International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)
Journal Home page: www.iscientific.org/Journal.html

Response surface methodology of subcritical water-reactive extraction of gingerol to shogaol ginger dregs through ultraviolet light pretreatment

[1,2*]*Mohamad Endy Yulianto*, [1]*Bakti Jos*, [1]*Budiyono*

[1]*Faculty of Engineering, Diponegoro University, Semarang, Indonesia*

[2]*Vocational School, Prof. Sudarto SH Street, Semarang, Indonesia*

Abstract

The increase of shogaol production through reactive extraction of subcritical water depends on the stages of the pretreatment process in destroying the cellular cells of ginger pulp. Pretreatment using ultraviolet B (UVB) is predicted to be able to damage cellular cell walls and outer cell membranes (plasmalemma). In addition, to initiate the degradation of gingerols to shogaols, the reaction conversion and selectivity of shogaols are increased. This study aimed to examine the optimum process conditions for the subcritical water-reactive extraction of gingerol to shogaol from the ginger pulp by utilizing UVB pretreatment: reactive extraction temperature (110 – 130ºC), ratio solvent/feed (22:1 – 32:1 b/b), and time (10 – 30 minutes). The extract of shogaol in solvent was separated from the raffinate with a separator funnel and analysed using HPLC-MS. Interpretation of experimental data to optimize the reactive extraction process used the Response Surface Methodology (RSM). The study results show that at 120°C, solvent:inlet ratio 27:1 for 20 minutes through ultraviolet B-light pretreatment for 10 minutes, the yield of shogaol increased from 177 µg/L (without UV) to 18646 µg/L. Moreover, the RSM model was very good at predicting shogaol acquisition responses with a determination coefficient of R^2 to be 0.8554. The optimum condition for reactive extraction through UV-B light pretreatment was reached at a temperature of 121.46°C and an inlet solvent ratio of 27.96 at 18.36 minutes, with a shogaol concentration of 18567.1 µg /L.

Keywords: Pretreatment, Reactive Extraction, Shogaol, Subcritical Water, UV.

Full length article **Corresponding Author*, e-mail: endy_y@yahoo.com Doi # https://doi.org/10.62877/21-IJCBS-24-25-19-21

1. Introduction

Ginger (*Zingiber officinale*) could be a restorative plant known to have different pharmacological impacts with fundamental bioactive components such as gingerol, shogaol, paradol and zingerone [1-5]. The bioactive components 6-gingerol and 6-shogaol have a comparable chemical structure which is more profitable, be that as it may, 6-shogaol incorporates a more grounded dynamic control. Some ponders have appeared that 6-shogaol has more prominent viability than 6-gingerol, such as antioxidant action [3,6-8], anti-inflammation activity [3,9,10], effect of anti-platelet aggregation [11], prevention of muscle contraction [12], prevention of colorectal cancer [13], ovarium cancer [14,15], breast cancer [16], and lung cancer [17,18]. The advancement of the 6 shogaol extraction handle coming about from the change of 6-gingerol from ginger mash has been carried out, including: extraction through a soxhletation device, subcritical water extraction [19], subcritical fluid extraction [20], hydrotropic extraction [21], microwave extraction [22], enzymatic extraction [23], ultrasonic extraction [24,25], and ionic liquid extraction [26]. Extraction of dynamic compounds by maceration, hydro-distillation, and filtering strategies utilizing solvents such as ethanol, methanol, acetone, dichloromethane, and hexane, is said to be a viable prepare. In any case, this handle has the potential to take off poisonous dissolvable buildups [20], long extraction time, tall dissolvable necessity, moo selectivity, and warm corruption of bioactive compounds. Subcritical extraction utilizing CO_2 and water is exceptionally particular and does not take off a buildup of destructive solvents. However, this process requires a high enough cost to supply fluid at high-pressure supercritical conditions [27]. Therefore, it needs extraordinary gear and taking care of with moderately costly generation costs, i.e., 223 US$/kg oleoresin extract [28]. In general, 6-shogaol is dehydrated 6-gingerol in ginger pulp through acid or heat catalyst. Therefore, reactive extraction of 6-gingerol to 6-shogaol using subcritical water is considered appropriate. Subcritical water is stated to be able to act as a catalyst in the acid dehydration process because, in subcritical conditions, water has a high number of ions as a result of the water ionization process. The tall number of subcritical water ionization items is caused by the powerless hydrogen holding of water at the over temperature of 150°C [29]. Powerless hydrogen bonds in water will result in autoionization of water to create hydronium particles (H_3O^+) which can work as corrosive catalysts and hydroxide particles (OH^-) which can work as an antacid catalyst [30].The part of subcritical water as a catalyst is due to the ionic products from auto-ionization of water additionally and subcritical water can lower the enactment vitality [31]. In the subcritical region (100-374°C), the ionisation constant (K_w) increases with the increasing temperature [32]. However, shogaol production through reactive extraction of subcritical water is considered to be limited because the cellular cell walls containing bioactive compounds are relatively thick [33]. For this reason, it is necessary to study the reactive extraction pretreatment of gingerol to shogaol through ultraviolet light to damage ginger pulp's cellular cell wall and outer cell membrane (plasmalemma). Various extraction studies have been carried out to help damage cellular cells, including using ultraviolet light. Photon energy wavelengths of ultraviolet B (UVB) and ultraviolet C (UVC) can cause significant cell damage [34,35]. UVB and UVC cause direct DNA damage by absorbing photons to produce chemical compounds forming pyrimidine dimers [36]. UVB exposure for 232 seconds at 1.5 W/cm^2 is able to extract algae oil and convert it into methyl ester from 45.3 mg/L (without UV) up to 79.9 mg/L [37].The degradation and conversion of organic compounds occur through the mechanism of forming superoxide radical anions, which are capable of converting existing protons into highly active hydroxylperoxyl radicals and then converting them into hydrogen peroxide. This hydrogen peroxide will initiate the degradation of organic compounds [38]. Exposure to UV light for 180 minutes was able to degrade 60% gallic acid and up to 50% vanilla acid. However, data on the optimum process conditions for the reactive extraction of ginger dregs shogaol by pre-treating the cellular cell wall and outer cell membrane (plasmalemma) with ultraviolet light are not yet available. For this reason, this study aimed to examine the optimum process conditions for reactive extraction of gingerols into shogaol of ginger dregs with subcritical water as a solvent through pretreatment of UVB [39].

2. Materials and methods

2.1. Materials

The materials needed in the research are: *emprit* ginger rhizome, nitrogen, demin water as subcritical water, and methanol. The main equipment used in the research includes a subcritical water extractor unit and a stirred tank equipped with ultraviolet light. Supporting tools for analytical purposes include UV-Vis spectrophotometry, centrifugation, magnetic stirrers, and glass tools.

