

Optimisation of sterilisation process for oil palm fresh fruit bunch at different ripeness

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Abstract

The optimisation of fresh fruit bunch (FFB) sterilisation process was studied using different degree of FFB ripeness (i.e. under-ripe, ripe, overripe) and loose fruits. This study was carried out with the application of Response Surface Methodology (RSM), based on the interrelation between process temperature (X_1 ; 100 to 120°C) and time (X_2 ; 20 to 80 min) used for FFB sterilisation process on Free Fatty Acid, FFA (Y_1 , underripe FFB; Y_2 , ripe FFB; Y_3 , Overripe FFB; and Y_4 , loose fruits). Thirteen experimental runs were conducted per degree of ripeness using laboratory scale steriliser with varying sterilisation temperature and time, as generated by Central Composite Rotatable Design (CCRD). Raw experimental data trend showed substantial FFA increment with the increment of FFB maturity. Four polynomial models were found appropriate to predict the responses within experimental regions. Analysis regarding factor influences on each response was performed using Analysis of Variance (ANOVA) and graphical analysis. For under-ripe and ripe FFB, the temperature exerted higher and significant ($p < 0.05$) influence on FFA values as compared to time. However, as the fruit ripen, both temperature and time were found similarly strong in affecting the FFA due to highly significant p-value of interaction model term, X_1X_2 for both overripe FFB and loose fruits. Optimum sterilisation operating conditions corresponding to FFA content were also successfully determined using numerical optimisation method. Results revealed the optimum conditions for underripe FFB and ripe FFB at the combination of 100°C for 20min and 100°C for 80min, respectively. Both overripe FFB and loose fruits recorded similar optimum condition, i.e. 120°C and 20 min. The computed optimum conditions have resulted relatively low FFA values at 0.619%, 1.052%, 1.751% and 3.160% for each Y_1 , Y_2 , Y_3 , and Y_4 . The verification experiments were performed and the results were found satisfactory. Small standard deviation values were calculated indicated the actual values was in close agreement with the model prediction. This study has showed promising potential of extracting good quality CPO from other degrees of FFB ripeness as long as the right combination of FFB sterilisation operating condition were used during the extraction process.

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Introduction

It is essential to produce crude palm oil (CPO) with excellent quality and stability, specifically on the acidity and oxidation (Berger and Martin, 2000), due to the fact that good-quality refined oils cannot be produced from CPO with poor quality (Gibon *et al.*, 2009). Previous studies have shown that there was an endogenous lipase (triacylglycerol acylhydrolase) in oil palm fruits, which is also acknowledged as the first enzyme to be involved in the degradation of triacylglycerols (Sambanthamurthi *et al.*, 2000). The action of lipase causes increment in free fatty acid (FFA) levels in CPO. The FFA is one of the most frequently determined quality indices during the production, storage, and marketing of palm oil products. At present, CPO produced by Malaysian palm oil mills are required to comply either with the trade specification (MPOB, 2008) or the revised

quality standard for CPO ex-bulking which have been published as Malaysian Standards (MS 814) (MPOB, 2008). In addition, specially processed CPO have already opened a market as new high-quality products like premium quality (PQ), low free fatty acid (low-FFA) and special quality (SQ) grades CPO (Berger and Martin, 2000). These types of CPOs are principally classified based on their FFA content, which bring to the facts that FFA dictates the oil price. Owing to this reason, research focusing on the CPO extraction process with regard to FFA content is of great concern to the producers and researchers.

