A Study on the Use of Water as a Medium for the Thermal Inactivation of Endogenous Lipase in Oil of Palm Fruit

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Abstract: The heat treatment of oil palm fruit using saturated steam (413 K) in conventional oil palm processing has been reported to be ineffective in terms of heat distribution and penetration into the fruit bunch inner layer; hence, there is a desire to explore other alternative processes. In this study, oil palm fruit was treated in water at temperatures between 308 K and 343 K. The effects of the treatment on the in vivo activity of the lipase, the abscission layer of the fruit, and the integrity of the oil globule membrane were observed. The results showed in vivo residual lipase activity to be almost completely inactivated after 40 min of heat treatment at 343 K. The micrograph of the fruit mesocarp exhibited disintegration of the oil globule membrane as well as dissolution of the pectin layer architecture of the abscission zone after the treatment at this temperature. A dynamic mathematical modeling of heat transfer was employed, and coupled with reaction kinetics of lipase inactivation. The inactivation kinetics was found to be a non-elementary reaction, and the initial rate constant, $k_{0,dec}$, and activation energy, $E_{dec}$, of the reaction were estimated to be $0.035 \text{U}^{-0.85/\text{kg-mes}^{-0.85}} \cdot \text{min}$ and $153,052 \text{kJ/kmol}$, respectively. The findings suggested the viability of water as a medium of heat treatment instead of the conventional steam treatment in oil palm processing.

Keywords: oil palm; endogenous lipase; inactivation; heat treatment; residual activity

1. Introduction

The mesocarp of a ripe oil palm fruit (elaeis guineensis) contains a large amount of oil comprising mainly of triacylglycerides (TAGs). The oil is a rich source of 50:50 saturated:unsaturated lipids, and plays an important role in the food industry worldwide [1]. The palm fruit contains endogenous lipase (Glycerol ester hydrolase, E. C. 3.1.1.3) within the mesocarp layer, which is extremely active and responsible for the lipolysis reaction within the fruit [2]. Lipolysis reaction involves several steps of hydrolysis to break down triacylglyceride, resulting in the production of free fatty acids (FFA) in the fruit [3]. The in vivo activity of lipase within mesocarp cells of palm fruit was reported by Sambanthamurthi et al. [4] to be accelerated by the damage of mesocarp tissue, over-ripening, improper handling, and ineffective sterilization process. Ripe unbruised fruit contains around 1% FFA at the time of harvest [5], but inappropriate handling may injure the fruit mesocarp thereby activating the endogenous lipase which results in increased FFA content. According to Pahoja and Sethar [6],
Ebongue et al. [3] and Ebongue et al. [1], the FFA contents increased to about 40% within 15 min of mesocarp damage.

The most important parameter in the quality assessment of crude palm oil (CPO) is FFA because of its influences on consumer decisions and trading of the commodity [7]. Oil of good quality usually contains more than 95% neutral TAGs and 0.5% or less FFA [8]. Gupta [9] reported that typical neutral oil loss as a result of the removal of FFA accumulated in vegetable oil is 1.5%–2% in the industry apart from losses from spills and degradation of the product. Kannan and Gundappa [10] reported even higher neutral lipid losses of 13.6% and 19.5%, after ethanol and sodium hydroxide de-acidification of CPO, respectively. Apart from the physical loss due to high FFA content of CPO, it also leads to some health implications, as oil with more than 5% FFA is considered to be unfit for human consumption [11]. FFA accumulation in CPO is inimical to palm oil producers, especially small-scale holders in Africa whose oil is essentially dedicated to direct consumption without further refining.

Typical palm oil processing uses heat sterilization to stop the lipolytic activity of lipase to avoid the increment of FFA content in the oil. The sterilization process of fresh fruit bunches (FFB) is normally performed at 140 °C (413 K) for between 75 to 90 min to inactivate the enzyme and to loosen the fruit on the bunch to facilitate stripping [12]. This process is also aimed to condition the oil-bearing cells (oil globules) in the mesocarp for ease of oil extraction. Nevertheless, thermal hydrolysis is a concern in this process, especially at the sterilization temperature above 120 °C (393 K), since oil–water mixture at elevated temperature could lead to hydrolysis of the TAGs. It is believed that treatment at a lower temperature (less than 373 K) can preserve some nutritional values such as β-carotene, vitamin E, phytosterols, and squalene in the extracted oil.

