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Enzymatic Inactivation of Oil Palm Fruits: Comparison of Microwave Irradiation and Steam Bath Process

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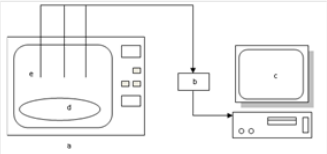
Graphical abstract



Abstract

The study on microwave irradiation and steam batch process to sterilize oil palm fruits is carried out to investigate their effectiveness on lipase inactivation. The inactivation parameters, palm oil quality, and stripping efficiency were evaluated. Evaluation on the inactivation parameters, such as decimal reduction time (D-value) and kinetic constant (k), were conducted to study the sterilization dependency on time and temperature. Microwave sterilization required only 14.085 to 16.949 minutes to inactivate lipase at temperature of 76.5°C (max), while steam batch sterilization required more than 90 minutes to obtained similar level of free fatty acid (FFA) at higher temperature (80 to 105°C). The quality of palm oil was indicated by the concentration of FFA in palm oil. Sterilization of either by microwave irradiation or steam batch sterilization reduced lipase's activity significantly, which is indicated by FFA concentration of below 1%. Stripping efficiency from microwave sterilization at various power level after 16 minutes were 27% (medium power level), 58.5% (medium high power level), and 61% (high power level), respectively.

Keywords: Microwave; steam batch; sterilization; oil palm fruits

Abstrak

Penyelidikan terhadap penyinaran mikro gelombang dan proses kelompok kukus untuk tujuan mensterilkan minyak buah kelapa sawit dan mengkaji kecekapannya atas pengaktifan lipase. Kajian ini dijalankan untuk menyelidik parameter pengaktifan kualiti minyak sawit dan kecekapan pelucutan. Penilaian terhadap parameter pengaktifan, seperti pengurangan masa perpuluhan (nilai-D) dan pemalar kinetik (k), dilakukan untuk mengkaji pensterilan yang bergantung pada masa dan suhu. Pensterilan mikrogelombang memerlukan hanya 14.085 hingga 16.949 minit untuk mengaktifkan lipase pada suhu 76.5°C (maksimum), sementara pensterilan kelompok kukus memerlukan lebih daripada 90 minit untuk mendapatkan tahap asid lemak bebas (FFA) yang hampir sama di dalam minyak sawit. Pensterilan dengan penyinaran mikrogelombang dan pensterilan kelompok kukus telah mengurangkan aktiviti lipase secara nyata sekali dengan ditunjukkan oleh kepekatan FFA yang di bawah 1%. Kecekapan pelucutan daripada pensterilan mikrogelombang selepas 16 minit adalah berada pada aras kuasa berbeza dengan mencapai 27% (aras kuasa medium), 58.5% (aras kuasa medium tinggi) dan 61% (aras kuasa tinggi) masing-masing.

Kata kunci: Mikro gelombang;, kelompok kukus; pensterilan; minyak buah kelapa sawit

1.0 INTRODUCTION

Milling sterilization of oil palm fruits is a steam batch process, which purpose is to inactivate lipase, soften the fruits and detach the fruits from the bunch [1]. It utilizes pressurized steam at 3 kg/cm^2 and temperature of 140°C [2] for about 80-90 minutes [1]. Microwave sterilization offers significant advantages, as this typical sterilization proceeds very quickly, as compared to milling process. Decimal reduction time (*D*-values) for this process is typically less than 17 minutes. It also requires lower energy to increase the temperature of the fruits during sterilization process [3, 4]. Oil palm is a dielectric material, which absorbs microwave energy and convert the energy into heat. The dielectric constant and dielectric loss factor of mesocarp are 16 to 17 and 3.5 to 4, respectively [5]. The dielectric constant expressed the fruit's ability to absorb microwave, while the dielectric loss factor is significant for heat generation.