Practically, CPO is the final product resulting from oil extraction process which its primary aims is to maximise oil recovery from oil palm fruits. In relation to this, fresh fruit bunch (FFB) and loose fruits are two main groups of oil palm products delivered to factories for oil extraction process

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Table 1. Experimental conditions of FFB sterilisation process

Runs	Process Parameters		Responses (FFA, %)			
	X_1 ($^{\circ}\text{C}$)	X_2 (min)	Y_1	Y_2	Y_3	Y_4
1	110.00 (0)	50.00 (0)	1.10	1.50	2.12	3.50
2	120.00 (+1)	80.00 (+1)	0.90	1.95	2.12	3.60
3	100.00 (-1)	20.00 (-1)	0.68	1.02	2.43	3.51
4	110.00 (0)	92.43 (+1.414)	0.82	1.46	1.92	3.56
5*	110.00 (0)	50.00 (0)	1.15	1.52	2.02	3.41
6*	110.00 (0)	50.00 (0)	1.07	1.48	2.16	3.46
7*	110.00 (0)	50.00 (0)	0.99	1.49	2.00	3.41
8*	110.00 (0)	50.00 (0)	1.05	1.54	2.00	3.43
9	110.00 (0)	7.57 (-1.414)	0.70	1.45	2.25	3.30
10	100.00 (-1)	80.00 (+1)	0.90	1.05	1.76	3.41
11	95.86 (-1.414)	50.00 (0)	0.65	0.89	2.12	3.51
12	124.14 (+1.414)	50.00 (0)	1.00	1.90	1.80	3.26
13	120.00 (+1)	20.00 (-1)	0.95	1.76	1.71	3.20

Each degree of FFB ripeness was subjected to the generated experimental runs obtained from the range and levels.

Average of two experiments for each run.

*Five replications at centre points

(Phorntipha *et al.*, 2009). Usually, factories will purchase loose fruits at higher prices than FFB due to its potential in contributing to higher oil extraction rate (Mohamad, 2008; Phorntipha *et al.*, 2009). According to the FFB ripeness classification established by Malaysian Palm Oil Board (MPOB), FFB ripeness can be classified into 5 main classes i.e. unripe, under-ripe, ripe, overripe and rotten. In the beginning of palm oil mill processing route, these products are usually subjected to sterilisation process. In current situation, the FFB received by palm oil mill for CPO production differ in degree of ripeness without stringent ripeness control and segregation practice employed onto the FFB (Phorntipha *et al.*, 2009). Thus, study in producing CPO from different ripeness of FFB is clearly needed since it might bring an additional value from a theoretical point of view.

The sterilisation process is carried out to accomplish numerous targets. The most important functions of the sterilisation process are to deactivate the biological factors which responsible for quality deterioration and loosen the fruit in the bunch for maximum fruit recovering during stripping and threshing process (Stork, 1960; Olie and Tjeng, 1982; PORIM, 1987; Whiting, 1990; Hamzah, 2008). Since sterilisation process is the earliest step or process in any scale of palm oil processing, it is unquestionably one of the critical operation in the processing, ensuring the success of other subsequent stages.

Currently, there are few issues arisen regarding sterilisation process such as high oxidation risks and over-sterilisation that may lead to poor bleachability

of the resultant oil. The process performance also was found limited due to inherent temperature gradient and difficult process control (Zaror *et al.*, 1993). In addition, Sivasothy *et al.* (1992) also reported that CPO quality is also influenced by heating parameters of the process. These drawbacks will definitely contribute to a great extent of deterioration of CPO quality and it will become worst if the operating variables of the process were not properly restricted and controlled. Hence, precise study should be carried out with special attention on the process modeling and optimisation with the aim of improving CPO quality. Although much work has been done and developed to date regarding the quality attributes of CPO, the interrelation between sterilisation process for different FFB ripeness and FFA content has not been thoroughly studied.

Response Surface Methodology (RSM) enables evaluation of the effects of many factors and their interactions on response variables. The main advantage of RSM is the reduced number of experimental runs needed to provide sufficient information for statistically acceptable results. Therefore, it is less laborious and time-consuming compared to full-factorial experimentation. The RSM was used as a statistical method in this study to explore the relationships between explanatory variables and response variables.

