Bioadsorption of Modified Empty Fruit Bunch Palm Oil for Reducing its 3-MCPD Compounds using Response Surface Methodology

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Abstract. Crude palm oil, consumed as a healthy food oil, contains a 3-monochloro-propane-1,2-diol (3-MCPD) ester in the range of 0.04-0.05 ppm. The 3-MCPD compound is one of the contaminants belonging to the chloropropanol group that is genotoxin carcinogen. It is therefore necessary to develop an integrated palm oil refining through adsorption with a modified palm empty fruit bunch bioadsorbent to reduce 3-MCPD ester (<0.02 ppm / Codex Standard). Response Surface Method applied in the optimization study of the modified empty fruit bunch of oil palm. The research was designed by using Central Composite Design. The parameter process studied were temperature (60-80^{0}C), time (20-40 minutes) and oil volume (400-600 ml). Response surface of the pressurized liquid water extraction of curcumin was expressed by a second-order polynomial. The research showed that temperature was the most influencing variable for the adsorption of 3-MCPD from modified empty fruit bunch of oil palm. The response surface contour plots of the RSM on the effect of temperature, time and oil volume have showed that the optimum condition for the adsorption of 3-MCPD from modified empty fruit bunch of oil palm were adsorption performed at temperature of 86.8^{0}C, 46.81 minutes and oil volume of 668.17 ml.

Keywords. RSM, Bioadsorbtion, 3-MCPD, Refining, Palm oil

1 Introduction

Crude palm oil is one of the fastest growing commodities in the last two decades. Despite the rapid growth of palm oil, the competitive advantages of crude palm oil (CPO) in international markets are still weak. Indonesia's CPO is only superior to the competitiveness of the on-farm (comparative advantages) level, but its competitive advantage or real competitiveness is very low [1]. Another potential concern for palm oil

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product development is the high level of consumer awareness of current health issues. Franke et al. [2] analyzing palm oil, found the 3-monochloro-propane-1,2-diol (3-MCPD) ester compound in the range of 0.04-0.05 ppm. The 3-MCPD ester compound is one of the contaminants belonging to the chloropropanol group which is genotoxin carcinogen. The chloropropanol group is a carcinogenic compound that can cause tumors and cancer in animals and humans [3]. The Scientific Committee on Food-Europe Commission in 2001 has established a maximum tolerance limit of 3-MCPD ester content in food products is 0.02 mg / kg or 0.02 ppm [4].

The 3-MCPD ester compound is believed to be a compound formed from the reaction between chloride ions and free glycerol present in the food product as a result of fat hydrolysis. Thus, theoretically, the reduction of 3-MCPD ester in palm oil product can be done in two ways, ie by avoiding the occurrence of a meeting between the precursor forming 3-MCPD ester (glycerol and chloride ion), or by removing 3-MCPD esters already formed by physical means, chemically as well as through microbiological means.

The negative impact of 3-MCPD has been widely investigated and the result proves that 3-MCPD is genetoxic carsinogen by testing in rats both in vivo and in vitro in animal cells. Cho et al., [5] conducted an in vivo assay study in mice for 2 years by administering 3-MCPD to a dose of 400 ppm / rat weight which results indicate that 3-MCPD is carcinogenic. The 3-MCPD compound is shown to stimulate the formation of cancer / tumors seen significantly after 72 weeks. A rapid 3-MCPD test has also been developed, using single cell / comet assay method in vitro or in vivo with observations based on cell DNA damage 24 hours after treatment [6]. The application of this method to test the impact of 3-MCPD and its derivative metabolites has been done by El Ramy et al., [7] whose results show that 3-MCPD and its metabolites cause DNA damage by in vivo and in vitro tests for 24 hours.

To date, the 3-MCPD ester content specification in palm oil is still a polemic, but some EU countries already use it as a barrier of palm oil trade in the European region. Therefore, it is necessary to develop purification techniques to reduce the 3-MCPD ester compound in palm oil. Refining palm oil through the adsorption of fixed beds with bioadsorbent oil palm empty bunches is potential to be developed, as it is capable of removing the chloride ion precursor and the 3-MCPD ester compounds.

Many researches have used Response surface methodology (RSM) in optimization of various process including in the optimization of acid hydrolysis of grasses [8], rice husk lignin extraction [9], fiber reinforcement [10], and biodiesel production [11]. RSM is an empirical statistical technique employed for multiple regressions analysis. It used multivariable-quantitative data to solve multivariable equation simultaneously. In this research, Response Surface Method has been applied in the optimization study of the bioadsorption of modified empty fruit bunch of oil palm for reducing its 3-MCPD compounds.