Recent studies on microwave irradiation of oil palm fruits discussed only time-temperature profile, dieletric properties of the fruits, palm oil quality and stripping efficiency [6-9]. So far, no

study has been reported on oil palm sterilization from lipase inactivation. Hence, this study investigates the parameters of lipase inactivation from oil palm fruit sterilization. The lipase inactivation is time and temperature depedent. Therefore, this study determined the *D*-value and kinetic constant (*k*). The *D*-value terminology for oil palm fruits sterilization is known as the time to reduce 90% of lipase's activity [10]. Since oil palm sterilization is mainly done by steam batch process, the *D*-value from the study is compared to laboratory scale of steam batch sterilization.

2.0 EXPERIMENTAL

2.1 Materials

The materials used in this study include oil palm fresh fruit bunch (FFB) (*Tenera* variety) obtained from UTM Plantation, Skudai, Johor Malaysia, microwave oven (Sharp Model: R-958A), data logger (Pico Temperature Data Logger, PT 104), hydraulic presser (fabricated), autoclave (Electric Steroclave), contactor (Fuji Electric FA series SC13AA) and digital temperature controller (Shimaden). Experimental rigs for microwave sterilization and steam batch sterilization are shown in Figure 1 and 2.

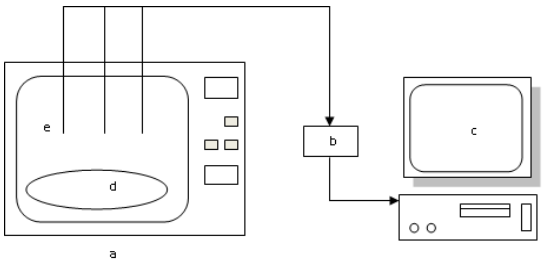


Figure 1 Experimental rigs for microwave sterilization: (a) microwave oven, (b) data logger, (c) computer, (d) tray, (e) thermocouple

2.2 Methods

2.2.1 Sample Preparation

FFB was cut into smaller desired sizes using chain saw (Tokai 3600) and 1.5 kg of each was weighed. The sample was kept in a dry place ready for sterilization.

2.2.2 Microwave Sterilization

The microwave oven was connected to a data logger and a computer to monitor the temperature profile of oil palm FFB during the sterilization process. Each sample was placed in the center of the microwave and exposed to microwave irradiation at high power level (600 W), medium-high power level (427 W) and medium power level (359 W). The temperature was recorded at every 4, 7, 10, 13 and 16 minutes intervals using a thermocouple type J. Each sterilization was carried out in duplicates. After the sterilization, the fruits were pressed using a hydraulic presser to squeeze the oil. Lipase assay and FFA test was then conducted immediately.

2.2.3 Steam Batch Sterilization

Sterilization was carried out by utilizing an autoclave complete with a contactor and digital thermometer controller. About 4 L of water was added into the bottom of the autoclave and the sample was then placed in the autoclave's basket. The sterilization time was adjusted ato 30 minutes, 1 hour and 1.5 hours, excluding the time required for pre-heating and cooling. Pressure and temperature recorded during sterilization. The fruits pressed after sterilization to obtain palm oil utilizing similar hydraulic presser, and lipase assay. The FFA test was conducted immediately after.

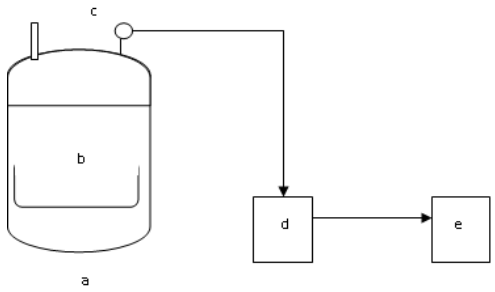


Figure 2 Experimental rigs for steam batch sterilization: (a) autoclave, (b) autoclave's basket, (c) pressure indicator, (d) contactor, (e) inverter

2.2.4 Lipase Assay

Lipase assay testing uses a mixture of acetone and ethanol (50:50 v/v) [11-13]. The palm oil (0.1 g) was dissolved and stirred for about 45 minutes and the temperature was adjusted to 37°C. 10 mL of acetone and ethanol mixture (50:50 v/v) was added to inactivate the lipase at the end of reaction and titrated with NaOH (0.1 N), using phenolpthalein as the indicator (until permanent pink colour appeared). Each test was conducted in duplicates. Blank test was also conducted.

