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Optimization of palm oil refining process for reduction of 3-monochloropropane-1,2-diol ester to obtain low MCPD palm oil

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■ **ABSTRACT :** The reduction in level of 3-monochloropropane-1, 2-diol esters in RBD palm oil is accomplished by optimizing the processing steps in the refining process. The main purpose of optimization of palm oil refining process is to get low MCPD palm oil with acceptable quality of refined palm oil. The refining process for palm oil is optimized by modifying four processing parameters: types of bleaching earths, bleaching earth dosage, bleaching temperature and deodorization temperature. For degumming step, temperature of 85°C and 0.02 per cent of phosphoric acid was carried out for 1 hour. Two different types of bleaching earths namely neutral bleaching earth and acidic bleaching earth at the dosage of 1 per cent, 2 per cent and 3 per cent were used. The bleaching of degummed oil is performed at different temperatures: 85°C, 90°C, 100°C, and 110°C for 1 hour. Deodorization of bleached oil was performed at 200°C, 220°C and 250°C for 1 hour. 3-MCPD levels were analyzed by GC/MS. 3-MCPD was not detected in the crude palm oil. Bleaching at 110°C temperature with 1 per cent acidic bleaching earth and deodorized at 250°C temperature reported to have highest levels (1.99 ppm) of 3-MCPD esters in RBD palm oil. The 3-MCPD esters were at lower levels (1.33 ppm) when the degummed oil is bleached at 90°C for 1 hour with 3 per cent neutral bleaching earth and deodorized at 250°C for 1 hour. Although study indicates that this optimized refining process also affect the physico-chemical properties of palm oil.

■ **KEY WORDS :** 3-MCPD esters, Bleaching, Bleaching earth, Degumming, Deodorization, GC/MS, RBP palm oil

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In order to eliminate unwanted substances the crude edible oils should refined before consumption. Some components including free fatty acids, pigments, phospholipids, oxidation products and other impurities are removed during refining. Commonly the refining of palm oil can be carried out in two ways physically and chemically. Chemical refining of palm oil is also known as alkali refining. In the current research we only focused on physical refining of palm oil because the process is time consuming as introduction of one more step in processing also which will be expensive. Gibon *et al.* (2007) conducted a research on palm oil refining and from the findings they states that about 30 per cent oil loss is expected when alkali refining is used (Muhammad

et al., 2011). Mostly the soft oils such as soybean oil, sunflower oil, peanut oil, cotton seed oil etc. are refined by alkali refining process. Rather than chemical refining process, physical refining process is inexpensive, as well as environment friendly. Oil losses are lower as compared to chemical refining due to requirement of minimal chemicals (Cmolik and Pokorný, 2000). Physical refining of palm oil includes three main processing steps, namely, degumming, bleaching, and deodorization (Musfirah *et al.*, 2013). Gums present in oil will interfere stability of final product; removal of such unwanted gums is the main objective of degumming. Degumming is the initial step of the refining process which decreases the amount of phospholipids (Matthäus, 2012). By treating crude palm oil with phosphoric acid the objective of degumming is accomplished. This degummed oil is treated with bleaching earth and heated with constant stirring, during bleaching process. This degummed and bleached oil is then passed through a deodorizer where bleached oil is heated at high temperature (above 200°C) under the vacuum, this process is called as deodorization which is the final processing step of refining of palm oil.