2.2. Variables

The research variables include a fixed variable of pre-treatment with ultraviolet B-light kept constant at 10 minutes. Variable changes include temperature, feed solvent ratio, and extraction time. The reactive extraction temperature is set at 110 – 130 °C because the solubility of gingerol and shogaol in subcritical water is quite large, and the hydration reaction of gingerol to shogaol occurs. The feed solvent ratio is set at 22:1 – 32:1 w/w, which is the range of solute crossing into the subcritical aqueous phase. Meanwhile, the extraction time is set at 10-30 minutes because the chance of contact between the phases was relatively sufficient so the hydration of gingerol to become shogaol is more selective.

2.3. Procedure

Clean the rhizome of ginger root measuring 5-8 cm from the earth and fertilize it using a grinding machine to urge ginger mash. Pound the dry ginger mash powder with a processor and sifter at 50 work measures. Light the ginger mash powder with bright B-light for 10 minutes. Nourish 100 g of ginger mash powder and 4000 ml of refined water into a stainless tube with a cover as an extraction cell. Stream N_2 gas into the extraction cell for 2 minutes to evacuate, discuss, and break up oxygen. Warm the subcritical water extractor for 3-5 minutes to reach the specified temperature. Begin the extraction preparation (t = 0) when the temperature comes beneath certain conditions agrees to the RSM test plan as presented in Table 1. Condition all runs at a certain weight as well. Afterwards, stream the extractant into the cooling cell at 25°C and 1 MPa for 1 miniature to allow brief cooling. During the extraction, take some tests at a certain time, agreeing to the method variable. Isolate the extricate within the shape of shogaol in dissolvable from raffinate with a separator pipe and analyse using HPLC-MS. Interpretation of the exploratory information to optimize the receptive extraction preparation used the Reaction Surface Strategy (RSM).

3. Results and Discussions

The process of optimizing the subcritical water reactive extraction of gingerol to shogaol ginger pulp through ultra-violet light pretreatment has been carried out using the response surface methodology. The experiment was designed using a central composite design with lower- and upper-level values for the variable temperature, inlet solvent ratio, and time set respectively at 110°C-130°C, 22-33, and 10-20 minutes. Shogaol levels in extracts obtained from subcritical water reactive extraction data are presented in Table 1. The study results show that at 120°C, solvent:inlet ratio of 27:1 for 20 minutes through ultraviolet B-light pretreatment for 10 minutes, the yield of shogaol increased from 177 µg/L (without UV) to 18646 µg/L. This happens because ultraviolet rays function as cellular cell destruction, degradation, and the conversion of organic compounds [38,39]. Thus, subcritical water would more easily infiltrate and penetrate the tonoplast membrane wall after cell damage by ultraviolet rays and phase contact occurred with gingerol, and shogaol compounds in the vacuole zone. The non-polar solvent then diffused out of the cell by dragging the ginger bioactive compounds due to differences in solubility and converting 6-gingerol to shogaol due to degradation by UV light and catalysed by hydronium ions (H_3O^+) of subcritical water autoionization [19,38,39]. Data of study on subcritical water reactive extraction of ginger pulp showed that the increasing temperature affected the increase of shogaol levels of the extract.

Table 1: Experimental Plan on Process Optimization of Reactive-Extraction

Run	Temperature (°C)	Solvent Ration (L)	Time (Minute)	Shogaol Concentration (µg /L)
1	110	22	10	797
2	110	32	10	3862
3	110	22	30	4444
4	110	32	30	2823
5	130	22	10	78
6	130	32	10	2449
7	130	22	30	17722
8	130	32	30	924
9	107.13	27	20	2153
10	132.87	27	20	2941
11	120	27	7.13	957
12	120	27	32.8	959
13	120	20.56	20	1083
14	120	33.40	20	1864
15	120	27	20	18646
16	120	27	20	18646
17 (without UV)	120	27	20	177

Table 2: Coefficient of Regression

Factor	Effect Estimates; Var: Shogaol Content (mg/L); R-sqr=,95537; Adj:,63843 (Shogaol Content with HPLC) 3 Factors, 1 Blocks, 16 Runs; MS Residual = 16,29026 DV: Shogaol Content (mg/L)									
	Effect	Std. Err	t(6)	p	-95 % Cnf.Limt	+95 % Cnf.Limt	Coeff	Std.Err Coeff	-95 % Cnf.Limt	+95 % Cnf.Limt
Mean/Interc.	18,3075	2,845603	6,43361	0,000667	11,3446	25,27043	18,30749	2,845603	11,34455	25,27043
(1)Temperature (C)(L)	1,5483	2,184332	0,70881	0,505038	-3,7966	6,89314	0,77414	1,092166	-1,89830	3,44657
Temperature (C)(Q)	-9,7493	2,652110	-3,67604	0,010379	-16,2387	-3,25977	-4,87463	1,326055	-8,11937	-1,62989
(2)Solvent Ratio (L)(L)	2,7430	2,184332	1,25576	0,255889	-2,6019	8,08787	1,37150	1,092166	-1,30094	4,04393
Solvent Ratio (L)(Q)	-10,8728	2,652110	-4,09970	0,006360	-17,3623	-4,38337	-5,43642	1,326055	-8,68116	-2,19168
(3)Time (Minute)(L)	-1,7090	2,184332	-0,78237	0,463744	-7,0538	3,63591	-0,85448	1,092166	-3,52692	1,81795
Time Minute (Q)	-10,5083	2,652110	-3,96225	0,007433	-16,9978	-4,01885	-5,25417	1,326055	-8,49891	-2,00943
1L by 2L	3,3778	2,853968	1,18353	0,281361	-3,6057	10,36116	1,68888	1,426984	-1,80283	5,18058
1L by 3L	-3,9677	2,853968	-1,39026	0,213831	-10,9512	3,01566	-1,98327	1,426984	-5,47558	1,50783
2L by 3L	-5,9638	2,853968	-2,08963	0,081640	-12,9472	1,01966	-2,98188	1,426984	-6,47358	0,50983