2.2.5 FFA Test

FFA testing was conducted according to MPOB test method. 50 mL of neutralized 2-propanol was sued to neutralize 0.5-2.0 g of palm oil sample. After regulating the temperature to 40°C, the sample was titrated with standard NaOH (0.1 M) using phenolphthalein 1% as the indicator [14,15]. FFA's concentration was calculated by multifying the volume of NaOH with its concentration divided by the number of sample.

2.2.6 Degumming Test

Degumming testing was conducted to determine the stripping efficiency of the sterilization process. The testing was carried out by using a finger press to detach the sterilized fruits from the bunch. Percentage of fruits released per bunch will give the stripping efficiency [7].

2.3 Kinetic of Inactivation

2.3.1 Destruction Rate

Destruction rate of lipase is expressed in terms of reduction on lipase activity [16-18],

$$\frac{dc}{dt} = -kc \qquad (1)$$

2.3.2 D-value Determination

The *D*-value was determined from *D*-*z* model [19], which was developed from the first order kinetics reaction of the destruction

rate of lipase. By integrating Equation (1), the limits, c_1 at t_1=0, and c at time t were calculated by:

$$logc = logc_1 - \frac{kt}{2.303} \quad (2)$$

The graphical expression of Equation (2) is a semi-logaritmic graph as a function of time at constant temperature. The kinetic constant, k, was obtained from the slope of the regression line. t is the D-value, and the remaining lipase (C) was about 10% of the initial lipase (C1) [20].

3.0 RESULTS AND DISCUSSION

3.1 Lipase Inactivation

The destruction rate of lipase, on either microwave irradiation and steam batch sterilization, reduced lipase activity significantly (Figure 3 and 4). The differences between the two methods are on the requirement of time to meet certain level of lipase activity or FFA concentration. Lipase activity model for both methods as a function of time is shown in Table 1.

In this investigation, the rate of lipase inactivation by microwave irradiation was higher and faster. The lipase activity measured from sterilization at medium, medium high and high power level were 0.1%, 0.22% and 0.18%, respectively, after 16 minutes of irradiation.

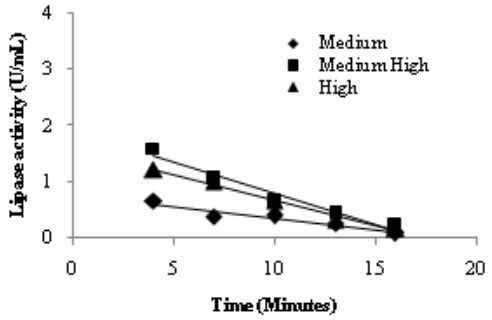


Figure 3 Lipase activity from microwave sterilization

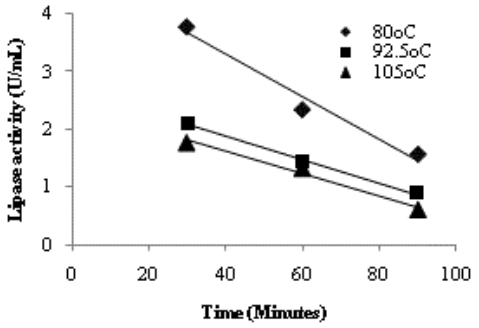


Figure 4 Lipase activity from steam batch sterilization

A simple model in Table 1 was used to compare the lipase inactivation effectiveness between microwave irradiation and steam batch process. The lipase activity of steam batch process was higher as compared to the microwave irradiation. This indicates that sterilization on steam batch process was less effective compared to microwave irradiation. The lipase activity after 16 minutes, for example, was 4.185% (T=80°C), 2.383% (T=92.5°C), and 2.103% (T=105°C). The lipase activity of steam batch process was less than 1% after being sterilized for 90 minutes. However, the lipase activity from sterilization at 80°C was below 1% by the model after 110 minutes.