The recent studies show that, refining processes will leads to the formation of 3-monochloropropane-1,2-diol esters (Pudel *et al.*, 2011 and Hrncirik and Van Duijin, 2011). Worldwide, contamination of refined oils with 3-monochloropropane-1,2-diol esters has becoming new safety issue (Musfirah *et al.*, 2013). Gardner *et al.* in 1983 were the first who published about presence of the esters in refined oils (Nuzul *et al.*, 2011). 3-MCPD belongs to chloropropanol compounds which comprised of 5 substances: 2-monochloropropane-1,3-diol, 2,3-dichloropropan1-ol, 1,3-dichloropropan-2-ol, 3-monochloropropan-1-ol and 3-chloropropane -1,2-diol (Lee and Khor, 2015). Chloropropanols area unit a well known cluster of contaminants found in many processed foods and food ingredients. These compounds area unit shaped through chemical reactions between lipids and chloride ions once subjected to high temperatures and may be found each in free and bound morpheme, the latter being known as chloropropanol esters. Though free chloropropanols were known in hydrolyzed vegetable macromolecule just about thirty years past (Davidek *et al.*, 1982), the incidence of sure forms, particularly 3-monochloropropane-1,2-diol (3-MCPD) esters, was rumored solely recently and at concentrations a lot of beyond those of their free forms (Svejkovská *et al.*, 2004). Chloride, levels of acylglycerols, pH, temperature and time influences the formation of 3-MCPD esters (Kuntom *et al.*, 2013). Toxicological studies on 3-MCPD show that, 3-MCPD is carcinogenic, nephrotoxic in nature and will cause tumors in various organs when consumed. Therefore, the main objective of this research work is to eliminate the 3-MCPD esters from refined palm oil by modifying the refining process. Preliminary studies concerning intake assessment, considering the amount of 3-MCPD esters found in foods and forward that 100 per cent of those esters area unit hydrolyzed throughout the digestion, showed that the exposure to free 3-MCPD might exceed the provisionary most tolerable daily intake (PMTDI) of two ug/kg weight (bw) presently established for this compound, suggesting a possible health risk (Abraham *et al.*, 2013). In this study, the crude palm oil is refined by physical method to avoid chemicals. And in the physical refining process, degumming; bleaching and deodorization steps was modified and therefore, optimized for reduction of 3-MCPD esters.

METHODOLOGY

Materials :

Crude palm oil was supplied by local oil mill located in Ahmedabad, Gujarat. The quality parameters of crude palm oil were analyzed and these were found to be: Deterioration of bleachability index (DOBI), 2.2 ± 0.25, free fatty acids (FFA), 4.32 ± 0.28 %, peroxide value (PV), 3.1 ± 0.21 meq/lit, phosphorus content, 9 ± 0.23 ppm. 3-MCPD esters were not detected in crude palm oil. Neutral bleaching earth and acidic bleaching earth were purchased from local market. Phosphoric acid was also purchased from local market. All standards required for analysis of 3-MCPD were purchased from Sigma Aldrich. All other chemicals and solvents were of analytical grade.

Refining process :

Refining of palm oil was carried out at laboratory scale bleacher and deodorizer having a capacity of 1.5 kg per batch. Before CPO transferred into degumming process, CPO was melted at 50°C. In the degumming process, CPO was heated to 85°C with continuous stirring. In this study, acid degumming was carried out in which a concentration of 0.02 per cent phosphoric acid (85%) was added to CPO and heated to 85°C with

continuous stirring for an hour. The whole degumming process was carried out under the vacuum of 760 mm Hg. The degummed oil was then transferred into the bleacher and heated to different temperatures of 85°C, 90°C, 100°C and 110°C for 1 hour. The neutral bleaching earth and acidic bleaching earth were added at the concentrations of 1 per cent, 2 per cent and 3 per cent, respectively. Bleaching process was also carried out under vacuum of 760 mm Hg. The degumming and bleaching assembly is shown in Fig. A. The bleached oil samples were then filtered to remove residues of bleaching clays and other impurities. Then each sample of bleached oil were deodorized under vacuum (760 mm Hg) at series of different temperatures 200^0C, 220^0C and 250^0C for 1 hour with injection of steam into deodorizer. Then the obtained product is known as refined, bleached and deodorized palm oil (RBD PO). The samples of RBDPO were collected for analysis. The schematic diagram of deodorizer is shown in Fig. B.

