RESPONSE SURFACE METHODOLOGY: AN EFFECTIVE TOOL FOR THE OPTIMIZATION OF THE EXTRACTION OF VITAMIN E FROM PALM FATTY ACID DISTILLATE BY ENZYMATIC HYDROLYSIS

Kevin Tochukwu Dibia[1*] | Philomena Kanwulia Igbokwe[1] | Christian Oluchukwu Asadu[2]

[1] Department of Chemical Engineering, Faculty of Engineering and Technology, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.

[2] Department of Chemical Engineering, Faculty of Engineering and Technology, Enugu State University of Science and Technology, P.M.B. 01660, Enugu State, Nigeria.

[*] Corresponding Author.

Kevin Tochukwu Dibia

E-mail addresses: tckevyn@gmail.com, tckevyn@yahoo.com

Abstract

Response surface methodology was applied as a valuable tool for the optimization of the extraction of tocopherols from palm fatty acid distillate. The extraction procedure and experimental set-up improved the yield of total tocopherol from an initial value of 0.39% to 4.67%. It was found that extraction by enzymatic hydrolysis followed by neutralization increased the yield of total tocopherol from palm fatty acid distillate. In the optimisation approach, the central composite design was employed as a means of experimental set-up, as analysis of variance and several correlation functions were statistical tools applied in the optimisation of process conditions of enzymatic hydrolysis of palm fatty acid distillate. Water

weight of 60.604 %(v/w), lipase weight of 7.130 %(w/w) and reaction time of 2.713 hrs gave the best (at optimized state) experimental protocol for the enzymatic hydrolysis PFAD in terms of yield of total tocopherol (as vitamin E) with a desirability of unity.

Keywords: Response Surface, Enzyme Hydrolysis, Tocopherols, Vitamin E, Palm Fatty Acid Distillate

1 Introduction

Palm fatty acid distillate is a by-product which is obtained from the deodorization step in the refinery of crude palm oil for human consumption. The deodorization stage is designed to handle the elimination of unwanted substances present in the crude vegetable oil as it operates by stripping of volatile compounds, components, and elements that causes the vegetable oil to deteriorate to a state of instability. Furthermore, this refinery stage removes compounds that confer odour, and make better the flavour of the product by thermal destruction of pigments (de Greyt, 2012). In the deodorization stage, steam distillation which hinders both oxidation of the vegetable oil by contact with atmospheric oxygen and oil hydrolysis, is the main unit operation that take place. The operating range of temperatures in this unit generally lies between 200 and 250℃, and column pressure is under vacuum as low as 10mmHg (Cibelem et al., 2014). Conventionally, distillation columns are designed to yield two product streams – top and bottom streams. The top product stream in this context is the deodorizer distillate while the bottom product stream contains the refined palm oil. Palm deodorized distillate contains mainly sterols, stanols, hydrocarbons, lipids and tocopherols. These substances have their respective uses and applications in science.

In clinical studies sterols and stanols have been discovered to have an effect in reducing plasma cholesterol concentration by inhibiting the absorption of cholesterol from small intestine (Jones et al., 1999; Sierksma et al., 1999).

Lipids are regarded as one of the most elemental nutrients by man because it produces several bioactive molecules that are fundamental mediators of multiple signalling pathways, and they are also indispensable compounds of the cell membranes of living organisms (Jana et al., 2015). The major constituent of lipids is fatty acids, which are grouped according to the presence of the type of bond each possesses in nature. Fatty acid is a hydrocarbon chain, saturated or not, with methyl group at one end and a carboxylic functional group at the other end. Saturated fatty acids have no double bonds, monounsaturated fatty acids have just one single bond, and polyunsaturated fatty acids have more than one bond, but up to six double bonds can be present in their chemical structure. Humans cannot synthesize polyunsaturated fatty acids with the first double bond on C3 and C6 from the methyl-end because of the absence of a proper enzymes for metabolism.

PFAD also contains another valuable product called tocopherols. Tocopherols are class of compounds with vitamin E activity. Until early 1940s, the main commercial sources of vitamin E were crude vegetable oils. Soybean and wheat-germ oil were considered to be the best sources of vitamin E but containing only minute amounts of the vitamin (Siew et al., 2017). Lately, other than crude vegetable oil sources, vitamin E can be extracted from a much cheaper, richer and non-competitive source - the fatty acid distillate (FAD), a residue from refining vegetable oils. This discovery was made by Hickman K.C.D. (Hickman, 1944). The vitamin E levels in FAD are many times greater than that of the original crude vegetable oils from which they are derived.

Free fatty acids and tocopherols can be obtained from palm fatty acid distillate by enzymatic hydrolysis - an extraction method that provides high yields of the product using an appropriate lipase to catalyse the reaction. According to Camilla et al. (2008), hydrolytic lipase-catalysed reaction provides the needed advantage of extracting these substances at lower energy cost and high efficiency with maximum yield. In addition, the application lipase-catalysed systems further concentrate tocopherols and fatty acids from PFAD, and this area have not received much study. In enzymatic operations, water-soluble substrates like proteins enzyme concentration, substrate concentration, temperature of the system, pH and metallic ions present are major factors that determine the rate of enzyme hydrolysis. However, in heterogeneous reactions such as the hydrolysis of FAD, water ratio, agitation speed, orientation and shape of the reactor and the presence of surfactants have large effects on the rate reaction and formation of stable suspension fatty acids and tocopherols in water (Tanaka et al., 1992). In this work, enzymatic hydrolysis was done before applying a neutralization method to pre-concentrate tocopherols by separating it from free fatty acid. Since process variables play a large role for an effective reaction to occur, there is need to optimize the variables in order to have optimum yield of the desired product.