Hence. with the same duration of time, microwave irradiation proved to be more effective in reducing the lipase activity as compared to steam batch sterilization. The amount of enzyme that liberates 1 µmol of butyric acid per minute at 30°C is 1 LU [21].

Table 1 Simple model of lipase inactivation

Sterilization process	Lipase activity (U/ml)	R^2
Microwave irradiation		
Medium Power Level	-0.041t + 0.779	0.903
Medium High Power Level	-0.112t + 1.921	0.958
High Power Level	-0.091t + 1.598	0.987
Steam batch sterilization		
T = 80°C	-0.037t + 4.777	0.970
T = 92.5°C	-0.020t + 2.703	0.997
T = 105°C	-0.019t + 2.407	0.981

t= time

3.2 Destruction Kinetic Parameters

Destruction kinetic parameters of this study comprised of D-value and k. Model of lipase inactivation by microwave irradiation and steam batch sterilization is shown in Table 2, while the D-value is given in Table 3. The model for inactivation by microwave irradiation gave the relationship between the inactivation rate and k. The k increased at elevated power level and affected the rate of inactivation. Lipase inactivation occured faster for treatment at medium high and high power level. Overall, the rate of lipase inactivation by microwave irradiation was faster as compared to steam batch sterilization.

The rate of lipase inactivation on steam batch sterilization was less influenced by k at elevated temperature. It was observed that the destruction rate of steam batch process remained constant at all temperature. It is also predicted by the model in Table 2, where steam batch sterilization at 80 or 140°C (commercially done in industry) would obtain a constant destruction rate.

By refering to Table 3, microwave irradiation requires only 14.085 to 16.949 minutes to inactivate 90% of the lipase activity. The D-value and k relationship is explained by Equation (3) and listed in Table 3. The power level increment of microwave sterilization reduces the D-value but increased rate of lipase inactivation and temperature of mesocarp were observed from temperature of 70 to 76.5°C.

The D-values observed for steam batch sterilization were 2 hours and 46 minutes (at T=80°C and 92.5°C, respectively) and 2 hours 23 minutes (at T=105°C). Hence, the D-value reduced with elevated temperature. Comparison of the D-value of microwave irradiation, laboratory scale steam batch sterilization and commercial palm oil milling is shown in Table 4.

This study observed that both microwave irradiation and steam batch sterilization are time and temperature dependent. The temperature's sensitivity, z-value, was investigated to study the process's dependency on time and temperature. For microwave

sterilization, *z*-value was 77 °C. The *z*-value indicates that the destruction rate was not temperature sensitive. The *z*-value for steam batch sterilization process was also not temperature sensitive as *z*- value measured was very high.

Table 2 Rate of lipase inactivation

Mode of sterilization		Rate of lipase Inactivation
Microwave irradiation	Medium power level	$\frac{dc}{dt} = -0.136c$
	Medium high power level	$\frac{dc}{dt} = -0.161c$
	High power level	$\frac{dc}{dt} = -0.164c$
Steam batch sterilization	T = 80ºC	$\frac{dc}{dt} = -0.014c$
	T = 92.5ºC	$\frac{dc}{dt} = -0.014c$
	T = 105ºC	$\frac{dc}{dt} = -0.016c$

3.3 Quality of Palm Oil

FFA's concentration was used as the quality indicator of the oil palm investigated in this study. The FFA's concentrations after treatment by microwave sterilization and steam batch sterilization are shown in Figure 5 and 6.

