7459; e-ISSN: 2040-7467

Submitted: October 19, 2013 Accepted: October 25, 2013 Published: June 25, 2014

Research Article

Optimization of Ginger (*Zingiber officinale*) Oil Yield from Malaysia in Different Hydrodistillation Physical Parameters via Central Composite Design of Response Surface Methodology (RSM)

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Abstract: Response Surface Methodology (RSM) with Central Composite Design face cantered (CCD) is the statistical analysis used for optimization process of ginger oil yield from Malaysia. In this study the three factors predictors (Water volume, extraction time and particle size) and one response criterion (Ginger oil yield) were optimized. The final optimization value for high yield of ginger oil is at water ratio of 2660 mL water to 100 g sample at 23.15 h of hydro distillation using medium size particles of dried ginger. Under these conditions the oil constitute majority of sesquiterpenes hydrocarbon which is 33.49% with alpha-zingiberene as a major compound.

Keywords: Design of experiment, ginger oil, hydrodistillation, optimization, response surface methodology

INTRODUCTION

The trend for Malaysia's export volumes of ginger had increased between years 2000 to 2007 suggesting gingers are among important herbs usage in Malaysia (Globinmed, 2012). The essential oil compound properties as antifungal, antibacterial and antioxidant were the major interesting medicinal benefits discovered. It is also approved by FDA as food additive. There are many studies in wide world on different source of ginger and different essential oil compound yield. The variation in compound constituents depends on geographical factor and extraction methods used.

In most literature it is agreed the essential oil in the rhizomes represent about 0.85-2% from dry weight (Noor *et al*., 2004; Sultan *et al*., 2005; Sasidharan and Menon, 2010). From several different paper, essential oil yield from ginger are differs according to origin where from China (0.98%), Thailand (1.58%), Nigeria (2.4%) and Eygpt (0.96-0.85%) (Sultan *et al*., 2005; Onyenekwe and Hashimoto, 1999; El-Baroty *et al*., 2010).

Sample preparation is crucial to obtain high yield of ginger oil. Factors involved include sample physical characteristic, drying methods and particle size. Sample physical characteristic can be either it is fresh or dried, peeled or unpeeled skin and young or old ginger. Upon drying, factors involved are time and methods (oven dried, microwave dried, sun dried, freeze dried and air dried). Particle sizes which involved slicing methods are as well important as others.

Unpeeled gingers were found to have highest yield of oil/oleoresin (Yiljep *et al*., 2005). Peeling or removal of skin may remove thick fibrous tissue of ginger suggesting loss of oil cells that may also reside at the cell parenchymal wall (Jayashree *et al*., 2012; Noor *et al*., 2004). Upon using fresh ginger in opposition to dried, it was found the chemical composition in fresh ginger are more than dried ginger due to drying process. Most monoterpene and sequiterpene alcohol compounds are decrease in ginger oil from dried ginger compared to fresh ginger (Sasidharan and Menon, 2010).

The particle size contributes on the yield of oil where it is suggested concentration of essential oil in ginger will decrease when there is increase in size (Okafor *et al*., 2009). However exact particle size requires for high yield of oil is undetermined.

To control several variables in an experiment is challenging upon acquiring a maximum and quality optimum response. Conducting One Factor at Time (OFAT) experiment per se failed to detect interaction between variables as the variables are not varied at one time. Design of Experiment is a statistical tool approaching controllable variables (input) and responses variables (output) allowing detection of possible interaction in a well designated structured and

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effective experiment plan. Response Surface Methodology (RSM) is suitable as it is an economical, robust and widely uses methods in optimization process of essential oil successfully (Tan *et al*., 2012; Rezzoug *et al*., 2005).

The objective of this study was to apply Central Composite Design (CCD) based RSM to analyze the effects of the process parameters on essential oil production and to search for the optimal values for attaining a higher essential oil yield.

MATERIALS AND METHODS

Plant material: The fresh rhizomes of ginger plant (*Zingiber officinale*) were purchased from herbal plantation (Kiza Herbs Sdn. Bhd) located in Pahang. The voucher specimens were identified and deposited at UKM Herbarium, Bangi (UKMB 30013). Ginger plants collected were washed and unpeeled. Dry gingers were obtained by slicing thin rhizome and were air dried for 3 days. The water content was determined using Dean's Stark apparatus. The dried rhizomes were then ground and sieved at 3 different sizes; coarse (>2000 µm), medium (2000-800 µm) and fine (<800 µm).