2 Methodology

2.1 Materials

The raw materials used to reduce 3-MCPD compound by the bioadsorption process were empty fruit bunch of palm oil, fresh fruit bunch of palm oil, and frying oil.

Chemical reagents used were standard 3-MCPD, sodium methoxide, hexane/isohexane, t-butiyl methyl ether, ethyl acetate, acetic acid, sodium chloride, distilled water, etanol, sodium hydroxide, aceton, tetradecyltrimethyl ammomium bromide, chloric acid dan

whatman filter paper, were purchased from Merc, an authorized chemicals distributor in Semarang-Indonesia.

2.2 Procedure

The main equipment used in the research were a batch adsorption with stirred tank and continuous adsorption towers, as shown in Figure 1.

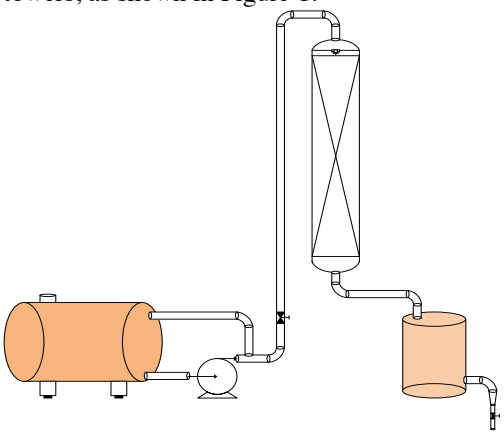


Fig. 1. Fixed bed adsorber

Supporting equipment include: oven, vacuum drying, pH meter, magnetic stirrer, isothermal shaker, AAS, Gas Chromatography-Mass Spectrometry (GC-MS) and other glassware.

2.2.1. Experimental Design using RSM Statistica 8

The experimental design was carried out to obtain useful data in determining the most influential process variables and the optimum process conditions that were modeled in the empirical equation. The experimental design using RSM statistic 8 presented in Table 1.

Table 1. Independent variables and their levels for central composite design in optimization of adsorption of 3-MCPD from modified empty fruit bunch of oil palm

Independent variables	**Coded variable levels**				
	A	**-1**	**0**	**+1**	**A**
Temperature (ºC)	53.18	60.00	70.00	80.00	86.82
Time (Minutes)	13.18	20	30	40	46.82
Oil Volume (ml)	331	400	500	600	668

Table 2. The experiments design of optimization of 3-MCPD adsorption

Temperature (°C)	Time (minute)	Oil Volume (ml)
60.00	20.00	400
60.00	20.00	600
60.00	40.00	400
60.00	40.00	600
80.00	20.00	400
80.00	20.00	600
80.00	40.00	400
80.00	40.00	600
53.18	30.00	500
86.82	30.00	500
70	13.18	500
70	46.82	500
70	30.00	331
70	30.00	668
70	30.00	500
70	30.00	500

2.2.2. Activation of zeolite and sodium aluminum silicate

Activation of zeolite and sodium aluminum silicate as adsorbents should be done before the adsorption process. Zeolite and sodium aluminum silicate are heated in the furnace at 400◦C for 4 hours. After the adsorbent has been activated, then it is used for the adsorption process.

2.2.3. Bioadsorption procedure

The modified bioadsorbent of empty fruit bunch of oil palm was weighed and put to the adsorption tower. Palm oil was heated at a certain temperature and flowed to the adsorption tower. While the heater was started, the adsorption time (t = 0) was calculated. Palm oil products with MCPD free and other polar compounds in oil (MAG, DAG) that come out of the top of the tower, measured every 10 minutes. Samples were measured for 3-MCPD. The measurement results were used to validate the adsorption kinetics model and adsorption isotherms.

2.2.4. 3-MCPD determination

The 3-MCPD ester identification method refers to DGF Standard Method C-III 18 (09) in 2009 using Gas Chromatography Mass Spectrometry (GC-MS) method [12].

2.2.5. Data interpretation

Data interpretation was carried out at Laboratory of Computation Process at Chemical Engineering UNDIP Semarang for 1 month. Data interpretation was used to obtain data which useful in determining the most influential process variables and the optimum process conditions that modeled in the empirical equation.