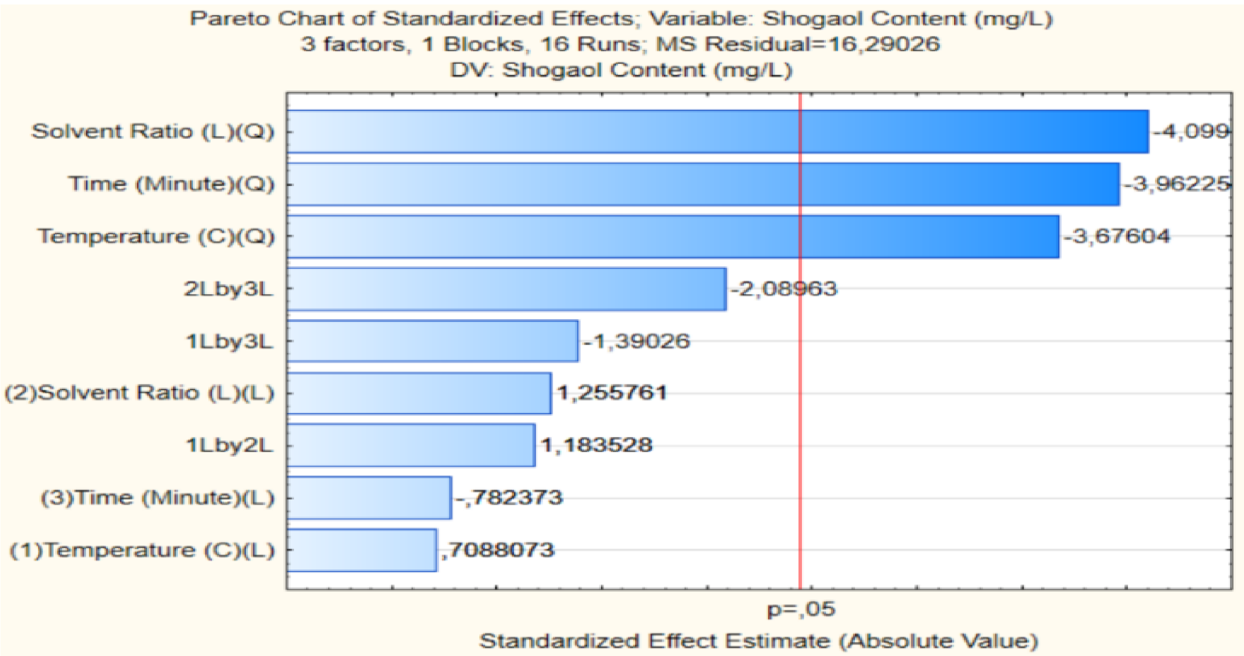
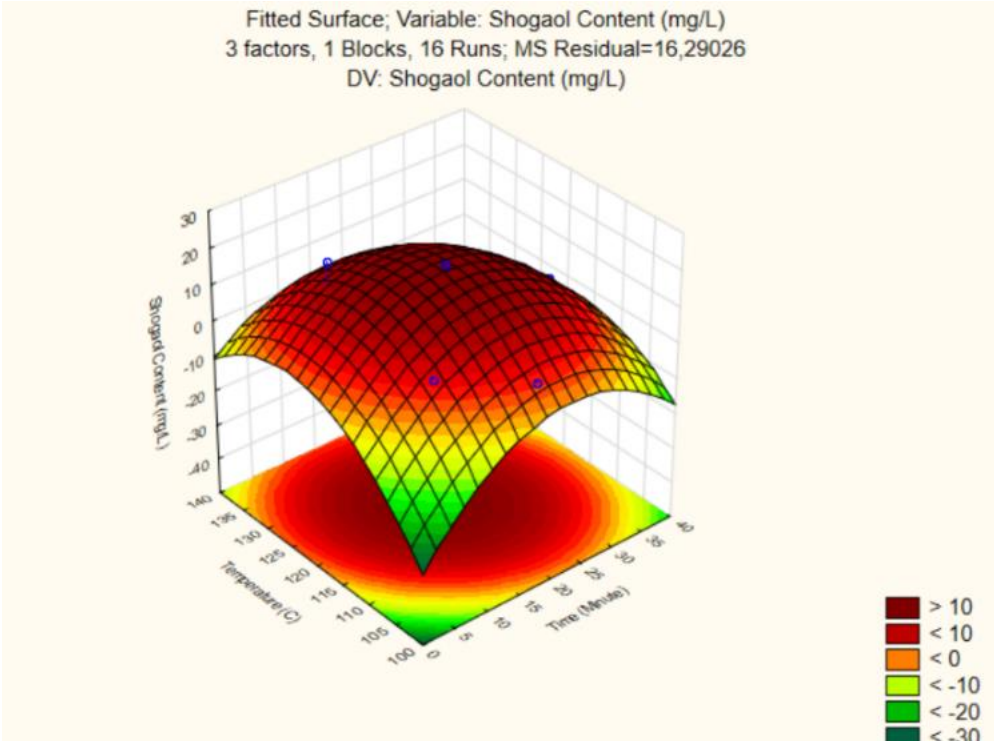


Figure 1: Pareto Diagram



(a)