Isolation of essential oil: The ground dried gingers (100 g) were hydro distilled using Clevenger apparatus at three different water-sample ratio content of 1000 mL: 100 g, 2000 mL:100 g and 3000 mL: 100 g at three different times of 8, 16 and 24 h, respectively. Temperature was remained constant at 40°C. The extracted oil was dehydrated with anhydrous sodium sulfate to isolate ginger oil.

Experimental design: RSM were employed in this study to improve the isolation process by optimizing the yield via CCD. A set of random experimental trial work with combination of factors involved in process for high and optimum production of desired results were effectively designed by Design of Experiment (DOE).

A central composite face centered design with was used. The design includes three variables factors (Water volume, extraction time and particle size) and 1 response (Ginger oil yield). There is 33 actual experiments with 3 factors (k = 3), 3 level with 3 centre points to form a central composite design. The experimental ranges of each variable factor were based on the results from preliminary trials and usual standard lab protocol practice in the laboratory. Variables, code, range and level of the experimental work are as in Table 1.

The suggested optimized values later were used to do a confirmation run to validate the model is fits and accurate.

Table 1: Experimental design variables, code, range and level

		Range and level		
Variables	Code	-1	0	+1
Water volume (mL)	A	1000	2000	3000
Extraction time (h)	B	8	16	24
Particle size	C	Coarse	Medium	Fine

Fig. 1: 3 different sample sizes of ginger

Essential oil analysis: The ginger oil extracted was dissolved in hexane and was analyzed using Shimadzu GC 14A using a FID detector and DB-5 stationary phase column (30 m×0.25 mm, 0.25 ||m film thickness) programmed from 75°C for 10 min, then 3°C /min to 230°C and held for 10 min). It was operated under nitrogen as carrier gas at 50 cm/sec, injector and detector temperatures were maintained at 250°C. The areas and retention times from chromatogram were used to measure linear retention indices of the compounds which were relative to *n*-alkanes (Adam, 2001).

For GC-MS analysis, it was operated using Hewlett Packard GC-MSD 5890 with combination of 70 Ev Electron Impact Ionisation (EI) zone and BPX5 column (30 m×0.25 mm, 0.25 µm film thickness) programmed similar protocol as in GC. Identification of the compound were compared with reference spectra in NIST library and confirmed via retention indices of compound from literature from GC spectrum (Adam, 2001).

RESULTS AND DISCUSSION

Ginger essential oil yield: The spectrum result of CG for analyzed ginger oil is as in Fig. 1. The ginger oil yield was average of 0.95% at 24 h. Upon comparing with other ginger from different location, the ginger oil yield from Malaysia ginger is among the lowest of all (Onyenekwe and Hashimoto, 1999; Sultan *et al*., 2005).

The major chemical constituents were listed in Table 2 sorted according to elution order. There are 14 compounds identified as monoterpenes (10.89%) and 19 compounds as oxygenated monoterpenes (15.46%) in the ginger oil using GC. Total monoterpenes compounds identified are 33 comprising 26.35% of total oil yield. For sesquiterpenes (19.97%), there are 19 compounds identified and 18 more compounds are group as oxygenated sesquiterpenes (13.52%). Total sesquiterpenes in the oil is 33.49%. The major compounds in sesquiterpenes are alpha-zingiberene