3 Results and Discussion

Many researches have used Response surface methodology (RSM) in optimization of various process including in the optimization of acid hydrolysis of grasses [8], rice husk lignin extraction [9], curcumin extraction [13], fiber reinforcement [10], biodiesel production [11], and optimizing waste heat recovery from the cement plant [14]. RSM is an empirical statistical technique employed for multiple regressions analysis. It used multivariable-quantitative data to solve multivariable equation simultaneously. In the design of experiments, the experimenter is often interested in the effect of some process or intervention (the "treatment") on some objects (the "experimental units"), which may be people, parts of people, groups of people, plants, animals, materials, etc [15].

In this research, a central composite design was employed the response, namely the acid number. The independent variables of adsorption of 3-MCPD from modified empty fruit bunch of oil palm were X_1, X_2, and X_3 temperature (°C), extraction time (minutes), and oil volume (ml), respectively. The data obtained by carrying out the experiment according to central composite design were analyzed by Statistica 8. The data obtained was tabulated on Table 3.

Table 3. The tabulated data of the adsorption of 3-MCPD from modified empty fruit bunch of oil palm

Temperature (°C)	Time (Minutes)	Oil Volume (ml)	Acid Number
60.00	20.00	400	0,495364238
60.00	20.00	600	0,242178771
60.00	40.00	400	0,380195783
60.00	40.00	600	0,345954613
80.00	20.00	400	0,351503759
80.00	20.00	600	0,423929471
80.00	40.00	400	0,423929471
80.00	40.00	600	0,41991018
53.18	30.00	500	0,415966387
86.82	30.00	500	0,414124016
70	13.18	500	0,15214497
70	46.82	500	0,220355823
70	30.00	331	0,3884273
70	30.00	668	0,30579782
70	30.00	500	0,354166667
70	30.00	500	0,151175894

The reducing of free fatty acids in cooking oil was done by activated carbon of palm shell. Optimization of the adsorption process was carried out by Response Surface Methodology. The parameters of the adsorption process were temperature, time and volume of oil. The analysis of the optimization study designed using Central Composite Design showed that the correlation between the acid number and adsorption process parameter (equation 1) is:

$$Y = 0.41870 - 0.00061X_1 - 0.009168(X_1)^2 + 0.04734X_2 - 0.02827(X_2)^2 + 0.04063X_3 - 0.05783(X_3)^2 + 0.06600\,X_1X_2 - 0.06759\,X_1X_3 + 0.04983X_2X_3 \quad (1)$$

Where Y is an acid number, X_1 is temperature, X_2 is time, and X_3 is the volume of oil.

Table 4. The value of effect estimates of the adsorption process parameter

Factor	Effect Estimates; Var: Acid Number; R-sqr=0,36795; Adj:0, (Spreadsheet6) 3 factors, 1 Blocks, 16 Runs; MS Residual=,0164413 DV: Acid Number									
	Effect	Std. Err.	t(6)	P	-95% Cnf. Limt	+95% Cnf. Limt	Coeff.	Std. Err. Coeff.	-95% Cnf. Limt	+95% Cnf. Limt
Mean/Interc.	0.4187	0.0904	4.6315	0.00357	0.19749	0..6399	0.41870	0.09040	0.19749	0.6399
(1)Temperature(L)	-0.00061	0,06939	-0,0088	0,99323	-0,17041	0,16918	-0,00030	0,03469	-0,08520	0,08459
Temperature(Q)	-0.09168	0,08425	-1,0882	0,31826	-0,29785	0,11447	-0,04584	0,04212	-0,14892	0,05723
(2) Time (L)	0.04734	0,06939	0,6822	0,52054	-0,12245	0,21714	0,02367	0,03469	-0,06122	0,10857
Time (Q)	-0.02827	0,08425	-0,3356	0,74856	-0,23444	0,17788	-0,01414	0,04212	-0,11722	0,08894
(3) Oil Volume (L)	0.04068	0,06939	0,5863	0,57903	-0,12911	0,21048	0,02034	0,03469	-0,06455	0,10524
Oil Volume (Q)	-0.05783	0,08425	0,5863	0,51806	-0,26400	0,14832	-0,02891	0,04212	-0,13200	0,07416
1L by 2L	0.06600	0,09066	0,728	0,49403	-0,15585	0,28786	0,03300	0,04533	-0,07792	0,14393
1L by 3L	-0,06759	0,09066	-0,7455	0,48411	-0,28945	0,15426	-0,03379	0,04533	-0,14472	0,07713
2L by 3L	0.04983	0,09066	0,5496	0,60240	-0,17202	0,27169	0,02491	0,04533	-0,08601	0,13584