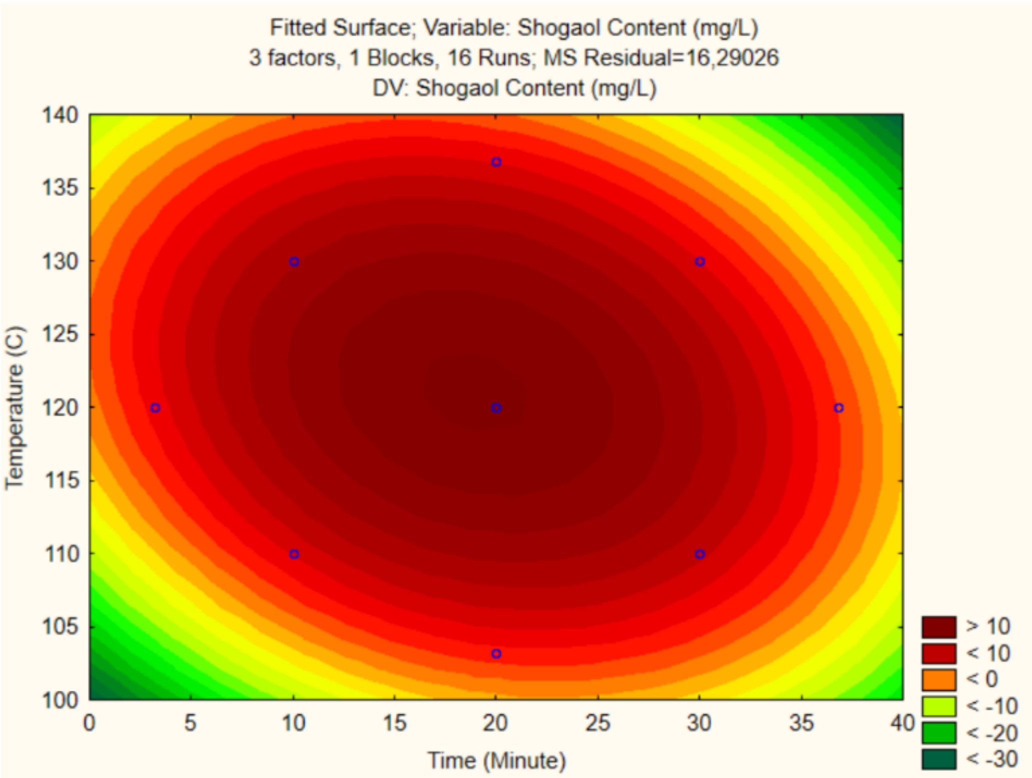
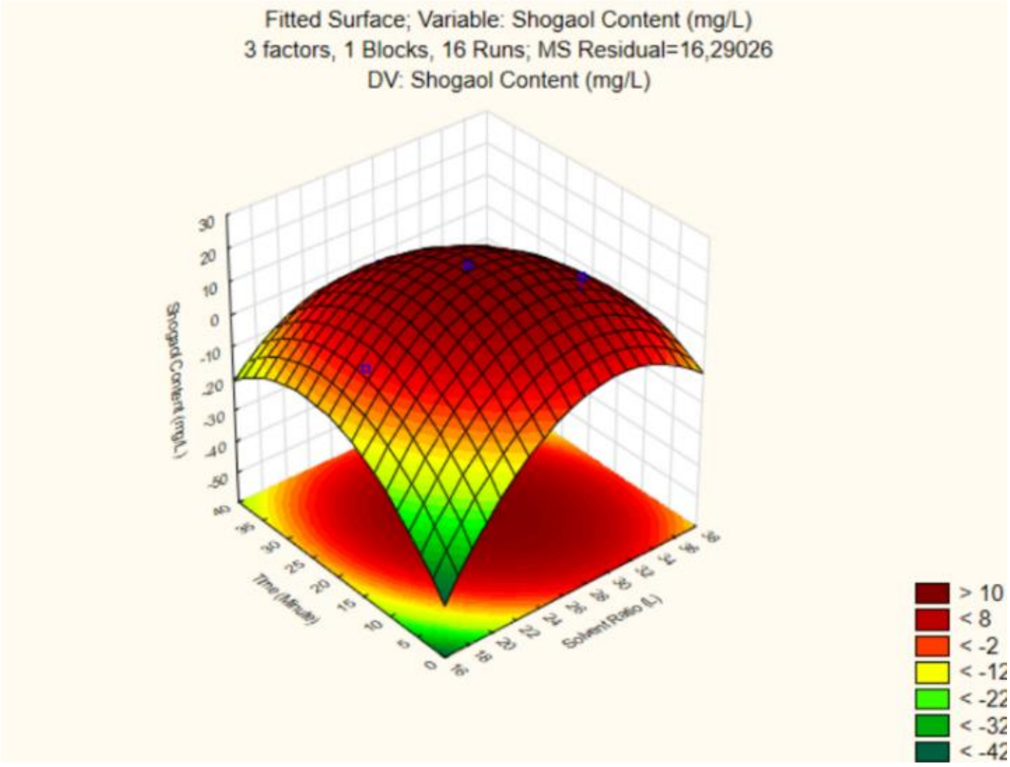
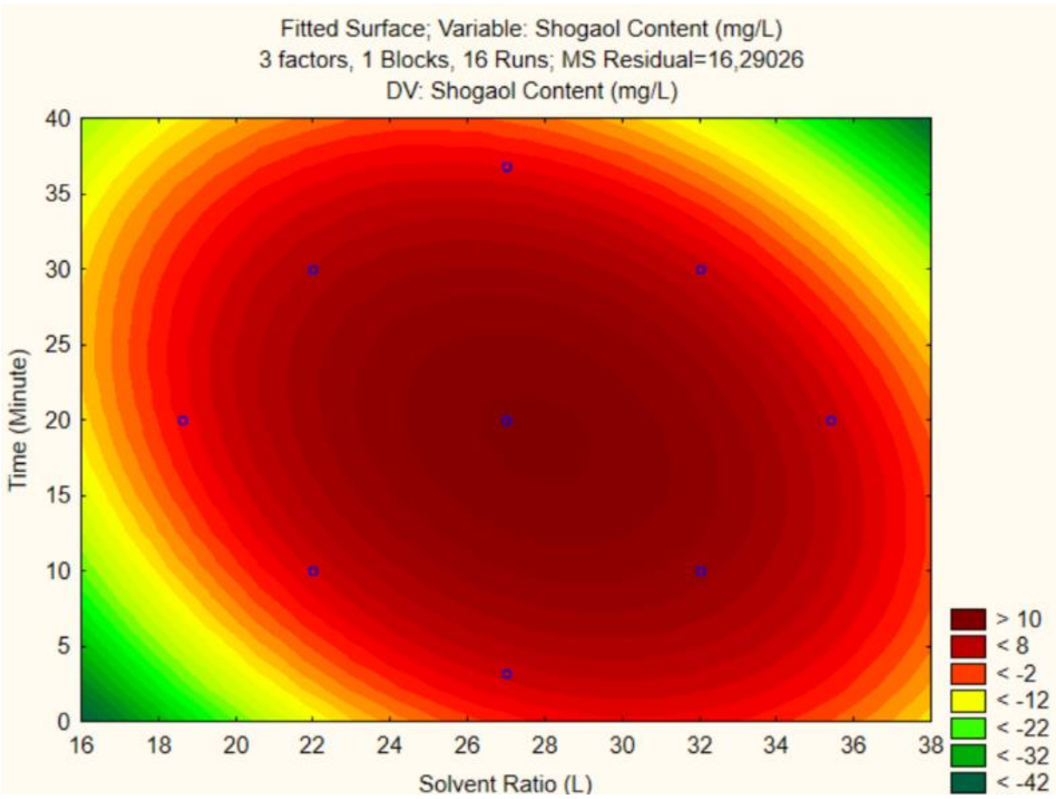


Figure 2: (a) Profile of fitted surface and (b) contour plot of subcritical water reactive extraction of gingerol to be shogaol from ginger pulp

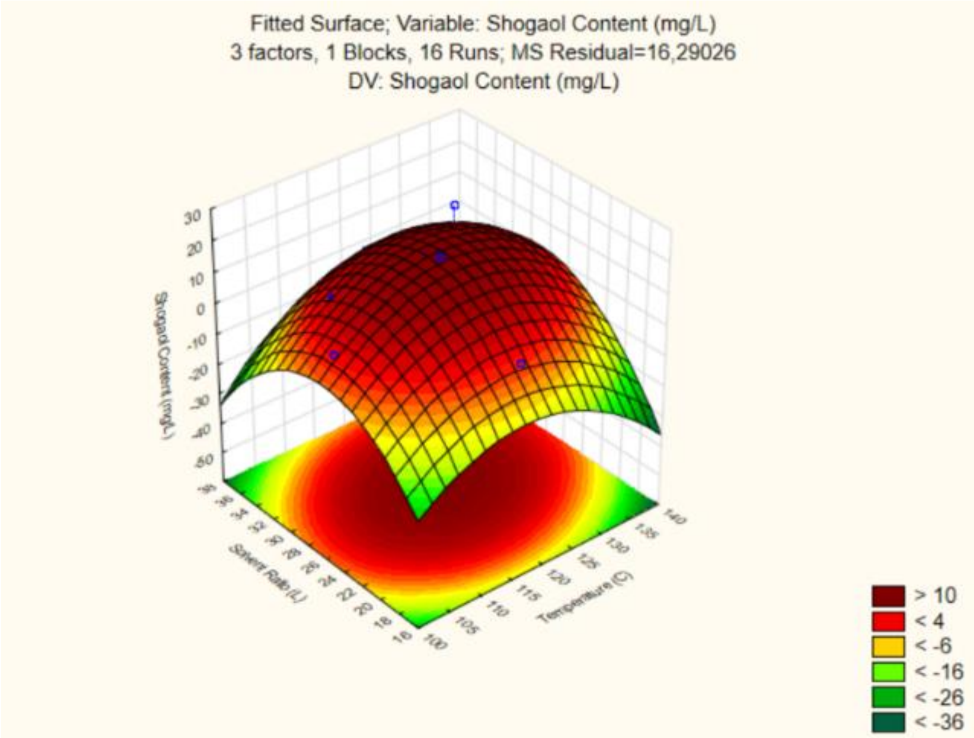


(a)