The objectives of this study are to investigate the relationship between CPO quality (in terms of FFA content) and degree of FFB ripeness, and to determine optimum operating condition of FFB

Table 2. Estimated coefficient values and statistical data obtained from ANOVA of the fitted models

Coefficients	Estimated Coefficient Values			
	Y ₁ (underripe)	Y ₂ (ripe)	Y ₃ (overripe)	Y ₄ (loose fruits)
Intercept	1.072	1.516	2.032	3.430
X ₁	0.096*	0.384*	-0.102*	-0.059*
X ₂	0.042	0.029	-0.091*	0.083*
X ₁ ²	-0.107*	-0.052*	-	-
X ₂ ²	-0.140*	-0.025	-	-
X ₁ X ₂	-0.068	0.04	0.270*	0.130*
R ²	0.9116	0.9838	0.8950	0.8906
Model (p-value)	0.0014*	<0.0001*	<0.0001*	0.0001*
Lack of Fit (p-value)	0.3654	0.0914	0.4973	0.3250

Subscripts: 1= temperature; 2= time

*Significant at 0.05 level

sterilisation process at different degree of ripeness which satisfies the FFA of extracted CPO.

Materials and Methods

Raw materials

The FFB were obtained from a local palm oil mill situated in Melaka, Malaysia. The samplings were performed according to MPOB FFB Grading Manual (MPOB, 2003) with the assistance from certified mill FFB grader. Different batches of fruits had to be used as the experiments were done over 6 months period. The chosen FFB was chopped into smaller form called spikelet to cope with usage of laboratory-scale steriliser.

Experimental design

With the aid of Design-Expert version 8.0.1 software (Stat-Ease Inc., Minneapolis, USA), two-variable Central Composite Rotatable Design (CCRD) was employed to study the effect of FFB sterilisation condition at different degree of ripeness on the response, namely FFA, Y₁₋₄ (Y₁, underripe; Y₂, ripe; Y₃, overripe; Y₄, loose fruits). The independent operating variables were heating parameters of the process; sterilisation temperature, X₁ and sterilisation time, X₂, which varies from 100 to 120°C and 20 to 80 min, respectively. Five replicates run at the center point were performed to allow the estimation of pure error. Thirteen experimental runs were generated for each degree of FFB ripeness (refer Table 1). The experiments were conducted in randomised order to minimise the effects of unexplained variability in the observed response due to extraneous factors (Liyana-Pathirana and Shahidi, 2005).

FFB sterilisation

For each experimental run, 20 spikelets (~3 kg)

were placed in swiftlock programmable autoclave, a laboratory-scale steriliser (Astell Acientific, 5000 series model, 240V AC, UK) and subjected to sterilisation process at varying times and temperatures, as per value generated during experimental design stage. The sterilised spikelets then were subjected to oil extraction to obtain CPO.

In-laboratory CPO extraction

The sterilised fruitlets were detached from the stalk and the mesocarp was manually peeled off from the nut using a stainless steel blade. The peeled mesocarp then was submerged (5-10 minutes) into boiling water before it was pressed using a coconut milk presser (obtained from the local market) to facilitate oil extraction. Subsequently, the mixture was transferred into separating funnel with surrounding temperature of approximately 60°C to aid oil clarification process. After 45 min, three distinctive layers consist of oil, water and sludge were formed at the upper, middle and bottom parts of the funnel, respectively. At this stage, the clarified crude oil was subjected to centrifugation for purification purpose, immediately after water and sludge layers carefully removed from the funnel. The centrifugation was performed at 10000 rpm and 45°C using centrifuge (Sorvall® RC 26 Plus, Kendo Laboratory, USA) for 10 minutes. Finally, the purified CPO was subjected to vacuum drying process using vacuum oven (15 inHg max; 80°C) for 150 min. The extracted CPO was immediately subjected to FFA analysis, without any prior storage.

FFA analysis

Determination of FFA was carried out according to MPOB Test Methods (MPOB, 2004).