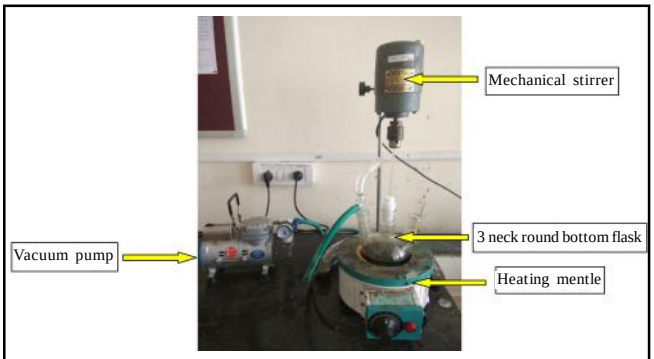

Fig. A : Degumming and bleaching assembly

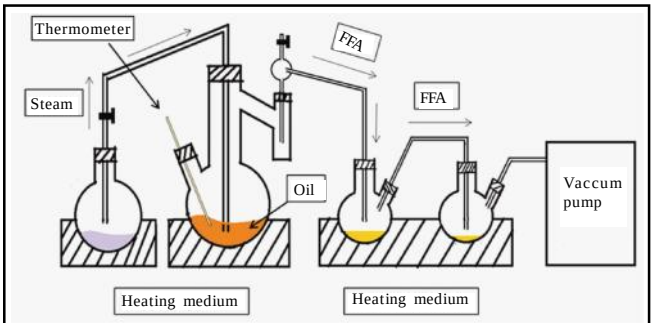

Fig. B : Deodorizer

MCPD esters analysis :

The 3-MCPD esters present in RBD palm oil were determined using AOCS official method by GC/MS. This method is based on alkaline-catalyzed ester cleavage, transformation of the released glycidol into monobromopropanediol (MBPD) and derived free diols (MCPD and MBPD) with phenylboronic acid (AOCS, 2013) Cd 29b-13. The quantitative determination of derived 3-MCPD esters was carried out in GC/MS. Weigh 100 mg completely melted sample of refined palm oil into a 2 ml screwcap vials and dissolve 100 µl of 3-MCPD-d5 diester and 2-MCPD-d5 diester standard working solutions. Completely dissolves the solution by adding 600 µl diethyl ether with shaking. After cool down to -22°C to -25°C, add 350 µl methanolic sodium hydroxide to the solution. Keep the solution at -22°C to -25°C for 16 hours to complete the ester cleavage. Addition of 600 µl acidified sodium bromide solution will stops the reactions. Leave the mixture at room temperature by adding 600 µl iso-hexane. This will leads to transformation of glycidol into MBPD. Add small amount of anhydrous sodium sulfate to the mixture to achieve derivatization. Dissolve the mixture in approximately 300 µl to 500 µl iso-octane and the determination of 3-MCPD was analyzed in GC/MS.

Analysis of oil quality :

Free fatty acids (*FFA*) :

Free fatty acids (FFA) content was determined by titrating palm oil sample with alkali (KOH) according to the AOCS official method Ca 5a-40 (AOCS, 2009a).

Deterioration of bleachability index (DOBI) :

Deterioration of bleachability index was determined according to PORIM P-2.9 test method (PORIM, 1995). The completely melted oil sample was weighted (0.1 g) in 25 ml volumetric flask and dissolved in *iso*-hexane or *iso*-octane. The mixture was measured in UV visible spectrophotometer at wavelengths of 269 nm and 446 nm. The DOBI value was calculated as the ratio of absorbance at 446-269 nm.

Peroxide value :

Peroxide value was measured in terms of meq/lit according to AOCS official method Cd 8-53 (AOCS, 2003). Weigh accurately 5 g completely melted oil sample into 250 ml conical flask and dissolve in 30 ml acetic acid-chloroform (3:2) solvent. Add 0.5 ml of saturated KI solution to it and allow it to stand for 1 min in dark

then add 30 ml distilled water to the solution and titrate solution with 0.1 N sodium thiosulfate until yellow colour almost gone. Then add 0.5 ml starch solution to it, the blue colour will observe. If blue colour does not appears, it was reported as absence of peroxide in sample. And if blue colour appears, titrate the solution with 0.1 N sodium thiosulfate until blue colour gone. The peroxide value can be calculated as product of titrate and normality of sodium thiosulfate per lit of solution.