Table 2: Chemical constituents of rhizome oils of *Zingiber officinale*

Compound	(%)	RI	Methods of identification
Monoterpene (C10)			
Camphene	3.05	954	RI, MS
Pinene <alpha->	0.29	937	RI, MS
Citronellene <beta->	1.21	950	RI
Thuja-2, 4 (10) -diene	0.96	960	RI
Verbenene	0.57	968	RI
Pinene <beta->	0.56	979	RI, MS
Myrcene	0.20	991	RI, MS
Phellandrene <alpha->	0.31	1003	RI, MS
Sylvestrene <iso->	0.67	1009	RI
Cymene <para->	0.70	1025	RI
Limonene	1.17	1029	RI
Ocimene <(Z) -beta->	0.62	1036	RI
Terpinene <gamma->	0.36	1060	RI, MS
Copaene <beta->	0.22	1432	RI
	10.89		
Monoterpene oxygenated			
Norbornen-2-ol <exo-5->	1.09	947	RI
Thiazole <2-ethoxy->	2.30	958	RI
Terpinene <alpha->	0.61	1017	RI, MS
Thiazole <2-acetyl->	0.56	1018	RI
Cresol	0.78	1021	RI
Linalool	0.01	1097	RI, MS
Camphor	0.02	1146	RI, MS
Isoborneol	0.02	1162	RI, MS
Terpineol <cis-dihydro-alpha->	0.47	1165	RI
Borneol	0.63	1169	RI, MS
Linalyl oxide <cis->	1.52	1174	RI
Terpinen-4-ol	0.82	1177	RI
Octane <1, 1-dimethoxy->	0.76	1179	RI
Cymen-8-ol <para->	0.96	1183	RI
Phenol <2-allyl->	1.06	1191	RI
Neral	0.47	1238	RI, MS
Geranial	1.69	1267	RI
Terpinen-7-al <gamma->	0.67	1291	RI
Isoeugenol <E->	1.02	1451	RI
	15.46		
Sesquiterpene (C15)			
Elemene <delta->	0.92	1338	RI, MS
Cubebene <alpha->	0.91	1351	RI, MS
Sesquithujene	0.51	1417	RI
Gurjunene <beta->	0.59	1434	RI
Farnesene < (Z) -beta->	0.16	1443	RI, MS
Cedrane	0.79	1444	RI
Farnesen < (E) -beta->	0.79	1457	RI, MS
Aromadendrene <allo->	0.65	1460	RI
Aromadendrene <dehydro->	0.73	1463	RI
Muurola-4 (14), 5-diene <cis->	1.28	1467	RI
Gurjunene <gamma->	0.96	1477	RI, MS
Curcumene <ar->	0.90	1481	RI, MS
Zingiberene <alpha->	2.17	1494	RI, MS
Bisabolene <beta->	1.04	1506	RI, MS
Germacrene A	2.10	1509	RI
Sesquiphellandrene <beta->	1.06	1523	RI, MS
Cadinene <alpha->	0.99	1539	RI
Selina-3, 7 (11) -diene	2.50	1547	RI
Germacrene <beta>	0.92	1561	RI, MS
	19.97		
Sesquiterpene oxygenated			
Sesquicineole <dehydro->	1.14	1473	RI
Menthyl isovalerate	1.11	1518	RI
Nerolidol <Z->	0.79	1534	RI, MS
Cadinene ether <cis->	0.59	1554	RI
Cadinene ether <trans->	0.81	1559	RI
Caryolan-8-ol	0.58	1573	RI
Spathulenol	0.51	1578	RI
Turmerol <ar->	0.66	1580	RI
Bisabolol <dihydro (10, 11) -ar-alpha->	0.53	1602	RI
Geranyl isovalerate	0.96	1607	RI
Tetradecanal	0.72	1613	RI

Table 2: Continue

Bisabolol-11-ol <Z->	1.21	1619	RI
Silphiperfol-6-en-5-one	0.77	1626	RI
Cadinol <epi-alpha->	0.75	1640	RI
Muurolol <alpha->	0.74	1646	RI
Eudesmol <beta->	0.80	1651	RI, MS
Atractylone	0.59	1659	RI
Nuciferal <E->	0.26	1729	RI
	13.52		
Non-terpene			
Heptanone <5-methyl-3>	1.26	944	RI
Cyclohexyl formate	0.56	962	RI
Furfural <5-methyl->	1.00	964	RI
Cryptone	0.84	1186	RI
Fenchyl acetate <exo->	0.59	1233	RI
Carvone	1.18	1242	RI
Carvacrol, methyl ether	1.16	1245	RI
Ethyl-oct- (2E-) -enoate	0.78	1249	RI
Thymoquinone	1.11	1252	RI
Sabinene hydrate acetate <trans->	1.63	1256	RI
Phenyl ethyl acetate <2->	1.20	1258	RI
Ethyl salicylate	3.95	1270	RI
Menthyl acetate <neo->	2.28	1274	RI
Nonanal	2.24	1279	RI
Thujyl acetate <neoiso-3->	1.61	1284	RI
Bornyl acetate	1.47	1289	RI
Menthyl acetate <iso->	0.54	1305	RI
Geranyl acetate	0.01	1381	RI, MS
Anthranilate <ethyl->	0.51	1416	RI
Carvyl propanoate <trans->	0.83	1456	RI
Vanillin acetate	2.02	1527	RI
Phenyl ethyl tiglate <2->	0.85	1586	RI
Citronellyl tiglate <E->	0.39	1668	RI
Muurol-5-en-4-one <cis-14-nor->	0.35	1689	RI
Mayurone	0.26	1710	RI
	28.62		