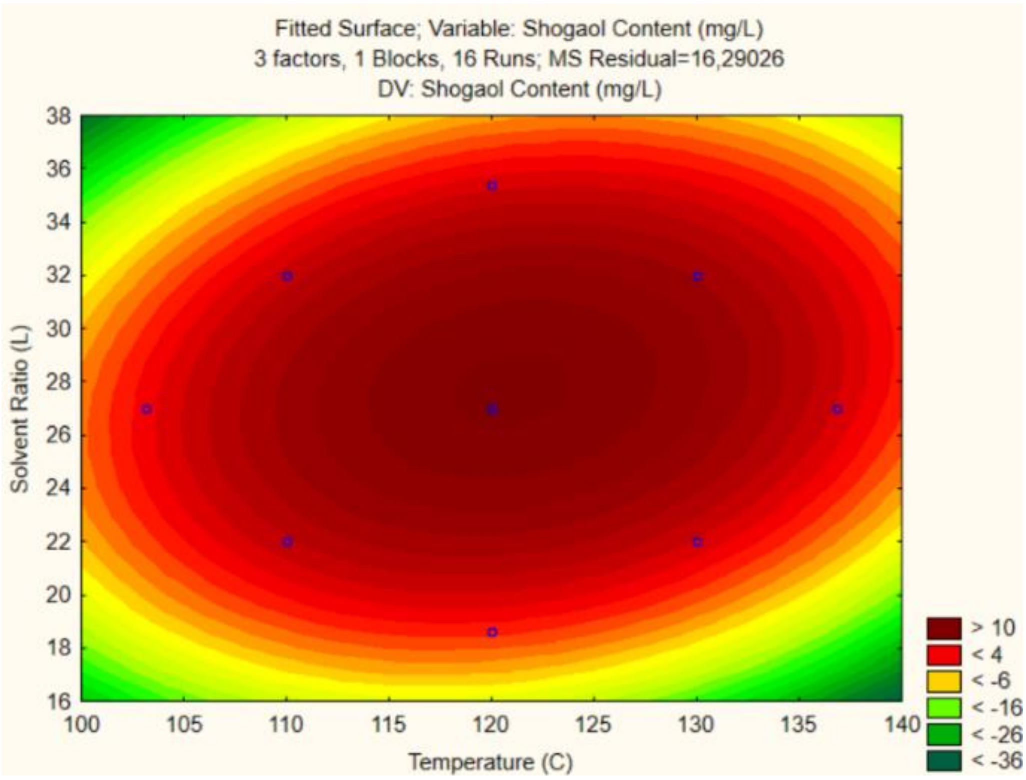


(b)

Figure 3: (a) Profile of fitted surface and (b) contour plot of subcritical water reactive extraction of gingerol to be shogaol from ginger pulp

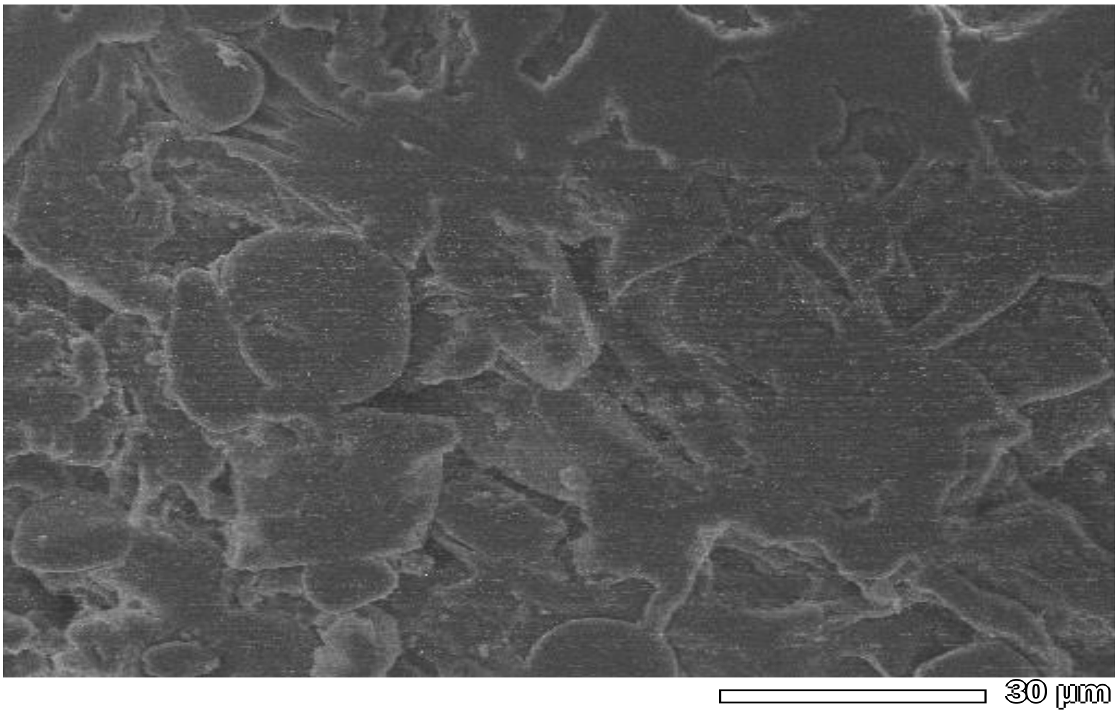


(a)