Phosphorus content :

Analysis of phosphorus content in palm oil samples were carried out according to the AOCS official method Cd 8-53 (AOCS, 2009b). Add 0.5 g zinc oxide to accurately weighted oil sample (3 g) and heat slowly on hot plate until sample thickens. After heating the sample until desired thickness achieved, the sample was placed in muffle furnace at temperature of 550-600°C for 2 hours. After 2 hours the sample was cooled down to room temperature and added 5 ml distilled water and 5 ml concentrated HCl followed by heating. The solution was then filtered and neutralized by dropwise addition of 50 per cent KOH also few drops of HCl was added to precipitate zinc oxide. Taken absorbance of solution at 650 nm with distilled water as blank by adding 8 ml hydrazine sulfate solution and 2 ml sodium molybdate solution. The amount of phosphorus was measured by preparation of curve and comparing with standard curve.

■ RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Optimized refining process :

The existing process of palm oil refining was optimized to eliminate the 3-MCPD esters from the refined palm oil. Application of acid in degumming plays a crucial role in removing most of gums and impurities from Palm oil. It is essential to remove the unwanted gums from palm oil during degumming because the presence of gums can affects the flavour, colour and shelf-life of the palm oil. In degumming step, 0.02 per cent phosphoric acid was used because higher amount of acid can lead to formation of 3-MCPD esters. Neutral bleaching earth and acidic bleaching earth were employed in bleaching process to remove carotene or pigments and other impurities. In this study, degummed palm oil was treated with both bleaching earths were used at dosage of 1 per cent, 2 per cent and 3 per cent and heated at temperatures of 85°C, 90°C, 100°C and 110°C for an hour. Deodorization is the process where the 3-MCPD esters formation occurs. Therefore, some modifications in deodorization were done. The bleached oil was deodorized at temperatures of 200°C, 220°C and 250°C for period of 1 hour. Each sample of RBD PO were taken for quantitative analysis of 3-MCPD esters and concluded the best processing conditions for palm oil refining having low MCPD content. Crude palm oil sample after degumming, bleaching; and deodorization is shown in Fig. 1.

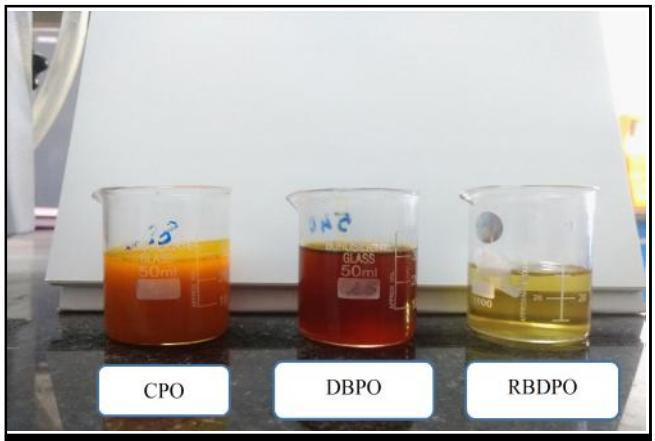

Fig. 1 : CPO after degumming, bleaching, and deodorization
CPO= Crude Palm Oil, DBPO= Degummed, Bleached Palm Oil, RBDPO= Refined Bleached and Deodorized Palm Oil

Effects of processing parameters on refined palm oil quality :

Free fatty acid is one of the important quality factors of refined palm oil. Standard specification requirement of FFA should between 0.05 per cent - 0.09 per cent. This study results the FFA in refined palm oil in the range of 0.08 per cent to 1.84 per cent. Deodorization temperature influences on the removal of FFA in refined palm oil. The main objective of this study is to remove 3-MCPD esters from palm oil. Scientific Committee on Food (SCF) has proposed the tolerable daily intake (TDI) for free 3-MCPD esters of 2μg kg^{-1} bw^{-1} day^{-1} (SCF, 1994). The CONTAM Panel established for 3-MCPDa tolerable daily intake (TDI) of 0.8 μg/kg bw per day and concluded that this TDI constitutes a group TDI for 3-