Ali et al. [13] reported that the nature of FFB and the medium (steam) of heat application currently used has contributed to the ineffectiveness of the sterilization process. The ineffectiveness of the process is due to the uneven heat distribution within the sterilization vessel and the heat transfer rate from the steam to the fruit causing incomplete inactivation of lipase in the mesocarp. Han et al. [14] reported that the current method of steam sterilization is also faced with prolonged steaming before sufficient heating of the inner layers of the bulky FFB can be achieved.

To overcome this issue, a few alternatives have been proposed, such as dry air sterilization, which was reported to be ineffective because it only heats up the outer layer of fruit in the FFB and the prolonged heating of this method severely damages the fruit [15,16]. Sarah & Taib [17], Umudee et al. [18] and Nokkaew and Punsuvon [19] reported the use of microwaves for the sterilization oil palm fruit (OPF) and obtained results that are comparable to the steam heating. However, the application of microwaves for commercial sterilization of FFB is still not economically feasible at this moment. This may be due to the nature of its physical properties, such as volume, density, and shape that affects the dielectric properties of foods and influences the absorption of microwave energy, which has been reported by Martins et al. [20]. The non-adoption of microwaves for the inactivation of the endogenous lipase in oil palm fruit at the industrial scale could also be as a result of non-uniform temperature distribution, resulting in hot and cold spots mainly in solid and semi-solid products [20]. Other factors limiting the application of microwaves in OPF processing are lack of experimental data needed for microwave heating modeling, analysis of energy and manufacturing costs, the necessity of technical expertise to understand and minimize heat leakage, and high initial investment are also considered limitations of the process [21,22].

Owing to the challenges associated with steam, hot air, and microwave sterilization of FFB, hot water treatment is a viable alternative. According to Wang et al. [23] and Hong et al. [24], hot water treatment is the most preferred method for many applications, since water is a more efficient heat transfer medium than air and steam. Apart from serving better heat transfer properties (i.e., higher specific heat capacity), water will guarantee deep and even heat distribution. Moreover, its physical cleansing effects on the fruit surface will alleviate public concerns for fungicide residues found in food.

There is scarce data in the literature on OPF lipase regarding in vivo activity and the inactivation kinetics of this enzyme. Therefore, a mechanistic understanding of the inactivation of the endogenous
lipase in OPF is imminently required to facilitate and enhance the economic feasibility of using hot water as a medium of heat transfer for the inactivation of the lipase. The knowledge of the mechanism is essential for the development of a rational approach for the inactivation of the endogenous lipase in OPF mesocarp. Hence, the aims of this work are to study the effect of hot water treatment on the in vivo activity of lipase in the mesocarp of OPF mesocarp, and to develop dynamic mathematical models of the enzyme inactivation.

2. Materials and Methods

2.1. Materials

Ripe FFB of the oil palm (*Elaeis guineensis* Jacq. var. tenera) was obtained from Universiti Putra Malaysia farm, Serdang, Malaysia. The fruit bunches were randomly taken from oil palm trees aged 10−11 years.

2.2. Determination of OPF Dimension

OPF is a spheroid shape and the dimensions required are the fruit, endocarp, and kernel layers (Figure 1). For the simulation proposed, OPF was assumed as a spherical, and therefore, the mean radius (*R*) was calculated using Equation (1) [25]. In this work, mean dimensions such as fruit radius (*Rm*), endocarp radius (*Rs*), and kernel radius (*Rk*) were measured.

\[ R = \frac{R_x + R_y + R_z}{3} \]  

\[ (1) \]

**Figure 1.** Geometry of oil palm fruit (a) Spheroid shape, (b) Section to measure mean radius for fruit, endocarp, and kernel.

2.3. Heat Treatment of Fresh Palm Fruit

OPF were hand-picked from the FFB and sorted (bruised and rotten fruitlets were removed), surface-sterilized in 95% ethanol for 10 min, and washed in sterile distilled water [26] to render microbe-free fruitlets. The fruitlets were gently shuffled with hand to avoid bruise and to give a homogeneous lot. The lot was randomly divided into 7 groups (A, B, C, D, E, F, and G), each composed of 104 fruitlets with an approximate weight of 1.35 kg.