Table 5. Anova Analysis

Factor	ANOVA; Var.:Acid Number; R-sq r=0.36795; Adj:0, (Spreadsheet) 3 factors, 1 Blocks, 16 Runs M S Residual=0.0164413 DV:Acid Number				
	SS	df	MS	F	p
(1)Temperature(L)	0.00000	1	0.00000	0.00007	0.99323
Temperature(Q)	0.01947	1	0.01947	1.18424	0.31826
(2) Time (L)	0.00765	1	0.00765	0.46543	0.52054
Time (Q)	0.00185	1	0.00185	0.11265	0.74856
(3) Oil Volume (L)	0.00565	1	0.00565	0.34377	0.57903
Oil Volume (Q)	0.00774	1	0.00774	0.47120	0.51806
1L by 2L	0.00871	1	0.00871	0.52998	0.49403
1L by 3L	0.00913	1	0.00913	0.55582	0.48411
2L by 3L	0.00496	1	0.00496	0.30211	0.60240
Error	0.09864	6	0.01644		
Total SS	0.15607	15			

The regression coefficient obtained (R^2) is 0.36 (Table 4). It can be interpreted that only 36% of the total variation of the model can be represented by the regression equation. Tables 4 and 5 also show that the p value of the parameters of the adsorption process is more than 0.05. This information of the process parameters was not significant to the adsorption process.

Furthermore, the optimization process using the Response Surface Methodology also produces the most influential process parameter data. Pareto graph (Figure 2) shows that temperature was the most influencing parameter to the adsorption process.

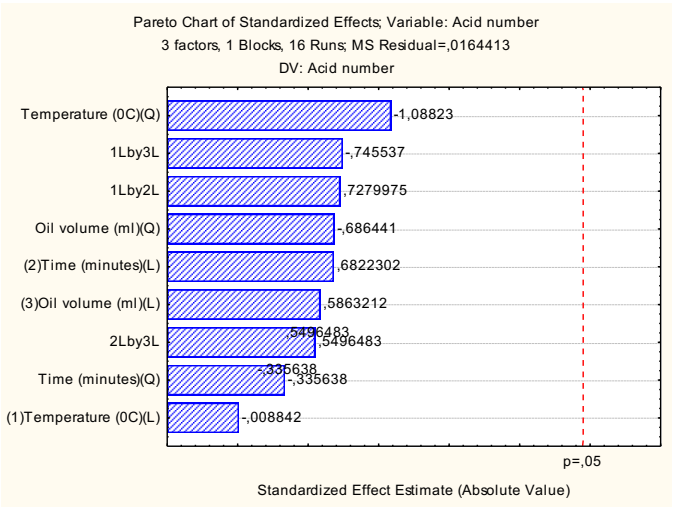
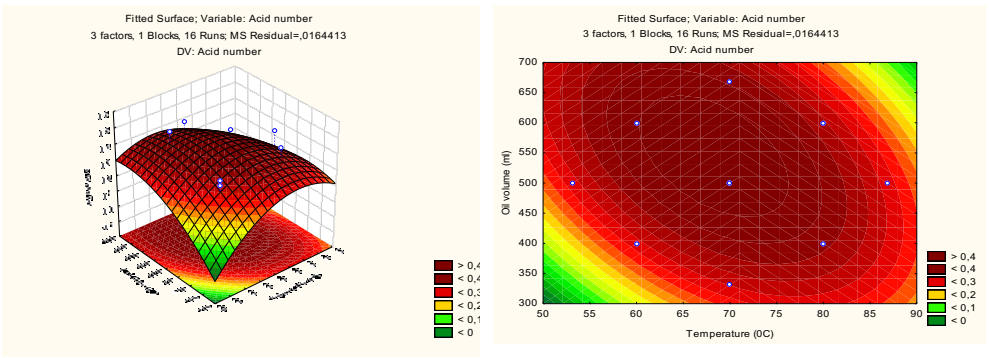