MCPD and its fatty acid esters (Jan, 2017). This study results the presence of 3-MCPD esters in refined palm oil in the range of 1.33 ppm to 1.99 ppm. All the results and observations are of two duplicates and substituted in a tabular form. Table 1 represents quality analysis of crude palm oil. Chloride and pH of water used for washing purpose has positive effect on formation of 3-MCPD esters. The formation of 3-MCPD esters could only be effected in the presence of chlorides in any food matrix (Ibrahim, 2015). Therefore, the analysis of water was also done (Table 2). Type of bleaching earth used in bleaching imparts negative effects on quality of refined palm oil hence, both bleaching earths were analyzed (Table 3). Table 4 and 5 represents the quality analysis of refined palm oil. The quality analysis of palm oil available in market is shown in Table 6.

Statistical analysis :

The data were analyzed statistically for analysis of variance and least significant difference tests using the software of minitab (MTB-Oneway). The data analysis by statistical analysis is given in Table 7. This P value represents there is a impact of bleaching temperature, bleaching earth concentration and type of bleaching earth on removal of 3-MCPD. But this P value also represents there is no impact of deodorization temperature on removal of 3-MCPD. Fig. 2, it show that the main effects of bleaching temperature, bleaching earth concentration and type of bleaching earth on the removal of 3-MCPD.

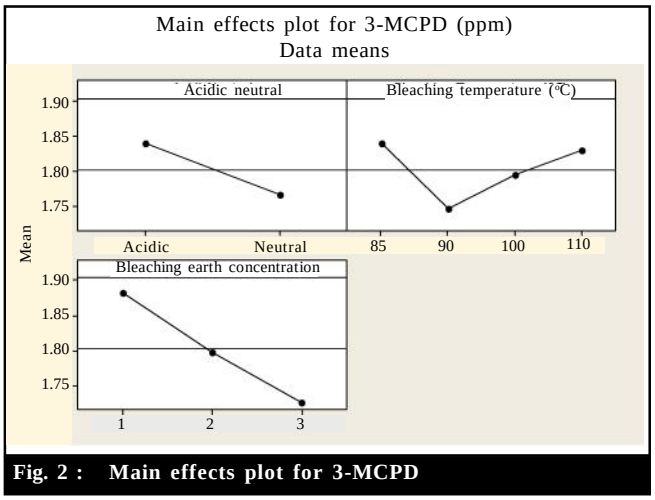

Fig. 2 : Main effects plot for 3-MCPD

Conclusion :

The formation of 3-MCPD esters is affected by higher temperatures. Changes in the bleaching

Table 1 : Quality analysis of crude palm oil

Parameters	CPO
FFA (%)	4.32±0.28
DOBI	2.2±0.25
PV (meq/lit)	3.1±0.21
Phosphorus (ppm)	9±0.23

Crude palm oil (CPO)

Table 2 : Analysis of water

Parameters	Water	
	Tap	Distilled
pH	6.64±0.25	7.05±0.06
Chloride (mg/lit)	425.4±3.03	135.98±3.83

Table 3 : Analysis of bleaching earths

Parameters	Bleaching earth	
	NBE	ABE
pH	6.5±0.21	4.3±0.10
Moisture (%)	8.2±0.15	10.8±0.25
Bulk density (g/ml)	0.67±0.05	0.61±0.03
WHC	1.092±0.05	0.909±0.07
OHC	1.021±0.03	0.836±0.05
Bleaching efficiency	79.4±2.08	71.9±2.65

*NBE = Neutral bleaching earth
*ABE = Acidic bleaching earth

temperature is found to be effective in removal of 3-MCPD esters as well as it affects the quality characteristics of refined palm oil. Bleaching earth concentration and type of bleaching earth used are also affects the removal of 3-MCPD (Fig. 2). Removal of 3-MCPD is more efficient if bleaching temperature was 90°C. There is no significant difference between 3-MCPD content in refined palm oil bleached with 2 per cent and 3 per cent of bleaching earths. So, it is concluded that use 2 per cent bleaching earth for bleaching purpose rather than 3 per cent bleaching earth it will be economical also. Use of neutral bleaching earth is found to be more effective than acidic bleaching earth. Neutral bleaching earth has higher water and oil holding capacity,