RI: Retention index/kovats index; MS: Mass spectrometer

Table 3: Validation of the data and model constructed

Std	Run	A water volume (mL)	B extraction time (h)	C particle size	Total oil yield (observed) (%)	Total oil yield (predicted) (%)
1	9	1	8	Coarse	0.30	0.32
2	25	3	8	Coarse	0.50	0.54
3	24	1	24	Coarse	0.70	0.74
4	17	3	24	Coarse	1.00	1.05
5	30	1	16	Coarse	0.50	0.59
6	12	3	16	Coarse	0.80	0.85
7	1	2	8	Coarse	0.75	0.74
8	26	2	24	Coarse	1.24	1.20
9	13	2	16	Coarse	1.07	1.03
10	20	2	16	Coarse	1.00	1.03
11	7	2	16	Coarse	1.25	1.03
12	18	1	8	Medium	0.20	0.16
13	6	3	8	Medium	0.80	0.79
14	5	1	24	Medium	0.60	0.59
15	14	3	24	Medium	1.32	1.31
16	28	1	16	Medium	0.40	0.44
17	23	3	16	Medium	1.10	1.11
18	33	2	8	Medium	0.80	0.78
19	10	2	24	Medium	1.30	1.26
20	27	2	16	Medium	1.05	1.08
21	19	2	16	Medium	1.10	1.08
22	15	2	16	Medium	1.00	1.08
23	31	1	8	Fine	0.40	0.39
24	16	3	8	Fine	0.50	0.49
25	21	1	24	Fine	0.70	0.61
26	32	3	24	Fine	0.84	0.80
27	2	1	16	Fine	0.60	0.56
28	4	3	16	Fine	0.80	0.71
29	8	2	8	Fine	0.72	0.75
30	11	2	24	Fine	0.87	1.01
31	3	2	16	Fine	0.82	0.94
32	29	2	16	Fine	0.95	0.94
33	22	2	16	Fine	0.93	0.94

(2.17%), selina-3, 7 (11) diene (2.50%), alpha-germacrene (2.10%), Muurola-4 (14), 5-diene (1.28%) and beta- sesquiphellandrene (1.06%) which compliments well a literature reported on zingiberene as highest compound in Chinese (31.1%, 8%), Guinean (19.89%), Nigerian (29.54%) and Thailand (30.81%) ginger rhizome (Toure and Xioming, 2007; Onyenekwe and Hashimoto, 1999).

The compound profile listed above shows good quality ginger oil. Ginger oil must be characterized by high sesquiterpene hydrocarbons (alpha zingiberene, ar-curcumene, beta-sesquiphellandrene) follows by other compounds (Sivasothy *et al*., 2011; Onyenekwe and Hashimoto, 1999; Wohlmuth *et al*., 2006; Sasidharan and Menon, 2010). The quality of oil was also contributed by the age of oil which is by determination ratio of zingiberene+beta-sesquiphellandrene to ar-curcumene (Zachariah and Gopalan, 1987). A ratio of 2:3 is the recommended ratio for a good quality of ginger oil (Zachariah, 2008).

Different physical characteristic and distillation parameter will provides different quality of ginger oil odor. In industry where aroma is essential, a mixture of alpha terpineol, neral, geraniol, beta sesquiphellandrene, ar-curcumene and nerolidol is characteristic of ginger oil aroma (Prabhakaran, 2013).

Optimization of ginger essential oil: The Table 3 shows the actual factorial experiment and response value that were designed by Design of experiment and were performed which comprised three factors predictors (Water volume, extraction time and particle size) and one response criterion (Ginger oil yield).

Result for Analysis of Variance (ANOVA) is shown in Fig. 2. The significant level of each coefficient is determined by p value <0.0001. From ANOVA, the model is statistically significant with F-value of 38.15, thus the model is accepted and there is only 0.0001% chances that this due to noise.