Statistical and graphical analysis of response surface

The assessment measured data was analysed using the Design-Expert version 8.0.1 software. The most appropriate polynomial regression models were chosen for predicting the interactive effects of heating parameters (X_1 and X_2) of sterilisation on the response (Y_{1-4}). The fitness of each developed model was evaluated by the Analysis of Variance (ANOVA). After this statistical analysis process, the fitted models were interpreted into two- and three-dimensional graphs (contour and response surface plots) to ease in the model evaluation.

Optimisation

Based on the results obtained from the models analyses, numerical optimisation method has been chosen to determine the optimum condition of each sterilisation operation at different degree of FFB ripeness. In this technique, the desired goals for each variables (X_1 and X_2) and response (Y_{1-4}) were chosen. Hence, the time and temperature were kept within the study range while the FFA was minimised, as good CPO quality was indicated by low FFA content. In order to search a solution maximising the response, the goal is combined into an overall composite function, $D(x)$, called as the desirability function (Montgomery, 2001). Verification experiments of the optimum conditions were done in triplicate for individual degree of ripeness and the models' accuracy was checked by calculating mean values and standard deviation as described by Pineiro et al. (2008).

Results and Discussion

Model fitting and statistical analysis

The experimental results were fitted to polynomial regression models which sufficiently explained each response. Accordingly, ANOVA was performed to examine the statistical significance of the model terms. The estimated coefficients values of the fitted models (in coded terms) and statistical data obtained from ANOVA have been summarised in Table 2. The effect of sterilisation heating parameters on the FFA of CPO extracted from different degree of FFB ripeness has been found sufficiently described by two different polynomial regression models which are quadratic (Y_1 and Y_2) and two factor interaction (Y_3 and Y_4). The Y_2 and Y_3 models were found highly significant with very low probability values ($p < 0.0001$) while Y_1 and Y_4 were found significant at probability 0.0014 and 0.0001, respectively. Hence, the terms in the model were found to have a significant effect on the response. The reported R^2 values were

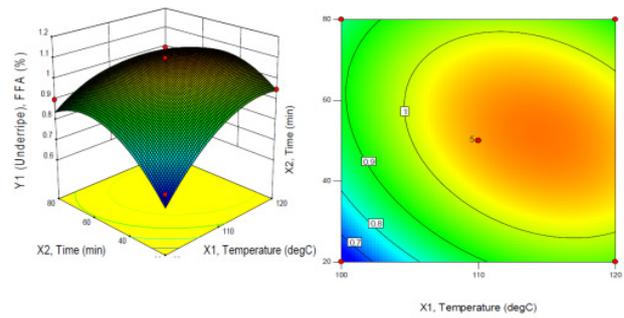


Figure 1(b). Y_1 , Underripe FFB.

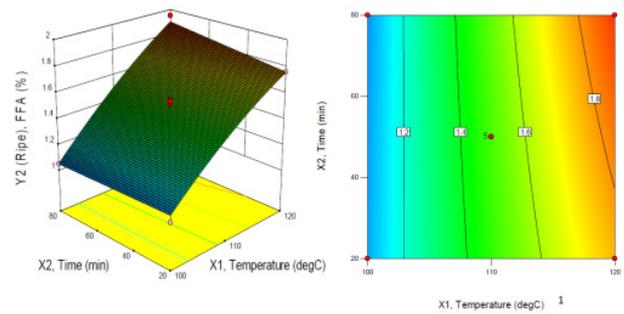


Figure 1(b). Y_2 , Ripe FFB.

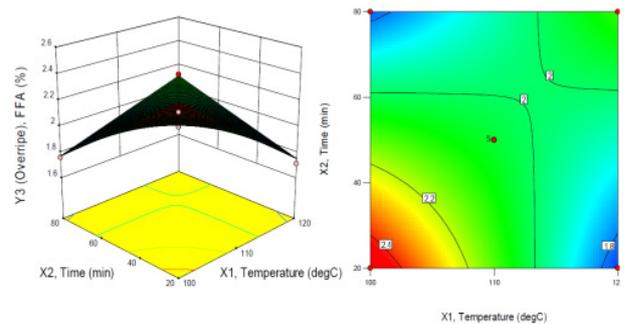


Figure 1(c). Y_3 , Overripe FFB.