In this study, the heat inactivation experiments of endogenous lipase were conducted in vivo of the OPF. Group A, B, C, D, E, and F fruitlets were heat-treated at 308 K, 313 K, 318 K, 323 K, 333 K, and 343 K, respectively in a water bath. Group G served as control (i.e., without heat treatment). During the heat treatment, 2 samples of the fruitlets (Sample 1 and Sample 2) were taken from the water bath
at an interval of 5, 10, 20, 30, 40, 50, and 60 min. Immediately, Sample 1 was heated in a microwave oven (Sharp model: R207EK) at medium power level (359 W) for 3 min to stop the enzyme activity. Each of the heat treatment was carried out in triplicates (n = 3).

2.4. In Vivo Lipase Assay

Wound-induced assay technique was used to initiate the residual activity of the endogenous lipase after the heat treatment. As illustrated in Figure 2, fruit in Sample 2 was cooled down to 310 K, in which wounding of the sample was initiated by mashing the mesocarp of the fruit as quickly as possible (i.e., to minimize the temperature drop) in a mortal. This action creates the required inter-phase (oil–water) for the lipase activity. The mashed mesocarp was immediately incubated in an incubator at 310 K for 10 min. After that, Sample 2 was removed from the incubator and straight away heated in a microwave oven to stop the activity of the residual enzyme completely. CPO was thereafter extracted from Sample 1 and Sample 2 using a laboratory scale screw press.

![Diagram](image-url)

**Figure 2.** Illustration of the procedure for the determination of in vivo activity of endogenous lipase activity using wounding technique.
The FFA accumulated in Sample 1 and Sample 2 were measured as $FFA_1$ and $FFA_2$, respectively. FFA content in group G was considered to be an initial value; it was also used to measure the initial lipase activity ($c_{E0}$). The residual lipase activity ($c_{Et}$) and the relative residual lipase activity ($RA$) were computed using Equations (2) and (3), respectively.

$$c_{Et} = \frac{FFA_2 - FFA_1}{t} \quad (2)$$

$$RA(\%) = \frac{c_{Et}}{c_{E0}} \cdot 100 \quad (3)$$

### 2.5. Determination of FFA Content

The FFA content of the CPO extracted was determined using the official methods and recommended practices of the American Oil Chemists Society Ca 5a-40 [27]. The percentages of FFA was calculated as palmitic acid considering palmitic acid to be the predominant fatty acid present in palm oil. Hence,

$$FFA (\%) = \frac{25.6 \cdot N \cdot V}{W} \quad (4)$$

where 25.6 is a constant based on molar mass of palmitic acid, $N$ is the normality of NaOH (0.25 mol/L), $V$ is the volume of NaOH used (mL) and $W$ is the mass (g) of the sample.

### 2.6. Histological Light Microscopy

With the aim of investigating the influence of heat on the cell wall integrity, the mesocarp fibers of a raw and heat-treated fruit were used for microscopy analysis. Samples were prepared according to the standard method and examined under a light microscope (LEICA ICC50 HD, Wetziar, Germany). The exocarp layer of OPF was removed using a scalpel and a thin slice of about 0.5 mm was excised carefully from the mesocarp with a razor blade as reported by Ho et al. [28]. The excised section was placed on a glass slide and wetted with a distilled water. The slide was then mounted on the microscope and the cells (oil globules) were examined.

### 2.7. Abscission Zone Preparation

In OPF, the abscission zone (AZ) composed of a morphologically distinct layer of pectin located between the mesocarp and the pedicel. Three spikelets were cut off from an FFB. One was heat-treated in water for 60 min at 343 K, the second one at 373 K, and the third one was left untreated. Transverse sections of the AZ were cut from the spikelets. The pectin layer was examined and photographed.