In this model, A (Water volume) B (Extraction Time), C (Particle size), AC (Water volume×Particle size) and A^2 (Water volume)^2 are significant model terms with significant p-value <0.0001. These factors have significant effect on response individually or via interaction. Non-significant lack of fit F-value of 0.99 indicate that the replication variation on the region and design point of the model are fit and there is that 0.52% chance the value occur due to noise.

Factor A, B and C when act independently preferred gained higher ginger oil yield at water ratio <1000 mL to 100 g, time >24 h and using medium particle sizes. However upon synergistic relationship, interaction between AC was significantly exist (F-value 17.68) where at water ratio 2000 mL to 100 g and using medium particle size, ginger oil are highly obtained. Here is where the desirability parameter was selected to optimize the process.

Individually, water ratios have a trend of at certain point, the ginger oil production will drop and further increase in ratio will not be beneficial (Teoh *et al*., 2012; Li *et al*., 2009). This may due to oil cells that are not rebuilt due to fully rupture of parenchyma cell wall (Noor *et al*., 2004).

Particle size wouldn't matter if the plant do not have secretory duct (Xu *et al*., 2011). When the size is big, there is longer diffusion or mass transfer between water and the plant particles. Thus the interaction between water ratio and particle size is important as stated in this studies to optimized ginger oil yield.

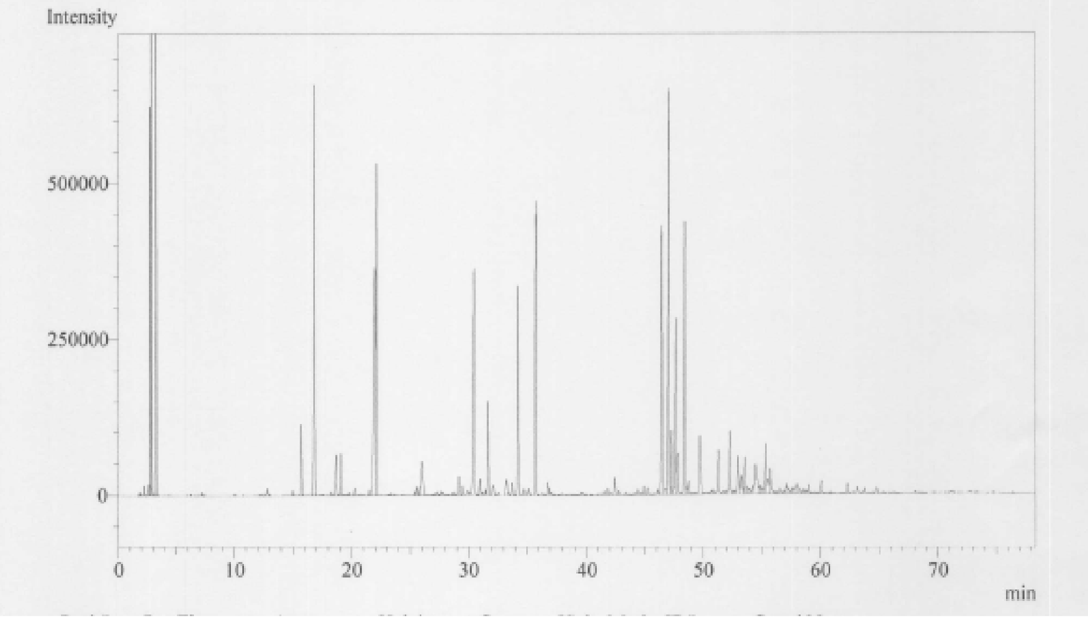
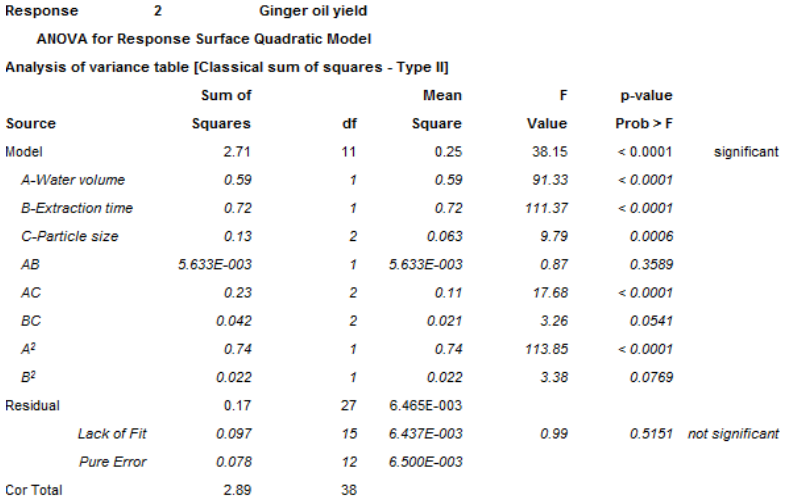