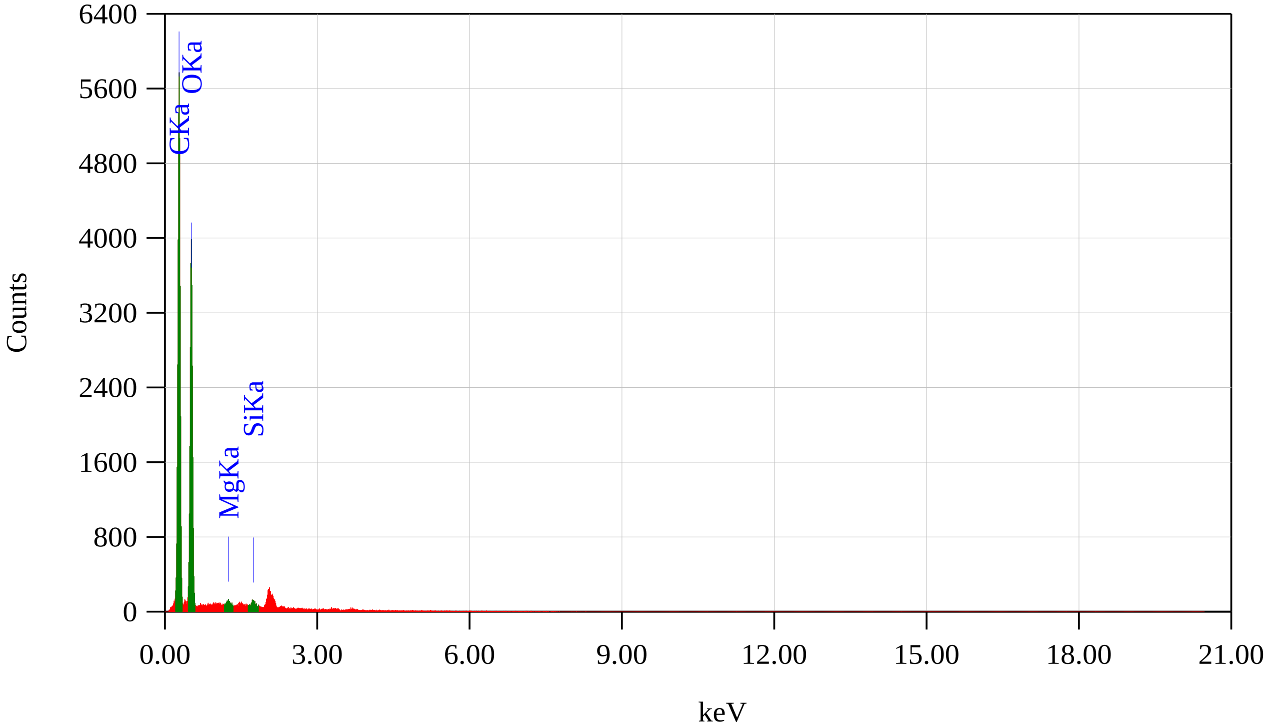


(b)

Figure 4: (a) Profile of fitted surface and (b) contour plot of subcritical water reactive extraction of gingerol to be shogaol from ginger dregs



(a)



(b)

Figure 5: (a) Morphology of ginger dregs after extraction and (b) components in ginger dregs after extraction

Table 3: Results of elemental characterization in ginger dregs after extraction

	Carbon (%)	Oxigen (%)	Mg (%)	Si (%)
Sample	55.61	43.80	0.22	0.37

Increasing the reactive extraction temperature up to 130°C with a ratio of 22L solvent for 30 minutes was able to increase the yield of shogaol content up to 74.92%, which as greater than at 110°C. This occurs because an increase in the reactive extraction temperature causes a decrease in the value of the dielectric constant of water [40] and an increase in the ionization constant (K_w).[35] This condition causes the weakening of the hydrogen bonds in water, and results in the auto-ionization of water into hydronium ions (H_3O^+) which functions as an acid catalyst in accelerating the dehydration of gingerols into shogaols. An increase in the yield of shogaol from hydrothermal extraction was also reported by [41] who studied the effect of extraction temperature (110°C-170°C), a ratio of ginger powder:water 1:10 at a pressure of 3.5 MPs for 60 minutes. The researcher stated that the higher the subcritical water extraction temperature, the higher the shogaol yield. However, the effect of increasing temperature on the increase of the shogaol yield was not shown in the extraction process with a large ratio of water:ginger pulp. Table 1 shows that when using a large ratio of water to ginger pulp, an increase in temperature resulted in a decrease in shogaol levels. It seems that the dehydration process of gingerol to become shogaol was hampered by the excess water causing some of the shogaol to turn into paradol. Cheigh et al. (2015) reported that hydroxyl group compounds, such as gingerol, were extracted at lower temperatures, resulting in a higher dielectric constant than in the water polarity of 6-shogaol. The subcritical water as a solvent causes some shogaol to turn into paradol. The use of RSM for process optimization aims to obtain equations that can describe the characteristics of the process being studied. Based on multiple regression analysis (Table 2), a second order polynomial equation was obtained to describe the relationship between the responses and the independent variables studied (table 3). The response was in the form of shogaol levels with the independent variables studied including temperature, time, and the ratio of solvent-ginger. The second order polynomial equation is presented in Equation 1.

$$Y = 18{,}3075 + 1{,}5483X_1 - 9{,}7493X_1^2 + 2{,}7430X_2 - 10{,}8728X_2^2 - 1{,}7090\ X_3 - 10{,}5083X_3^2 + 3{,}3778X_1X_2 - 3{,}9677X_1X_3 - 5{,}9638X_2X_3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(F.1)}$$

Where Y represents shogaol content (mg/L), while X1, X2, X3 consecutively represent temperature (°C), time (minutes), and ratio of solvent-inlet. To verify whether the regression model obtained can provide reliable predictions, a study was conducted on the acquisition of the coefficient of determination (R2). The gingerol subcritical water reactive extraction model became shogaol from ginger pulp with an acquisition R2 value of 85.54%. This value indicates that the model has an independent variable that describes the variability of the response variable. In general, the acquisition of a value greater than 85% indicates that the model has a fairly good correlation. The significance of three process variables can also be seen from the Pareto Diagram as presented in Figure 1. The Pareto Diagram presents the absolute value of the effect of each factor. The length of each bar on the Pareto Diagram indicates the effect of each factor on the response. The length of the bar chart that extends beyond the boundary line indicates the extent to which the factor influences the response. Figure 1 shows that the inlet solvent ratio (Q), time (Q), and temperature (Q) significantly influence the shogaol content resulting from the subcritical water reactive extraction process. The significance of the temperature variable for the shogaol extraction process was also reported by [28] who studied the optimization of the shogaol extraction process using ionic liquid solvents and utilizing the microwave heating mechanism. However, the researcher stated that temperature had the most dominant influence on the ILMAE process followed by concentration and irradiation time [28]. Response surface contour plots of the RSM on the effect of solvent volume, time, and temperature on the shogaol concentration are shown in Figures 2 to 4. The study results show that the optimum conditions for reactive extraction through UV-B light pretreatment were reached at a temperature of 121.46°C and an inlet-solvent ratio of 27.96 at 18.36, with shogaol concentration of 18.5671 mg/L. Figure 2 appears that expanding the temperature and extraction time can increment the concentration of shogaol. The longer the stage contact time between the scattered stage and the nonstop stage (subcritical water), the longer it is, causing the scattered stage to be dragged increasingly to the ceaseless stage. Hence, the longer the extraction time, the more noteworthy the lack of hydration change of gingerol to shogaol. Previous study [33] stated that, at temperatures over 150°C, the hydrogen bonds in water are weaker, which is able result in auto ionization of water into hydronium particles (H3O+) which can work as corrosive catalysts and hydroxide particles (OH^-) which can work as base catalysts. Be that as it may, after the extraction time surpassed 21 minutes at a temperature of over 121°C, the shogaol concentration started to diminish, conceivably changing over to paradol. Figure 3 shows that increasing the solvent and extraction time can increase the concentration of shogaol. This phenomenon occurs because the solvent increases with aqueous phase, so the dehydration of gingerol to shogaol goes faster. Subcritical water also acts as a catalyst due to the ionic products resulting from autoionization of water and because subcritical water can lower the activation energy [30]. However, after the solvent passed 28L with an extraction time of 19 minutes, the shogaol concentration tended to decrease to form paradol. Figure 4 shows that expanding the dissolvable and extraction temperature can increment the concentration of shogaol. This happens since subcritical water capacities as a dissolvable within the parchedness response of 6-gingerol to shogaol, considering that subcritical water encompasses higher solvency due to changes within the physical properties of water, such as the esteem of the dielectric steady of water diminishes as the temperature increases [40]. As a lack of hydration response medium, subcritical water will increment the chances of a response happening in a homogeneous stage, where on the off chance that the response is carried out beneath routine working conditions, and the response will take put in a heterogeneous phase. Be that as it may, after the extraction temperature surpassed 121°C with more than 28L of dissolvable, the shogaol abdicate diminished. This made it conceivable for shogaol to be thermally corrupted into paradol and to be dragged into the dissolvable stage. Paradol, which actually happens in ginger, is additionally delivered from gingerol change due to warming of gingerol [29]. Beneath acidic conditions and tall temperatures, gingerol, which turns into shogaol, can be further changed to paradol [1]. The morphology of the extracted ginger dregs was seen using SEM-EDX to analyze the image after UV treatment of the components in the ginger pulp. The morphology of ginger pulp can be seen in Figure 5.