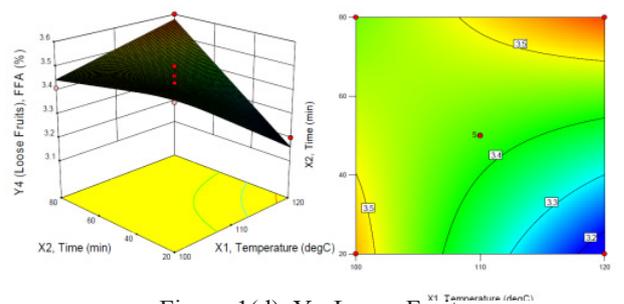


Figure 1(d). Y_4 , Loose Fruits.

Figure 1. Three dimensional response surfaces and their respective two dimensional contour plots for the effects of sterilisation process on FFA of CPO extracted from different degree of FFB ripeness, i.e. (a) Y_1 , Underripe; (b) Y_2 , Ripe; (c) Y_3 , Overripe, and (d) Y_4 , Loose Fruits

0.9116, 0.9838, 0.8950 and 0.8906 for Y_1 , Y_2 , Y_3 and Y_4 , respectively. High R^2 values (approximating 1) indicate high proportion of variability explained by the data and it also show that the RSM models were adequate for prediction purposes. According to Table 2, the lack of fit were found as not significant in all of the models, indicating that these models are

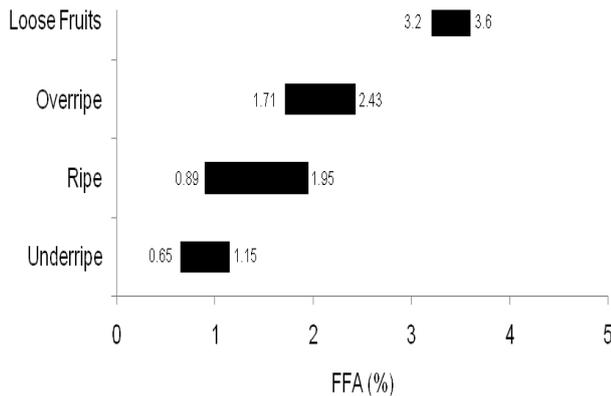


Figure 2. FFA Content (in range) for CPO extracted from different degree of FFB ripeness

sufficiently accurate for predicting those responses.

Effect of sterilisation process on FFA of CPO extracted from different degree of FFB ripeness

Figure 1 (a) to (d) illustrates the relationship between independent and dependent variables in three-dimensional representation of response surfaces and two-dimensional contour plots generated by the models. These figures will be useful in explaining the effects of time and temperature of sterilisation on the FFA of CPO extracted from different degree of FFB ripeness. From the overall assessment on the raw experimental data, the FFA content in the CPO was found increased as the FFB ripen (Figure 2). The lowest FFA content was recorded by underripe FFB's, i.e. 0.65%. It was quite expected since more than 75% of the fruitlets in underripe FFB were still attached to the bunch prior to CPO extraction process and thus, reduce the risk of spontaneous autocatalytic- and microbial- hydrolysis reactions (Sambanthamurthi *et al.*, 2000). Ripe FFB has recorded the widest minimum-to-maximum FFA range, from 0.89% to 1.95%, followed by overripe FFB (1.71% to 2.43%), underripe FFB (0.65% to 1.15%) and loose fruits (3.2% to 3.6%). The upward trend shown by the figure has proven the assumption made by Chong and Sambanthamurthi (1993) that there is an explicit relation between hydrolytic activity and degree of ripeness of the palm fruits. The observation is also found aligned with study conducted by Stork (1960) who reported FFA deterioration occurs with the increment of FFB ripeness. This might be due to physical condition of riper fruits that are softer and therefore has less resistance to rough handling than immature fruits. Accordingly, the fruits will be easily exposed to bruising effect which may break the oil bearing cell walls and start the fat splitting action by the presence of lipase enzyme in the fruits (Stork, 1960; Tan *et al.*, 2009). This situation might also explain the appreciable amount of FFA content