Fig. 2: Spectrum of ginger oil using GC



Response 2 Ginger oil yield

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Classical sum of squares - Type II]

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	2.71	11	0.25	38.15	< 0.0001	significant
A-Water volume	*0.59*	*1*	*0.59*	*91.33*	*< 0.0001*	
B-Extraction time	*0.72*	*1*	*0.72*	*111.37*	*< 0.0001*	
C-Particle size	*0.13*	*2*	*0.063*	*9.79*	*0.0006*	
AB	*5.633E-003*	*1*	*5.633E-003*	*0.87*	*0.3589*	
AC	*0.23*	*2*	*0.11*	*17.68*	*< 0.0001*	
BC	*0.042*	*2*	*0.021*	*3.26*	*0.0541*	
A^2	*0.74*	*1*	*0.74*	*113.85*	*< 0.0001*	
B^2	*0.022*	*1*	*0.022*	*3.38*	*0.0769*	
Residual	0.17	27	6.465E-003			
Lack of Fit	*0.097*	*15*	*6.437E-003*	*0.99*	*0.5151*	*not significant*
Pure Error	*0.078*	*12*	*6.500E-003*			
Cor Total	2.89	38				

Fig. 3: Analysis of Variance (ANOVA)

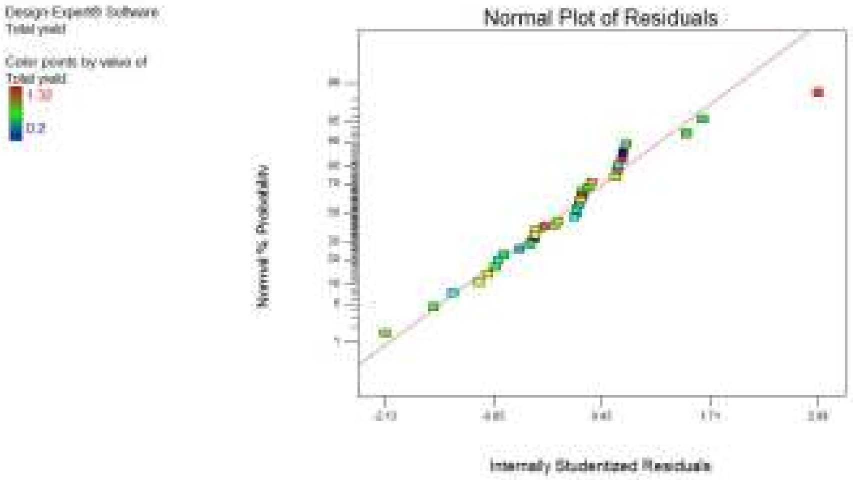


Fig. 4: Normal probability plot versus residuals

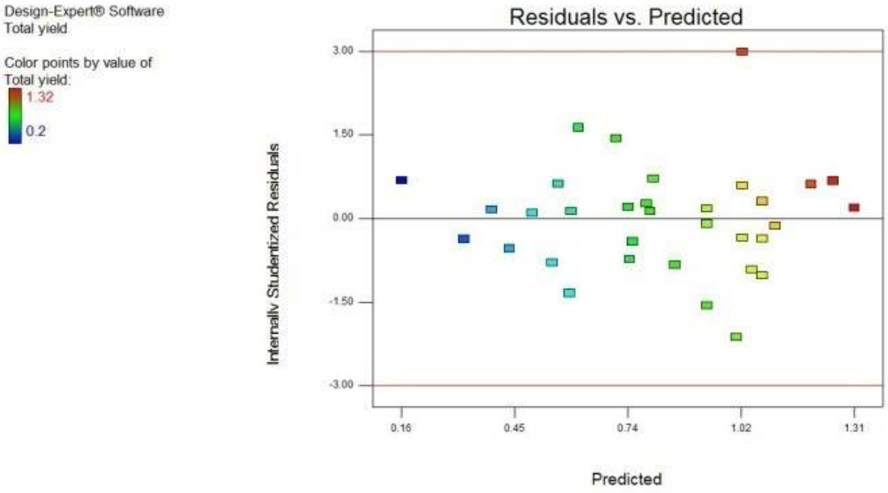


Fig. 5: Residual versus predicted plot

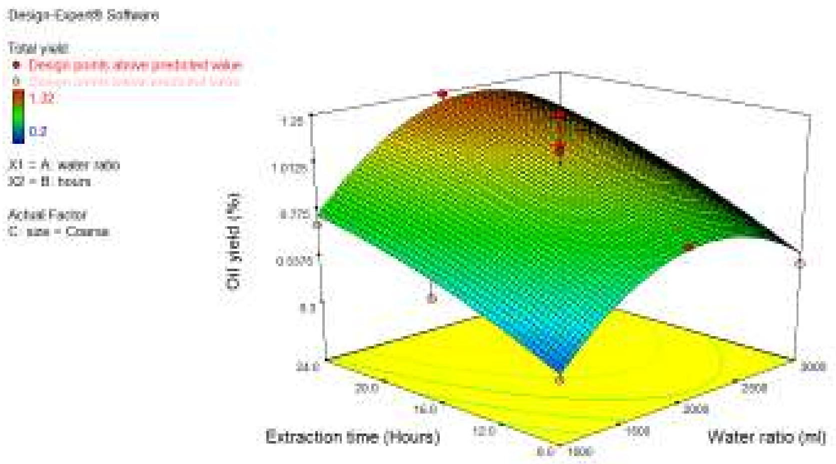


Fig. 6: Response surface method contour plot

The regression analysis was performed besides for prediction; it is also to understand or explained the relationship of three inputs to one output response. The coefficient of determination R^2 value is 0.95 suggesting there is a good prediction of the model. The prediction R^2 of 0.89 is also in reasonable agreement with adjusted R^2 of 0.92.

Model measurement for accuracy can be achieved by adequate signal of "Adeq Precision" more than 4. The ration of 23.396 in this model is adequate. Stability of this model to predict well in other sample can be evaluated from PRESS (Predictive Residual-Error Sum of Squares). A smaller value is desired thus 0.30 in this model is accepted (Palmer and O'Connell, 2009).