Table 4 : Quality analysis of refined palm oil

Bleaching temperature (°C)	Bleaching earth concentration (%)	Deodorization temperature (°C)	FFA (%)	PV (meq/kg)	PO_4 (ppm)	3-MCPD (ppm)
85	1	200	0.33±0.06	1.8±0.25	1.84±0.15	1.9±0.07
		220	0.13±0.05	1.7±0.19	1.9±0.21	1.92±0.05
		250	0.098±0.01	0.9±0.07	1.88±0.26	1.89±0.07
90	1	200	0.13±0.07	Nil	1.56±0.12	1.85±0.08
		220	0.19±0.05	0.6±0.04	1.28±0.08	1.88±0.05
		250	0.16±0.05	Nil	1.08±0.1	1.91±0.04
100	1	200	0.17±0.06	Nil	1.3±0.09	1.87±0.06
		220	0.12±0.01	Nil	1.21±0.09	1.9±0.07
		250	0.19±0.02	Nil	1.18±0.08	1.93±0.05
110	1	200	0.09±0.00	Nil	1.32±0.21	1.84±0.03
		220	0.087±0.00	Nil	1.28±0.16	1.93±0.05
		250	0.085±0.01	Nil	1.21±0.12	1.95±0.08
85	2	200	0.62±0.03	1.3±0.17	0.99±0.07	1.85±0.07
		220	0.4±0.04	1.2±0.15	0.89±0.05	1.87±0.05
		250	0.099±0.01	0.8±0.08	0.88±0.06	1.9±0.04
90	2	200	0.1±0.05	Nil	0.83±0.05	1.77±0.06
		220	0.085±0.01	Nil	0.67±0.07	1.78±0.04
		250	0.08±0.01	Nil	0.48±0.05	1.53±0.02
100	2	200	0.092±0.02	Nil	0.98±0.08	1.76±0.05
		220	0.087±0.01	Nil	0.91±0.05	1.79±0.02
		250	0.088±0.00	Nil	0.85±0.07	1.84±0.05
110	2	200	0.096±0.00	Nil	0.99±0.1	1.8±0.06
		220	0.095±0.01	Nil	0.89±0.07	1.83±0.03
		250	0.086±0.00	Nil	0.88±0.05	1.71±0.04
85	3	200	0.09±0.00	1.03±0.15	0.8±0.04	1.64±0.08
		220	0.33±0.07	0.5±0.02	0.62±0.03	1.7±0.07
		250	0.15±0.04	0.36±0.08	0.58±0.06	1.72±0.05
90	3	200	0.085±0.01	0.2±0.07	0.48±0.06	1.51±0.06
		220	0.08±0.00	0.1±0.06	0.28±0.2	1.5±0.02
		250	0.09±0.01	Nil	0.2±0.02	1.33±0.01
100	3	200	0.28±0.06	Nil	0.91±0.07	1.6±0.04
		220	0.19±0.08	Nil	0.92±0.1	1.63±0.03
		250	0.11±0.05	Nil	0.79±0.05	1.61±0.01
110	3	200	0.17±0.03	Nil	0.84±0.04	1.71±0.05
		220	0.08±0.00	Nil	0.9±0.08	1.69±0.04
		250	0.083±0.00	Nil	0.81±0.05	1.73±0.07