### 3. Mathematical Modeling of the Heat Inactivation of Endogenous Lipase in Palm Fruit

Mathematical models are indispensable in the quantification of effects of thermal treatment of fruit and vegetables. These are formidable tools for making computational process control possible. According to Silva et al. [29], modeling of the heat sterilization of foods requires a mathematical description of heat flow into the product, the inactivation (degradation) kinetics of microorganisms or enzymes and quality factors. According to the authors, the energy consumed by the thermal destruction of cells and food quality indices is negligible and therefore the above-mentioned phenomena can be solved independently. Generally, enzyme activities are dependent on temperature and heating time [30]. In this study, palm oil fruit was assumed to be spherical in shape (Equation (1)) and water was used as a medium of heating the fruit to inactivate the endogenous lipase.

Ordinary differential equations (ODE) for heat transfer and it effect on in vivo activity of endogenous lipase was applied and combined with the kinetics of the thermal inactivation. The oil cell distribution can be clearly seen under a microscope. It is believed, lipase that plays a significant role in the hydrolysis of CPO, is within the cell wall that house the oil globules. Therefore, once oil cell raptures as results of mashing, the released oil will mix with the cytoplasm fluid (water) to form...
an emulsion an interface ideal for the catalytic reaction of the lipase which is also present in the cell wall/lipid membrane.

3.1. Heat Transfer

The partial differential equation (PDE) of energy transfer was applied to represent the temperature distribution profile within mesocarp region, shell region, and kernel region, which was written as in Equation (5), Equation (6) and Equation (7), respectively. The values for density and thermal properties of each region were referred to Table 1.

\[
\rho_m \cdot C_{pm} \cdot \frac{r}{2} \cdot \frac{\partial T}{\partial t} = k_m \left( \frac{r}{2} \cdot \frac{\partial^2 T}{\partial r^2} + \frac{\partial T}{\partial r} \right) \quad R_s < r < R_m
\] (5)

\[
\rho_s \cdot C_{ps} \cdot \frac{r}{2} \cdot \frac{\partial T}{\partial t} = k_s \left( \frac{r}{2} \cdot \frac{\partial^2 T}{\partial r^2} + \frac{\partial T}{\partial r} \right) \quad R_k < r \leq R_s
\] (6)

\[
\rho_k \cdot C_{pk} \cdot \frac{r}{2} \cdot \frac{\partial T}{\partial t} = k_k \left( \frac{r}{2} \cdot \frac{\partial^2 T}{\partial r^2} + \frac{\partial T}{\partial r} \right) \quad 0 \leq r \leq R_k
\] (7)

where the boundary condition was written as:

\[
-k_m \left. \frac{\partial T}{\partial r} \right|_{r=R_m} = h \left( T_{r=R_m} - T_w \right)
\] (8)

Table 1. Density and thermal properties of OPF.

| Property                      | Symbol | Value     |
|-------------------------------|--------|-----------|
| Density (kg/m³)               | \( \rho_m \) | 611 \(^{a}\) |
|                               | \( \rho_s \) | 560 \(^{a}\) |
|                               | \( \rho_k \) | 1203 \(^{b}\) |
| Specific heat capacity (kJ/kg K) | \( C_{pm} \) | 2.816 \(^{b}\) |
|                               | \( C_{ps} \) | 2.291 \(^{b}\) |
|                               | \( C_{pk} \) | 1.980 \(^{a}\) |
| Thermal conductivity (kJ/m·K-min) | \( k_m \) | 0.02082 \(^{b}\) |
|                               | \( k_s \) | 0.04080 \(^{b}\) |
|                               | \( k_k \) | 0.03174 \(^{a}\) |
| Heat transfer coefficient (kJ/m²·K·min) | \( h \) | 150 \(^{c}\) |

\(^{a}\) This study, \(^{b}\) Hadi et al. [31], \(^{c}\) Noerhidajat et al. [32].

3.2. Inactivation Kinetics

During the thermal treatment, the endogenous lipase will be inactivated at elevated temperatures. The lipase only located within the mesocarp layer of OPF. The PDE of the inactivation kinetic of lipase activity in mesocarp region is written as:

\[
\frac{\partial c_E}{\partial t} = -k_{dec} \cdot c_E^{n_d} \quad R_k < r \leq R_m
\] (9)

where the kinetic rate is temperature dependent based on the Arrhenius equation, as shown in Equation (10), which is its value will remain constant when reaching a temperature higher than 340 K.