in the loose fruits, i.e. 3.2% to 3.6%. While in the bunch, only the exterior part is exposed and the fruit is well protected from bruising. However, when the fruits are detached from the bunch, they are not effectively shielded from damage. If they have fallen out from the bunch and are allowed to remain in contact with ground, they will form an ideal basis for the development of mould which can speed up the development of microorganisms that cause significant FFA increment. Above all, the FFA values measured in this study are still considered relatively lower as compared to other studies (Orji and Mbata, 2008) and surprisingly, none of the measured FFA was found to exceed the 5.0% value, i.e. the maximum allowable FFA value for production of Standard Quality CPO. This might be caused by careful handling throughout the study, especially with the procedures involved during in-laboratory CPO extraction.

Figure 1 shows the effect of sterilisation heating parameters (X_1 , temperature; X_2 , time) on the FFA (Y) of CPO at different degree of FFB ripeness classification (Y_1 , under-ripe; Y_2 , ripe; Y_3 , overripe; Y_4 , loose fruits). At the early stage of ripeness level (under-ripe FFB and ripe FFB), temperature exerted greater influence as compared to time as only X_1 model term was found to be significant at $p < 0.05$. It is also supported by Figure 1(a) and (b) where only slight FFA increment could be observed as a result of prolonged time as compared to temperature elevation during the sterilisation process. Nevertheless, slight different trend was observed in the overripe FFB (Figure 1c) and loose fruits (Figure 1d). Strong interaction effect between both time and temperature was noticed as justified by high significant p-values for all X_1 , X_2 and $X_1 X_2$ model terms for both Y_3 and Y_4 models. Despite of showing two different response predictions' trends as the FFB ripeness increases, it can be concluded that sterilisation temperature plays more significant role in affecting the FFA content in view of consistency of significant p-values recorded by X_1 model term at all degree of FFB ripeness.

As shown in Figure 1 (a) and (b), at constant time and increased temperature, there is an increment in FFA value of CPO extracted from under-ripe and ripe FFB. This is possibly due to the hydrolysis reaction with the presence of sufficient water (saturated steam used for sterilisation process). This agrees with by Chong and Gapor study (1983) who reported that in the existence of moisture under thermal treatment, the hydrolysis reaction heavily depends on the temperature. It is very important to consider the effects of hydrolysis because apart from FFA increase and the associated increases in refining losses, hydrolysis also could cause partial glycerides

Table 3. Optimum sterilisation processing conditions for different Degree of FFB ripeness based on the FFA content of extracted CPO

FFB Classification	Process Variables		Responses (FFA)	Desirability
	X_1 (°C)	X_2 (min)	Y_{14} (%)	
Underripe	100	20	$Y_1 = 0.619$	0.973
Ripe	100	80	$Y_2 = 1.052$	0.877
Overripe	120	20	$Y_3 = 1.751$	0.722
Loose Fruits	120	20	$Y_4 = 3.160$	0.409

Table 4. Verification Experiments at Optimum Conditions

FFB Classification	Process Variables		Predicted Responses (FFA)	Actual Responses*
	X_1 (°C)	X_2 (min)	Y_{14} (%)	
Underripe	100	20	$Y_1 = 0.619$	0.63 ± 0.024
Ripe	100	80	$Y_2 = 1.052$	1.09 ± 0.084
Overripe	120	20	$Y_3 = 1.751$	1.68 ± 0.126
Loose Fruits	120	20	$Y_4 = 3.160$	3.20 ± 0.163

*Expressed as means \pm SD ($n=3$)

formation which would significantly influence the crystallisation behaviour and stability of emulsions (Jacobsberg, 1983).