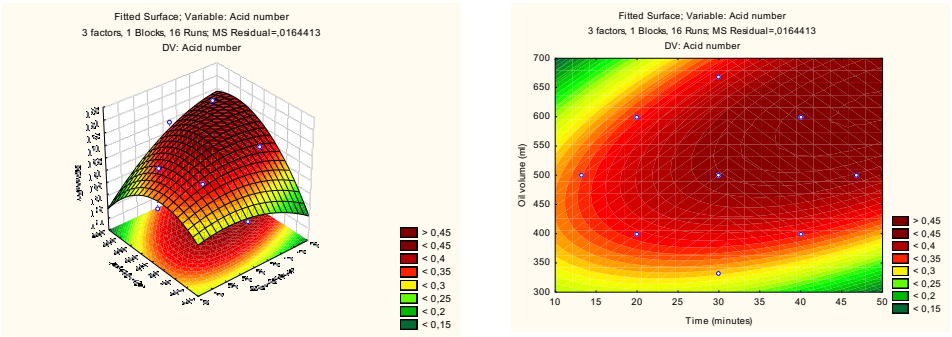
Fig. 2. Pareto chart

Figures 3, 4 and 5 illustrate the surface contour and plot contour of the parameters on acid numbers. Figures 3, 4 and 5 show the optimum adsorption process at temperature of 86.8°C, 46.81 minutes and oil volume of 668.17 ml.



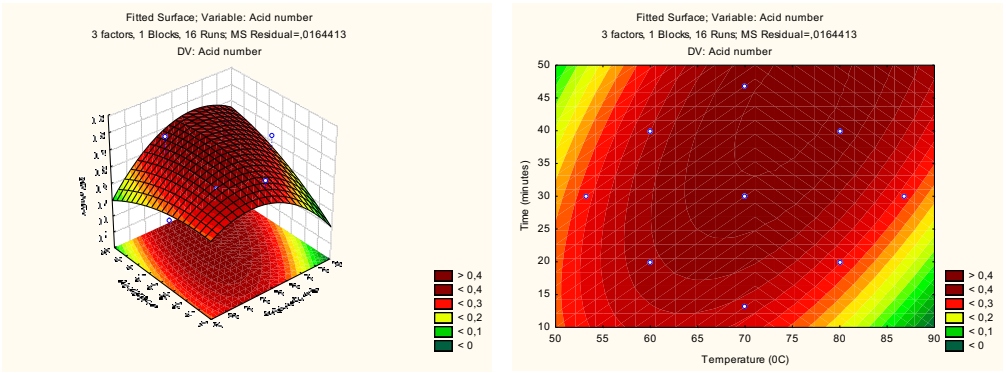
(a) (b)

Fig. 3. Plot contour (a) dan surface contour (b) profiles of temperature and oil volume



(a) (b)

Fig. 4. Plot contour (a) dan surface contour (b) profiles of time and oil volume



(a) (b) (b)

Fig. 5. Plot contour (a) dan surface contour (b) profiles of time and temperature

The reaction of the formation of 3-MCPD and 3-MCPD esters from fat / oil can be seen in Figure 6. From Figure 6 can be seen that 3-MCPD or 3-MCPD esters can only be formed if triglycerides have undergone hydrolysis and lose at least one chain of fatty acids. The concentration of 3-MCPD ester will increase if hydrolysis of triglycerides produces free fatty acids.

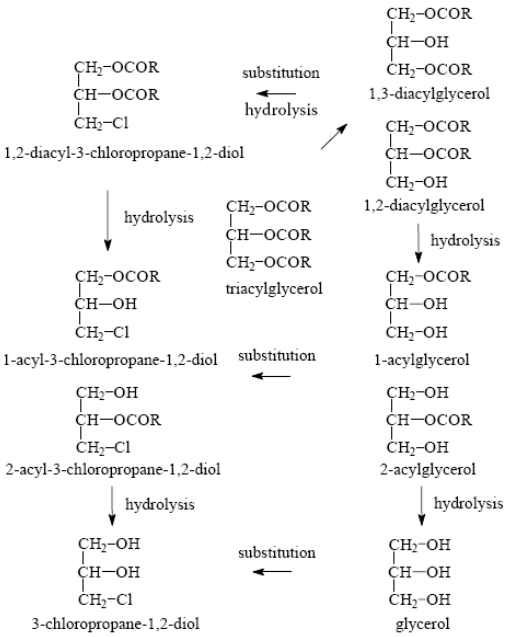


Fig. 6. Scheme of formation of 3-MCPD and 3-MCPD esters from fat / oil [16]

Mono-acyl glycerol is easier to form 3-MCPD than tri-acylglycerol or di-acylglycerol [16]. The more free fatty acids can affect the more likely 3-MCPD formation. This is because the more the free fatty acids mean the more the mono-acylglycerol and di-acylglycerol.

The formation of 3-MCPD ester compounds is influenced by the content of mono-acyl glycerol, di-acyl glycerol and tri-acyl glycerol and free fatty acids. This happens because the substitution reaction of glycerol and HCl. 3-MCPD esters is believed as a compounds formed from the reaction between chloride ions and free hydroxyl groups on glycerol which found on food products as a product of fat hydrolysis.

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