*Neutral bleaching earth is used as bleaching agent

Table 5 : Quality analysis of refined palm oil

Bleaching temperature (°C)	Bleaching earth concentration (%)	Deodorization temperature (°C)	FFA (%)	PV (meq/kg)	PO_4 (ppm)	3-MCPD (ppm)
85	1	200	0.24±0.022	1.23±0.23	1.84±0.25	1.79±0.07
		220	0.16±0.02	1.02±0.31	1.8±0.2	1.88±0.08
		250	0.08±0.00	Nil	1.77±0.23	1.81±0.06
90	1	200	0.15±0.01	0.58±0.08	1.8±0.15	1.85±0.09
		220	0.082±0.00	Nil	1.7±0.11	1.68±0.04
		250	0.12±0.02	Nil	1.56±0.09	1.79±0.07
100	1	200	0.25±0.07	Nil	1.88±0.1	1.89±0.08
		220	0.092±0.01	Nil	1.86±0.2	1.96±0.06
		250	0.091±0.01	Nil	1.79±0.19	1.92±0.05
110	1	200	0.91±0.01	Nil	1.62±0.25	1.87±0.03
		220	0.089±0.00	Nil	1.56±0.18	1.98±0.08
		250	0.088±0.00	Nil	1.32±0.08	1.99±0.09
85	2	200	0.09±0.00	0.97±0.07	1.48±0.12	1.9±0.1
		220	0.084±0.01	0.85±0.06	1.26±0.08	1.86±0.09
		250	0.13±0.05	Nil	1.03±0.07	1.84±0.08
90	2	200	0.28±0.04	0.45±0.08	0.98±0.07	1.87±0.04
		220	0.99±0.07	Nil	0.92±0.06	1.88±0.05
		250	0.097±0.01	Nil	0.87±0.05	1.96±0.1
100	2	200	0.1±0.04	Nil	0.99±0.05	1.76±0.03
		220	0.094±0.01	Nil	0.89±0.04	1.75±0.05
		250	0.085±0.01	Nil	0.48±0.03	1.63±0.02
110	2	200	0.13±0.04	Nil	0.88±0.07	1.82±0.01
		220	0.093±0.01	Nil	0.45±0.03	1.56±0.01
		250	0.091±0.00	Nil	0.82±0.08	1.9±0.05
85	3	200	0.18±0.03	0.36±0.07	0.98±0.07	1.92±0.08
		220	0.99±0.07	0.31±0.07	0.91±0.06	1.89±0.04
		250	0.91±0.05	Nil	0.85±0.05	1.82±0.06
90	3	200	0.1±0.01	0.26±0.06	0.98±0.07	1.76±0.04
		220	0.089±0.00	Nil	0.92±0.07	1.77±0.07
		250	0.087±0.00	Nil	0.87±0.04	1.8±0.1
100	3	200	0.099±0.01	Nil	0.9±0.07	1.85±0.09
		220	0.094±0.02	Nil	0.89±0.06	1.8±0.07
		250	0.088±0.00	Nil	0.83±0.05	1.82±0.05
110	3	200	0.092±0.01	Nil	0.85±0.04	1.96±0.1
		220	0.082±0.00	Nil	0.78±0.02	1.84±0.04
		250	0.080±0.00	Nil	0.76±0.05	1.82±0.05

*Acidic bleaching earth is used as bleaching agent

Table 6 : Quality analysis of palm oil available in market

Parameters	Market oil		
	S1	S2	S3
FFA (%)	0.07±0.01	0.09±0.01	0.07±0.01
PV (meq/kg)	Nil	Nil	Nil
PO_4 (ppm)	0.37±0.04	0.5±0.05	0.4±0.04
3-MCPD (ppm)	3.2±0.07	3.52±0.05	3.4±0.07

S1- Sample 1, S2- Sample 2, S3- Sample 3

Table 7 : Analysis of variance for 3-MCPD (ppm), using adjusted SS for tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Bleaching temperature (°C)	3	0.09628	0.09628	0.03209	3.05	0.035
Bleaching earth concentration (%)	2	029508	0.29508	0.14754	14.01	0.000
Deodorization temperature (°C)	2	0.00077	0.00077	0.00038	0.04	0.964
Type of bleaching earth	1	0.09534	0.09534	0.09534	9.05	0.004
Error	63	0.66358	0.66358	0.01053	-	-
Total	71	1.15104	-	-	-	-

and bleaching efficiency than acidic bleaching earth. In this study, it is concluded that the levels of 3-MCPD esters are removed more efficiently if the degummed oil is bleached with 2 per cent and 3 per cent neutral bleaching earth at 90°C for 1 hour and deodorized at 250°C for 1 hour. If the degummed oil is bleached with 2 per cent of acidic bleaching earth at 100°C and 110°C, respectively for 1 hour and deodorized at 250°C and 220°C, respectively are found to be best processing conditions for refining of palm oil.

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