As can be seen in Figure 1 (c) and (d), at higher temperature (110°C to 120°C), the addition of steaming time to the process could increase the FFA. This observation is consistent with study by Lau *et al.* (2006) that the percentage of FFA reflects the degree of hydrolysis of oil, which is possibly caused by the presence of moisture under prolonged heating (extended heating time) and enzymatic hydrolysis of oil before sterilisation. However, at lower temperature range, the plots discovered an opposite impact. At lower temperature, the addition of steaming time to the process might slightly reduce the FFA values. However, there are two similarities that could be highlighted in overripe FFB and loose fruits, i.e. i) possibility of extracting the badly FFA-deteriorated CPO at extreme heating parameters combination (either between low temperature and time, or between high temperature and time), and ii) minimum FFA value of CPO could be obtained if they are sterilised at combination of extremely high temperature (120°C) and lowest time, i.e. 20 min.

Optimisation of sterilisation process

Table 3 shows the optimum condition of FFB sterilisation process with the predicted values of

FFA. Though several solutions were suggested by the software, only a solution which possesses the maximum desirability value was selected (for each FFB ripeness) as optimum condition of FFB sterilisation. The obtained optimum conditions were statistically acceptable as the overall desirability scores were found within desirable value, i.e. positive and approximating one (Routara *et al.*, 2007; Ribeiro *et al.*, 2010). The optimum temperature suggested by the software increase by the fruit maturity, i.e. 100°C (under-ripe and ripe) and 120°C (overripe and loose fruits). On the contrary, the suggested optimum time is 20 min for all type of FFB except for ripe FFB which was suggested as 80 min. As expected, the predicted response values increased with the fruit maturities which are 0.619%, 1.052%, 1.751% and 3.16%, each for under-ripe, ripe, overripe FFBs and loose fruits, respectively. Table 4 presents the results of the conducted verification experiments. Small standard deviation values indicated the actual values are in close agreement with the model prediction and the results were considered satisfactory.

Conclusion

Based on the results, FFA deterioration substantially increases with the increment of FFB ripeness, with the minimum and maximum FFA values were recorded at 0.65% (underripe FFB) and 3.6% (loose fruits), respectively. Through application of RSM strategy, four appropriate polynomial models with high R^2 values, i.e. 0.9116 for Y_1 , 0.9838 for Y_2 , 0.8950 for Y_3 , and 0.8906 for Y_4 were successfully developed for analysing the simultaneous effects of oil palm FFB sterilisation heating parameters on the FFA content of the extracted CPO. The results revealed that FFA content was generally influenced by temperature and time of sterilisation process in palm oil processing. However, stronger temperature effect was noticed due to highly significant p-values at 0.05 level recorded by X_1 model term at all degree of FFB ripeness. The sterilisation process conditions were optimised by numerical optimisation method and the optimum conditions of sterilisation have been successfully obtained at 100°C and 20 min, 100°C and 80 min, 120°C and 80 min, and 120°C and 80 min for underripe FFB, ripe FFB, overripe FFB and loose fruits, respectively. Accordingly, FFA values were computed at 0.619%, 1.052%, 1.751% and 3.160% for each Y_1 , Y_2 , Y_3 , and Y_4 . Verification experiments of the optimum conditions were performed in triplicate for individual degree of FFB ripeness. The results were satisfactory as very small standard deviation values recorded, i.e. 0.024, 0.084, 0.126

and 0.163 for Y_1 , Y_2 , Y_3 and Y_4 , respectively. These small values simply indicate that the predictive and experimental values were in close agreement. This study however requires further works on the models verification using larger scale steriliser, either pilot or industrial scale. From the present study, it is evident that the use of statistical optimisation approach, RSM, has helped to identify the interactive effects of heating parameters of sterilisation condition on CPO quality and their optimum levels with minimum effort and time.

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