3.3.1 Quality of Palm Oil Obtained from Microwave Sterilization

The levels of FFA concentration at various power level and time of exposure are shown in Figure 5. Overall, FFA'S concentration was observed at below 3.5% or below standard requirement for commercial palm oil [22]. Microwave irradiation at medium and medium high power level showed similar performance. The concentration of FFA decreased very rapidly from 3.2% (at 3 minutes of exposure) to 1.2% after 10 minutes of exposure. Then the concentration remained constant at 1-1.2% after being irradiated for 13 minutes.

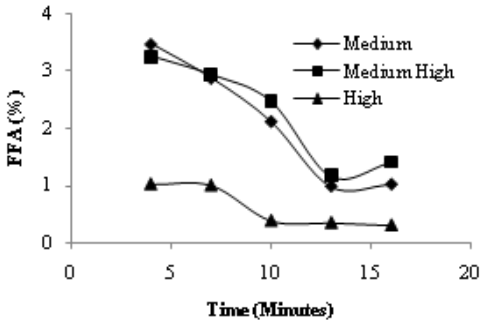


Figure 5 The FFA's concentration from microwave sterilization

Meanwhile, treatment at high power level is more effective in reducing FFA's concentration. The FFA'S concentration was recorded at below 1% at 3 to 10 minutes of irradiation. The correlation between the power level and FFA's concentration was the temperature. Treatment at high power level showed higher temperature, as compared to treatment at medium high and medium power level. Inactivation of lipase at higher temperature occured very quickly due to higher value of *k* (Table 3). The highest temperature for treatment at medium, medium high and high power level are 70, 75 and 76.5 °C, respectively.

3.3.2 Quality of Palm Oil Obtained from Steam Batch Sterilization

Milling sterilization is usually carried out for a duration of 80 to 90 minutes at the temperature of 140 °C. This temperature could not be achieved due to the limitations of the autoclave. Because of this, the temperature was adjusted to 80, 92.5 and 105°C, respectively, in order to get the trend of FFA's reduction, as shown in Figure 6.

The FFA observed from sterilization at temperature of 80°C indicates the corelation between FFA and time. Time increment from 30 to 60 and 90 minutes reduced the concentration of FFA. The FFA's concentration measured were 2.9%, 1.5% and 1.7% after sterilization for 30, 60 and 90 minutes, respectively. Meanwhile, the sterilization time was less significant to the reduction of FFA's concentration for sterilization at 92.5°C and it was insignificant for sterilization at 105°C. It observed that temperature and sterilization time had less corelation with the level of the FFA's concentration.

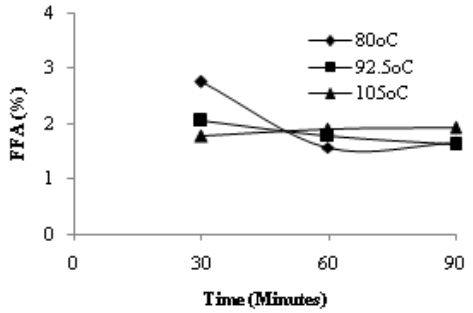


Figure 6 The FFA concentration from steam batch sterilization

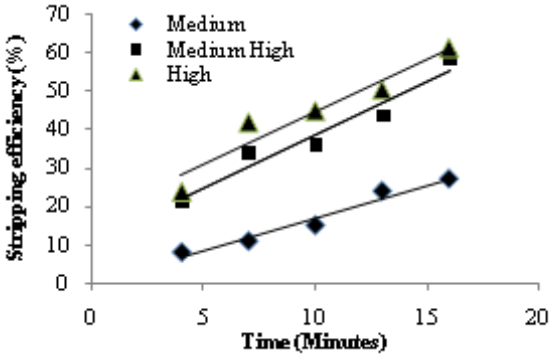
3.4 Stripping Efficiency

Gums that are present in the oil palm fruits as minor components (only 300 ppm) are responsible for cells' bonding. Gums consist mainly of phosphatides substances which are water soluble at high temperature. These gums can be evaporated together with water when the oil palm fruits are heated either by microwave irradiation or steam. The heating process facilitates the fruits' detachment from the bunch, which is known as degumming process. The degumming performance of palm oil milling is always represented by stripping efficiency. Stripping efficiency using microwave irradiation has been reported by several researchers [5, 7, 23].