4. Conclusions

At 120°C, solvent:inlet ratio 27:1 for 20 minutes through ultraviolet B-light pretreatment for 10 minutes, the yield of shogaol increased from 0.177 mg/L (without UV) to 18.646 mg/L. The regression model with a coefficient of determination of 85.54% was good enough to predict the reactive shogaol extraction of ginger pulp through subcritical water. The optimum condition for reactive extraction through UV-B light pretreatment was at a temperature of 121.46°C and an inlet solvent ratio of 27.96 at 18.36 minutes, with a shogaol concentration of 18.5671 mg/L.

References

[1] Q.-Q. Mao, X.-Y. Xu, S.-Y. Cao, R.-Y. Gan, H. Corke, T. Beta, H.-B. Li. (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). Foods. 8(6): 185.

[2] M.E. Yulianto, B. Jos, B. Budiyono. (2023). Kinetic Modelling of Liquid-Solid Extraction of Bioactive Compounds from Ginger Waste using Subcritical Water. Sainteknol: Science and Technology Journal. 21(1): 28-35. Available from: https://journal.unnes.ac.id/nju/index.php/sainteknol .

[3] S. Dugasani, M.R. Pichika, V.D. Nadarajah, M.K. Balijepalli, S. Tandra, J.N. Korlakunta. (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol,[8]-gingerol,[10]-gingerol and [6]-shogaol. Journal of ethnopharmacology. 127(2): 515-520.

[4] S. Kumar Gupta, A. Sharma. (2014). Medicinal properties of Zingiber officinale Roscoe-A review. Journal of Pharmaceutical and Biological Sciences. 9: 124-129. Available from: http://www.iosrjournals.org/iosr-jpbs/papers/Vol9-issue5/Version-5/S0955124129.pdf.

[5] J. Dhanik, N. Arya, V. Nand. (2017). A review on Zingiber officinale. Journal of Pharmacognosy and phytochemistry. 6(3): 174-184.

[6] A. Ghasemzadeh, H.Z. Jaafar, A. Rahmat. (2015). Optimization protocol for the extraction of 6-gingerol and 6-shogaol from Zingiber officinale var. rubrum Theilade and improving antioxidant and anticancer activity using response surface methodology. BMC complementary and alternative medicine. 15: 1-10.

[7] E. Farombi, I. Adedara, B. Ajayi, T. Idowu, O. Eriomala, F. Akinbote. (2018). 6-Gingerol improves testicular function in mice model of chronic ulcerative colitis. Human & Experimental Toxicology. 37(4): 358-372.

[8] S.R. Gundala, R. Mukkavilli, C. Yang, P. Yadav, V. Tandon, S. Vangala, S. Prakash, R. Aneja. (2014). Enterohepatic recirculation of bioactive ginger phytochemicals is associated with enhanced tumor growth-inhibitory activity of ginger extract. Carcinogenesis. 35(6): 1320-1329. Available from: http://carcin.oxfordjournals.org/http://carcin.oxfordjournals.org/

[9] Pan MH, Hsieh MC, Hsu PC, Ho SY, Lai CS, Wu H, et al. 6-Shogaol suppressed lipopolysaccharide-induced up-expression of iNOS and COX-2 in murine macrophages. Mol Nutr Food Res. 2008 Dec;52(12):1467–77.

[10] I. Bischoff-Kont, R. Fürst. (2021). Benefits of ginger and its constituent 6-shogaol in inhibiting inflammatory processes. Pharmaceuticals. 14(6): 571.

[11] H.-C. Shih, C.-Y. Chern, P.-C. Kuo, Y.-C. Wu, Y.-Y. Chan, Y.-R. Liao, C.-M. Teng, T.-S. Wu. (2014). Synthesis of analogues of gingerol and shogaol, the active pungent principles from the rhizomes of Zingiber officinale and evaluation of their anti-platelet aggregation effects. International journal of molecular sciences. 15(3): 3926-3951.

[12] L.R. Pancho, I. Kimura, U. Ryoichi, M. Kurono, M. Kimura. (1989). Reversed Effects between Crude and Processed Ginger Extracts on PGF2a-lnduced Contraction in Mouse Mesenteric Veins. Japanese journal of pharmacology. 50(2): 243-246.

[13] A.A. Aloliqi. (2022). Therapeutic potential of 6-gingerol in prevention of colon cancer induced by azoxymethane through the modulation of antioxidant potential and inflammation. Current Issues in Molecular Biology. 44(12): 6218-6228.

[14] J.S. Kim, S.I. Lee, H.W. Park, J.H. Yang, T.-Y. Shin, Y.-C. Kim, N.-I. Baek, S.-H. Kim, S.U. Choi, B.-M. Kwon. (2008). Cytotoxic components from the dried rhizomes of Zingiber officinale Roscoe. Archives of pharmacal research. 31: 415-418.

[15] J. Rhode, S. Fogoros, S. Zick, H. Wahl, K.A. Griffith, J. Huang, J.R. Liu. (2007). Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC complementary and alternative medicine. 7: 1-9.