The residual analysis emphasizing on different from actual observed and predicted value would address the appropriateness of the model. From normal probability plot in Fig. 3, the residuals falls on a straight line suggesting the random error from experiments are normally distributed.

The residual versus predicted plot as in Fig. 4 shows that there were no unusual or skewed data suggesting no possible outliers. The RSM model final code equation is:

$$\text{Total ginger oil yield} = +\,0.16 - 2.07^{*}A + 0.24^{*}B - 0.083^{*}C\,[1] + 0.37^{*}C\,[2] + 0.043^{*}AB - 0.096^{*}AC\,[1] + 0.31^{*}AC\,[2] + 0.031^{*}BC\,[1] + 0.037^{*}BC\,[2] - 1.22^{*}A^{2} - 0.058^{*}B^{2}$$

Upon optimization, a solution combining three factors above suggest at desirability value of 1, to obtained maximum ginger oil yield of 1.34%, the optimal water ratio to sample value is 2660 mL to 100 g, at 23.15 h using medium size particles. The 3D surface graph is as showed in Fig. 5. A confirmation run to validate the model were done at chosen suggested optimal parameter and revealed results of average ginger oil yield of 1.34% (Fig. 6).

CONCLUSION

From the analysis, we can conclude interaction of water ratio and particle size interaction contributes significantly for production of high ginger oil. From the optimize value, to obtained maximum ginger oil yield of 1.34%, the optimal water ratio to sample value is 2660 mL to 100 g, at 23.15 h using medium size particles via hydro-distillation. A confirmation run have validate the model is accurate.

ACKNOWLEDGMENT

The author would like to acknowledge postgraduate students and staff of Faculty of Pharmacy, UKM for guidance and help throughout the project.

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