For microwave sterilization, the stripping efficiency at medium, medium high and high power level are 27%, 58.5% and 61%, respectively, after 16 minutes of exposure time (Figure 7). The stripping efficiency increased at elevated power level. However, the stripping efficiencies in this study were lower compared to other studies using similar period of exposure time, as it was reported that the stripping efficiency was 81% after heating for 14 minutes [5].

For steam batch process, it was impossible to do any degumming testing since all the fruits were already detached from the bunch at the end of sterilization. The fruits' colour were dark brown as shown in Figure 8 (a), while the fruits' colour from microwave sterilization turned from light brown to a darker brown, as shown in Figure 8 (b). To compare the ability in detaching the

fruits by these two processes, prediction of degumming by microwave sterilization after 30 minutes to 90 minutes was done. The prediction was carried out using a simple model obtained from regression equation shown in Figure 7.



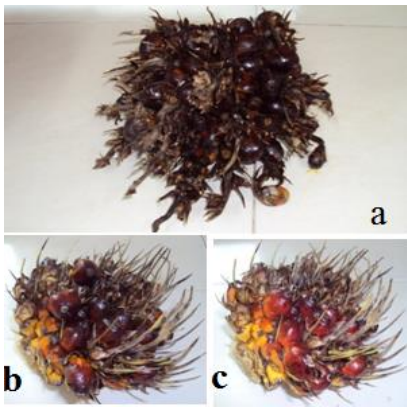


Figure 8 Oil palm fruits after sterilization by: (a) steam batch, (b) microwave irradiation and (c) un-sterilized oil palm fruits

Table 3 The *D*-value for microwave sterilization and steam batch sterilization

Microwave Sterilization				Steam Batch Sterilization			
***D*-value (min)**	**Tmax on fruits (ºC)**	**k**	**R^2**	***D*-value (hours)**	**T autoclave (ºC)**	**k**	**R^2**
16.949	70 (Medium*)	0.136	0.858	2.77	80	0.014	0.997
14.286	75 (Medium High*)	0.161	0.987	2.77	92.5	0.014	0.995
14.085	76.5 (High*)	0.164	0.959	2.38	105	0.016	0.933

Table 4 Performance of microwave sterilization, steam sterilization and commercial palm oil milling

Parameters	Microwave Sterilization	Steam Batch Sterilization	Commercial Palm Oil Milling
D-value	14 to 17 minutes	2 hours 23 minutes to 2 hours 46 minutes	80 – 90 minutes (not including loading and unloading)
Temperature	Average in70ºC to 76.5ºC	More than 80ºC (in the study was 80ºC to 105.5ºC)	140ºC (steam pressure is 3 to 3.5 kg/cm^2)
Quality after sterilization	FFA below 1%	FFA below 2%	NA**)
Stripping efficiency	61% after 17 minutes (nearly 100% after 29 to 58 minutes)***)	Nearly 100% after 2 hours and 46 minutes	NA**)
Colour of sterilized fruits	Light brown to brown	Dark brown	NA**)
Produced condensate	No	Yes	Yes

**) Not available
***)Predicted using regression equation

Complete detachment by microwave sterilization was predicted at 58 minutes, 31 minutes and 29 minutes using medium, medium high and high power level, respectively.

4.0 CONCLUSION

From all the parameters, microwave sterilization showed better advantages than steam batch process, with the exception of the stripping efficiency. Faster sterilization process can be achieved, as microwave sterilization only requires 14.085 to 16.949 minutes to destruct lipase activity. Furthermore, it only requires relatively low temperature (70 to 76.5 °C) using medium high power level (427
Watt) or high power level (600 Watt). The palm oil produced is also of higher quality as the FFA's concentration measured was below 1%. Microwave sterilization is also more environmental friendly as no condensates are discharged to the environment.

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