\[
k_{dec} = k_{dec}^{0} \cdot \exp \left[ -\frac{E_{dec}}{R} \cdot \left( \frac{1}{T} - \frac{1}{340} \right) \right] \quad |T| \leq 340K
\]

\[
k_{dec} = k_{dec}^{0} \quad |T| > 340K
\] (10)
Lipase activity measurement that was obtained from Equation (2) was assumed as the residual average lipase activity in the mesocarp layer, and it was related in the simulation modeling as:

$$c_{Et} = \frac{4 \cdot \pi \cdot \rho_m \cdot \int_{R_s}^{R_m} c_E \cdot r^2 \cdot dr}{M_m}$$  \hspace{1cm} (11)$$

where total mass of mesocarp is formulated as:

$$M_m = \frac{4}{3} \cdot \pi \cdot (R_m^3 - R_s^3) \cdot \rho_m$$  \hspace{1cm} (12)$$

3.3. Model Performance and Sensitivity Analysis

Relative root means square error (rRMSE) was used to evaluate the performance of the model. rRMSE measures the error between two datasets. In other words, it compares the predicted value with the observed value, and it was calculated using Equation (13):

$$rRMSE = \frac{1}{N} \sqrt{\frac{\sum_{j=1}^{N} (P_j - O_j)^2}{N_E}}$$  \hspace{1cm} (13)$$

A sensitivity analysis was also conducted to examine the influence of selected parameters (especially the kinetic parameter model) on overall performance of the dynamic simulation. Two important parameters were selected for this study, representing the inactivation reaction; initial rate constant, $k_{0dec}$, and reaction order, $n_d$. Their values increased and decreased (i.e., ±25%) from their nominal values.

4. Results and Discussion

4.1. Experimental Results

4.1.1. Geometrical Dimension of OPF

The measured dimensions required for modeling the geometry of OPF fruit are shown in Table 2. The values are in close agreement with the reports by Owolarafe et al. [33] and Davies [34]. These dimensions are very important to simulate the radial heat transfer through the fruit.

| Part              | Dimension (m)                     | Standard Deviation |
|-------------------|-----------------------------------|--------------------|
| Fruit radius ($R_m$) | $1.495 \times 10^{-2}$            | ±4 $\times 10^{-3}$ |
| Endocarp radius ($R_e$) | $7.1 \times 10^{-3}$              | ±2 $\times 10^{-4}$ |
| Kernel radius ($R_k$) | $6.1 \times 10^{-3}$              | ±1 $\times 10^{-4}$ |

4.1.2. Effect of Hot Water Treatment on the Integrity of Oil Cell/Globule Membrane

Neutral lipids, mainly triacylglycerol (TAG) and sterol esters, are stored in intracellular organelles of the mesocarp and are often termed as oil bodies, lipid droplets, oil globules, liposomes, and spherosomes [12]. As shown in Figure 3A, a very firmed and organized cell structure can be observed in the microstructure of the mesocarp. The oil globules were well encapsulated by the cell wall and large part of cell corner middle lamella can be seen. According to Owolarafe and Faborode [35], the effect of processing conditions on the structure of oil-bearing material can be easily identified by analyzing the retention degree of the normal features of the structure involved.
were heat-treated in water at 343 K, the pectin dissolved and completely disappeared (Figure 4B). The water treatment is an advantage as the non-firm surface of the fiber obtained after the failure of the wall [36]. The loose pattern of the oil globules and the melting of the oil globule membrane after hot water treatment at the range of 343 K to 373 K was able to weaken the integrity of the oil globule membrane. As it can be observed in Figure 3B, the oil globule membrane stated to lose their firmness and the organized cell structure after the treatment at 343 K. When the fruit were heated at 373 K for 60 min, the morphology turned bright yellow and cell walls almost vanished (Figure 3C). This was as a result of the destruction of the pectin architectures that binds the cells together. Pectin architectures in the middle lamella plays a vital role as intercellular glue and cell adhesive for the porosity of cell wall [36]. The loose pattern of the oil globules and the melting of the oil globule membrane after hot water treatment is an advantage as the non-firm surface of the fiber obtained after the failure of the pectin matrix will aid the diffusion and penetration of oil out of the cells. The structural modification of the fiber clearly demonstrates the effect of hot water treatment on the mesocarp tissue of oil palm.