[16] C.-H. Wu, B.-H. Hong, C.-T. Ho, G.-C. Yen. (2015). Targeting cancer stem cells in breast cancer: potential anticancer properties of 6-shogaol and pterostilbene. Journal of agricultural and food chemistry. 63(9): 2432-2441.

[17] Y.-L. Hsu, J.-Y. Hung, Y.-M. Tsai, E.-M. Tsai, M.-S. Huang, M.-F. Hou, P.-L. Kuo. (2015). 6-shogaol, an active constituent of dietary ginger, impairs cancer development and lung metastasis by inhibiting the secretion of CC-chemokine ligand 2 (CCL2) in tumor-associated dendritic cells. Journal of agricultural and food chemistry. 63(6): 1730-1738. Available from: http://pubs.acs.org.

[18] R.F. Warin, H. Chen, D.N. Soroka, Y. Zhu, S. Sang. (2014). Induction of lung cancer cell apoptosis through a p53 pathway by [6]-shogaol and its cysteine-conjugated metabolite M2. Journal of agricultural and food chemistry. 62(6): 1352-1362.

[19] M. Yulianto, V. Paramita, I. Hartati, R. Amalia. (2018). Response surface methodology of pressurized liquid water extraction of curcumin from curcuma domestica val. Rasāyan Journal of Chemistry. 11: 1564-1571.

[20] M.C. Mesomo, M.L. Corazza, P.M. Ndiaye, O.R. Dalla Santa, L. Cardozo, A. de Paula Scheer. (2013). Supercritical CO2 extracts and essential oil of ginger (Zingiber officinale R.): Chemical composition and antibacterial activity. The Journal of Supercritical Fluids. 80: 44-49.

[21] D. Handayani, D. Ikhsan, M.E. Yulianto, M.A. Dwisukma. (2015). Mass transfer coefficient in ginger oil extraction by microwave hydrotropic solution. AIP Conference Proceedings. AIP Publishing.

[22] J.-b. Guo, W.-j. Zhang, H. Wu, L.-m. Du. (2015). Microwave-assisted decomposition coupled with acidic food condiment as an efficient technology for ginger (Zingiber officinale Roscoe) processing. Separation and Purification Technology. 146: 219-226.

[23] D. Handayani, R. Amalia, M.E. Yulianto, M. Murni. (2018). In Nanoemulsion production of ginger oil from enzymatic extraction of isolated cow rumen enzyme. AIP Conference Proceedings. AIP Publishing.

[24] M.D. Supardan, A. Fuadi, P.N. Alam, N. Arpi. (2012). Solvent extraction of ginger oleoresin using ultrasound. Makara Journal of Science. 15(2): 26. Available from: https://scholarhub.ui.ac.id/scienceAvailableat:https://scholarhub.ui.ac.id/science/vol15/iss2/10.

[25] X. Kou, X. Li, M.R.T. Rahman, M. Yan, H. Huang, H. Wang, Y. Su. (2017). Efficient dehydration of 6-gingerol to 6-shogaol catalyzed by an acidic ionic liquid under ultrasound irradiation. Food chemistry. 215: 193-199.

[26] J.-B. Guo, Y. Fan, W.-J. Zhang, H. Wu, L.-M. Du, Y.-X. Chang. (2017). Extraction of gingerols and shogaols from ginger (Zingiber officinale Roscoe) through microwave technique using ionic liquids. Journal of food composition and analysis. 62: 35-42.

[27] C.C. Teo, S.N. Tan, J.W.H. Yong, C.S. Hew, E.S. Ong. (2010). Pressurized hot water extraction (PHWE). Journal of Chromatography A. 1217(16): 2484-2494.

[28] A.C. de Aguiar, J.F. Osorio-Tobón, L.P.S. Silva, G.F. Barbero, J. Martinez. (2018). Economic analysis of oleoresin production from malagueta peppers (Capsicum frutescens) by supercritical fluid extraction. The Journal of Supercritical Fluids. 133: 86-93.

[29] M. Yulianto, R. Amalia, V. Paramita, Q. Nisa. (2020). Preliminary study of auto catalytic palm oil hydrolysis into fatty acid through hydrothermalysis process, Journal of Physics: Conference Series.

[30] L. Zhao, H. Liao, M.-D. Tsai. (2004). The catalytic role of aspartate in a short strong hydrogen bond of the Asp274–His32 catalytic dyad in phosphatidylinositol-specific phospholipase C can be substituted by a chloride ion. Journal of Biological Chemistry. 279(31): 31995-32000.

[31] A.A. Peterson, F. Vogel, R.P. Lachance, M. Fröling, M.J. Antal Jr, J.W. Tester. (2008). Thermochemical biofuel production in hydrothermal media: a review of sub-and supercritical water technologies. Energy & Environmental Science. 1(1): 32-65.

[32] D. Lachos-Perez, F. Martinez-Jimenez, C. Rezende, G. Tompsett, M. Timko, T. Forster-Carneiro. (2016). Subcritical water hydrolysis of sugarcane bagasse: An approach on solid residues characterization. The Journal of Supercritical Fluids. 108: 69-78.

[33] M.-J. Ko, H.-H. Nam, M.-S. Chung. (2019). Conversion of 6-gingerol to 6-shogaol in ginger (Zingiber officinale) pulp and peel during subcritical water extraction. Food chemistry. 270: 149-155.

[34] D. Karentz. (1991). Ecological considerations of Antarctic ozone depletion. Antarctic Science. 3(1): 3-11.

[35] Z. Kuluncsics, D. Perdiz, E. Brulay, B. Muel, E. Sage. (1999). Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct or indirect mechanisms and possible artefacts. Journal of Photochemistry and Photobiology B: Biology. 49(1): 71-80.

[36] R.P. Rastogi, A. Kumar, M.B. Tyagi, R.P. Sinha. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. Journal of nucleic acids.

[37] T. Sydney, J.-A. Marshall-Thompson, R.V. Kapoore, S. Vaidyanathan, J. Pandhal, J.P.A. Fairclough. (2018). The effect of high-intensity ultraviolet light to elicit microalgal cell lysis and enhance lipid extraction. Metabolites. 8(4): 65.

[38] C.M. Sabliov, C. Fronczek, C. Astete, M. Khachaturyan, L. Khachatryan, C. Leonardi. (2009). Effects of temperature and UV light on degradation of α-tocopherol in free and dissolved form. Journal of the American Oil Chemists' Society. 86: 895-902.
[39] I. Volf, I. Ignat, M. Neamtu, V.I. Popa. (2014). Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. Chemical papers. 68: 121-129.
[40] M.H. Eikani, F. Golmohammad, M. Mirza, S. Rowshanzamir. (2007). Extraction of volatile oil from cumin (Cuminum cyminum L.) with superheated water. Journal of food process engineering. 30(2): 255-266.
[41] N.I. Anisa, N. Azian, M. Sharizan, Y. Iwai. (2014). Temperature effects on diffusion coefficient for 6-gingerol and 6-shogaol in subcritical water extraction, Journal of Physics.