4.1.3. Effect of Hot Water Treatment on Abscission of OPF

The OPF has two types of AZ—one large multilayer primary AZ (Figure 4A) and up to four adjacent AZ that are less distinguishable [37]. The primary AZ lies between the pedicel and mesocarp tissues at the base of the fruit, while the adjacent AZ are at the periphery of the primary AZ. The primary AZ of ripe oil palm fruit has high levels of un-methyl esterified pectin that cements or binds the mesocarp cells with the pedicel. As shown in Figure 4A, the mesocarp was rigidly glued to the pedicel in a freshly harvested OPF spikelet. However, pectin is soluble in water [38] and when the fruit were heat-treated in water at 343 K, the pectin dissolved and completely disappeared (Figure 4B). The dissolution of the pectin left a cleavage between the mesocarp and pedicel, signaling the detachment of the fruit. Similar disappearance of the pectin layer was also observed in Figure 4C which is a sample treated in boiling water (373 K) for the same duration of 60 min.

4.1.4. Effects of Heat Treatment on FFA Accumulation in OPF

Heat treatment of OPF is primarily aimed at inactivation of the endogenous lipase in the mesocarp of the fruit and to ease the detachment of the fruit during the threshing operation. The treatment also helps in weakening the mesocarp fiber to ease in oil extraction process. Because of the presence of high percentage of water (30%) [39] along with TAG in the mesocarp, it is often expected that a
heat-induced thermal hydrolysis will lead to the accumulation of FFA in the CPO. However, this was not the same case as in OPF since no significant FFA accumulation has occurred. This explanation can be supported by Figure 5, showing that there is no increment of FFA in OPF after heat treatment for 60 min. Therefore, heat treatment in water at a temperature between 298 K and 372 K does not generate FFA and its values lie consistently between 0.9 and 1.1%.

![Image](https://via.placeholder.com/150)

**Figure 5.** FFA accumulation ($FFA_1$) in OPF mesocarp after heat treatment for 60 min.

### 4.1.5. Effects of Heat Treatment on in Vivo Activity of the Residual Lipase

After mashing the mesocarp of OPF, the oil leached out of the cell and reacted with lipase in the presence of water. Based on the assay, the lipase activity was measured as the rate of change of FFA during the incubation at 310 K for 10 min. Figure 6 shows an increase trend of lipase activity lost with the increment of the temperature of the hot water. The enzyme completely lost its activity after 60 min treatment at temperature of 333 K and above. At high temperature treatment, the oil globule membrane loss its firmness, thus, the oil is released as previously discussed, but no FFA increase was observed. From here, it is confirmed that lipase has already been inactivated by the hot water treatment especially at temperature above 333 K. This finding established evidence that OPF can be effectively treated at a temperature below the current sterilization process in the palm oil mill, which is typically carried out at 413 K (3 bar).

![Image](https://via.placeholder.com/150)

**Figure 6.** Endogenous lipase activity lost after the treatment at different temperatures (K) for 60 min.

### 4.2. Simulation Results

#### 4.2.1. Parameter Estimation and Model Performance

Parameter estimations for the above presented kinetic models were conducted with the parameter estimation module of gPROMS®. The respective solution algorithm of gPROMS® applies the solution
of the inactivation model for each respective operating condition and compares the simulated lipase activity to the experimental values. The numerical solver for parameter estimations then varies the model parameters according to a combined search and gradient method, and resolves the model again until the maximum likelihood values were obtained.

A typical simulation of the differential equation model (Equation (9)) of residual lipase activity in OPF after heat treatment is shown in Figure 7. PDE of kinetic is temperature dependent model in which PDE for heat transfer to represent the temperature distribution in OPF was incorporated in the dynamic simulation. The activity of lipase was almost zero after 40 min of heat treatment at 343 K. The lipase cannot return to its active state after heating has been discontinued and the fruit returned to environmental temperature (310 K). Furthermore, it can be stated that the residual activity of endogenous lipase OPF can be qualitatively described with this simple model.

![Figure 7. Simulated (line) and measured (points) profiles of relative residual lipase activity in the mesocarp of OPF heat-treated at different temperatures.](image)

The lack of fit test gave a weighted residual of 100.06 and the chi-squared of 107.52. The fact that the weighted residual is less than the chi-squared value is an indication of a good fit. Table 3 exhibits the computed rRMSE between the measured and predicted residual activity. The rRMSE values between 1.57% and 7.70% further confirmed that the experimental data were accurately predicted by the model at 95% confidence level, indicating the model was reliable. The close resemblance of the simulated profile with the experimental data, reveals the adequacy of the physical (shape and size) and thermal (thermal conductivity of mesocarp and kernel) properties of OPF used in the model.

| Water Temp (K) | 308 | 313 | 318 | 323 | 333 | 343 |
|---------------|-----|-----|-----|-----|-----|-----|
| rRMSE (%)     | 1.57| 4.56| 3.17| 5.83| 5.62| 7.70|

The estimated parameters are initial reaction rate constant \(k_{0\text{dec}}\), reaction order \(n_d\) and the inactivation energy \(E_{\text{dec}}\). The optimum values of the parameters are shown in Table 4. The estimated inactivation energy \(E_{\text{dec}}\) of 153,052 kJ/kmol is in close range with 170,000 kJ/kmol reported by Owusu et al. [40] The variation might be as result of the method used (in-vitro) and the aqueous state of the reaction medium. The estimated \(n_d\) of 1.85 shows that the reaction is a non-elementary reaction.
4.2.2. Reaction Rate Constant

In this work, reaction kinetic of the lipase inactivation is temperature dependent and this was represented by reaction rate constant, $k_{dec}$. By using the estimated parameters value, as shown in Figure 8, the $k_{dec}$ was generated by the simulation depicting how it values changing at different temperatures through the Arrhenius model (Equation (10)). Between 300 K and 340 K, $k_{dec}$ shows as an exponential increase, but when reaching above 340 K, its value becomes constant, which is equal to estimated initial reaction rate constant ($k_{0dec}$), 0.035 $U^{-0.85}$/kg-mes$^{-0.85}$/min. The temperature distribution within mesocarp region will influence the $k_{dec}$ value, and therefore, the reaction rate of inactivation of lipase will be affected.

4.2.3. Temperature and Residual Lipase Activity Distribution

A graphical representation of a typical temperature distribution obtained by the simulation using estimated parameters (Table 4) is presented in Figure 9. As it can be observed, the temperature distribution within the fruit attained uniformity 5 min after the commencement of heating irrespective of the set temperature of water. This is very important to ensure uniform and total inactivation of the lipase. The dynamic profile of temperature distribution show that within mesocarp region (7.1×10$^{-3}$ m < $R_m$ < 1.495×10$^{-2}$ m), which is very important region that contain lipase to be inactivated. Very fast temperature distribution in region to reach equilibrium temperature to the set temperature treatment is crucial for the efficiency of sterilization process.

Enzyme activity is measured as the amount of some specific substrate converted or product formed per unit time. This activity, as observed by activity measurements, is a combination of a true concentration of the enzyme, multiplied by its specific reaction rate constant [41]. After the optimum temperature of the enzyme is exceeded, it starts to denature, thereby reducing the amount or concentration of the active enzyme configuration. Eventually, the enzyme loses its activity entirely. The contour plots of the simulated in vivo activity of the endogenous lipase in the mesocarp fiber of OPF immersed in hot water are shown in Figure 10. However, the effect of heat can be observed with variation in the in vivo activity as temperature increases from 318 K to 347 K. The time-effect of

Table 4. Estimated parameters for the kinetic of thermal inactivation of lipase.

| Parameter                  | Value       | Unit                      |
|----------------------------|-------------|---------------------------|
| Initial reaction rate constant ($k_{0dec}$) | 0.035       | $U^{-0.85}$/kg-mes$^{-0.85}$/min |
| Reaction order ($n_d$)     | 1.85        | -                         |
| Inactivation energy ($E_{dec}$) | 153,052    | kJ/kmol                   |
treatment can also be seen, especially in Figure 10D where the in vivo activity in the mesocarp tends towards zero as treatment time increases from 0 to 60 min.

Figure 9. Contour plot of simulated heat distribution within OPF immersed in water at (A—318 K, B—323 K, C—333 K and D—347 K).

Figure 10. Contour plot of the simulated profile of in vivo residual activity of lipase (U/kg-mes·min) in OPF after heat treatment at (A—318 K, B—323 K, C—333 K and D—347 K).

4.2.4. Sensitivity Analysis

The sensitivity analysis was carried to evaluate the sensitivity of the models by changing some selected parameters. Two parameters were investigated, namely; the initial reaction rate constant ($k_{0\text{dec}}$)
and the reaction order \((n_d)\). The nominal value of \(k_{0dec}\) (0.035) was adjusted ±25% and the effect of the changes on the predicted residual activity was plotted in Figure 11. It can be observed that a change as small as 25% in these parameters caused a visible change in the predicted residual activity. A one-way analysis of variance (ANOVA) between the predicted lipase activity when the simulation was run with the nominal value of \(k_{0dec}\) and when adjusted ±25% shows that there is statistically significant difference \((P < 0.05)\) between the 3 sets of data. Hence, the model can be said to be very sensitive to this parameter.

![Figure 11](image1.png)

**Figure 11.** Sensitivity analysis of the developed model to changes in value of \(k_{0dec}\) with respect to predicted in vivo residual lipase activity inside OPF after immersion in hot water at 343 K for 60 min.

The model estimated the reaction order \((n_d)\) to be 1.85. This is a non-integer value hence; the reaction was taken to be a non-elementary reaction. Further investigation was carried out to see if the model will be sensitive to any other values of order. The simulation was re-run with \(n_d\) altered with ±25 from nominal value (1.85) and the resultant lipase activity compared with the nominal lipase activity. Figure 12 is the comparison of the simulated profile of lipase activity with the nominal value and altered values. A one-way ANOVA conducted, and the results reveal that there is statistically significant difference \((P < 0.05)\) between the profiles. This therefore implies that the model is sensitive to any change in reaction order

![Figure 12](image2.png)

**Figure 12.** Sensitivity analysis of the model to changes in \(n_d\) with respect to predicted in vivo lipase activity inside OPF after immersion in hot water at 70 °C for 60 min.
5. Conclusions

The effect of hot water treatment on the oil globules in the mesocarp fiber and pectin layer of the abscission of OPF was studied within a temperature range of 308 K to 373 K. The integrity of the lipid membrane was highly compromised at a temperature between 343 K and 373 K. Within this same range of temperature, the pectin layer was found to have been dissolved completely, leaving a cleavage between the fruit and the pedicel. FFA accumulation within this range of heat treatment was found to be insignificant. However, hot water treatment of OPF has great effect on the residual activity of the endogenous lipase. The kinetics reveals heat inactivation of OPF lipase followed a non-elementary reaction order. Hot water treatment is a viable option for lipase inactivation of OPF, especially within the range of 343 K to 373 K as well as for the destruction of the oil globule membrane and dissolution of pectin architecture in the abscission zone of the fruit.

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Nomenclatures

- $c_E$: Lipase activity (U/kg-mes)
- $c_{E0}$: Lipase activity (U/kg-mes)
- $c_E$: Lipase activity (U/kg-mes)
- $C_{pm}$: Specific heat capacity of mesocarp (kJ/kg·K)
- $C_{ps}$: Specific heat capacity of shell (kJ/kg·K)
- $C_{pk}$: Specific heat capacity of kernel (kJ/kg·K)
- $E_{dec}$: Activation energy (kJ/kmol)
- $FFA$: Free fatty acid (%)
- $h$: Convective heat transfer coefficients (kJ/m$^2$·K·min)
- $k_{dec}$: Inactivation rate constant (U$^{-0.85}$/kg-mes$^{-0.85}$·min)
- $k_{0,dec}$: Initial inactivation rate constant (U$^{-0.85}$/kg-mes$^{-0.85}$·min)
- $M_m$: Mass of mesocarp (kg-mes)
- $N_E$: Number of measured data
- $n_d$: Reaction order
- $P_j$: Value of measured data $j$
- $P_j$: Average value of measured data $j$
- $RA$: Relative activity (%)
- $R_g$: Gas constant (kJ/kmol·K)
- $R_m$: Radius of fruit (m)
- $R_k$: Radius of kernel (m)
- $R_s$: Radius of shell (m)
- $T$: Temperature of fruit (K)
- $T_w$: Temperature of water (K)

Subscripts

- $j$: number of data
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