Response surface methodology (RSM) is an acceptable statistical technique that applies quantitative data from appropriate experimental designs to determine and simultaneously solve multivariate equations in order to optimize processes or, increase or decrease the yield of products (Camilla et al. 2008) as the case may be. In practise, the RSM is a design constructed in a way that correlations between the chosen variables are either minimised or totally eliminated thereby allowing the independent estimation of variable effects and their potential interactions. According to Mikko (2017), a response surface model is based on approximating the true behaviour of a response:

$$y = f\ (\beta_1, \beta_2, \dots, \beta_k) + \ \delta \qquad (1)$$

where y is referred the measured response as a function of $(\beta_1, \beta_2, \ldots, \beta_k)$ variables and δ represents other sources of variabilities. The variables are coded to compare their effects within the design range:

$$\theta_i = \frac{(\beta_i - \beta_{min})}{\Delta\beta/_2} - 1 \tag{2}$$

where θ_i denotes a coded value and β_i, β_{min} and $\Delta\beta$ the respective variable value and variable range in their original units. A quadratic regression equation is mainly used to approximate the response y:

$$y = \varphi_0 + \sum_{i=1}^{k} \varphi_i x_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} \varphi_{ij} x_i x_j + \sum_{i=1}^{k} \varphi_{ii} x_i^2 + \varepsilon \tag{3}$$

where φ_0 describe the mean value of y, φ_i, φ_{ij} and φ_{ii} the first order, interaction and quadratic coefficients, respectively, x_i the coded factors and ε the model residual. Most programs operate the matrix notation, and this is expressed as:

$$y = Xb + e \tag{4}$$

where y is an n×1 vector response value, X an n×p matrix of coded values, b is a p×1 vector coefficients and e is an n×1 vector of model residuals. The design matrix X concludes columns for determining the interaction and quadratic coefficients included in Eq. (3).

In terms of the application of RSM, a mathematical prediction of the model for the yields of tocopherol has been developed with regards to the selected experimental parameters. The effect and description of these parameters on the yield of tocopherols have been investigated. In studying oil and fat applications, RSM is well adapted for the determination of the coefficients of a second order model that describes the system parameters and is used as a valuable tool for design optimisation of the extraction of tocopherols and improve its yield.

In this study, RSM was applied to evaluate usefulness in optimizing the effects of several variables pertaining enzymatic hydrolysis of PFAD. Furthermore, the investigation of the relationship among selected process parameters – water weight, lipase weight and reaction time – affecting the yield of tocopherols as the response, and the determination of the optimal conditions for enzymatic hydrolysis with Aspergillus niger lipase for maximum yield of tocopherols are the objectives of this study. The Aspergillus niger is among the most well-known lipase produced and its enzymatic activity is suitable for many industrial applications. In addition, the effect of process parameters (reaction time, water content, lipase concentration, and temperature for the release of FFA and tocopherol during hydrolysis using a lipase were studied as these areas have received very little attention in the course of research.

2 Materials and Method

2.1 Reagents and Sample Collection

All chemicals, reagents, tocopherol and fatty acid methyl ester (FAME) standards, and solvents used were from the Multi-Purpose laboratory, Ahmadu Bello University, Zaria. Fatty acid distillate from palm oil (PFAD) was purchased from Presco Palm Oil Processing Industry located at Obaretin, Edo State in Nigeria. The PFAD sample was stored in the refrigerator at a temperature of ≤4℃ till further analyses.

2.2 Physicochemical Parameters

Physicochemical parameters such as moisture content (MC), acid value (AV), iodine value (IV), peroxide value (PV), saponification value (SV), unsaponifiable matter (USM) of the PFAD samples were determined by the official AOCS methods regarding lipids.

2.2.1 Iodine Value

The traditional method of determining the average number of double bonds in fat or oil makes use of carbon tetrachloride as solvent. This parameter was determined according to the AOCS official method Cd 1-25. 5g of PFAD sample was put in a conical flask which contained 30ml of carbon tetrachloride, and a solution of 10% potassium iodide. The mixture was kept in the dark for about 30mins and the liberated iodine was titrated against 0.1N standard sodium thiosulfate solution using 1% starch solution as indicator.

$$Iodine\ value = \frac{(S - B)\ .N\ .12.69}{W}$$

where S is the sodium thiosulfate solution for back-titration of sample (ml), B is the sodium thiosulphate solution for back-titration of blank (ml), N is the normality of sodium thiosulfate solution, and W is the weight of the sample (g).

2.2.2 Saponification value

The AOCS official method Cd 3-25 was followed for the determination of the saponification value of the FAD sample. About 5g of PFAD was weighed into a round bottom flask and refluxed for 60mins in the presence of 50ml of 95% ethanoic potassium hydroxide. In the end, titration was carried out against standard solution of 0.5N hydrochloric acid using phenolphthalein as indicator.

$$Saponification\ value = \frac{(S - B)\ .N\ .56.10}{W}$$

where S is the 0.5 N HCl solution for titration of the sample (ml), B is the 0.5 N HCl solution required for titration of the blank (ml), N is the normality of HCl solution, and W is the weight

of sample (g). The PFAD sample was saponified with excess of alcoholic potassium hydroxide. Titration with HCL indirectly delivers the amount of KOH consumed.

2.2.3 Determination of Unsaponifiable Matter

Determination of unsaponifiable matter is a rigorous experiment that required a level of accuracy. Experimental procedure according to Lester (1928) was adopted, and this is in conjunction with the ACOS official method Ca 6a-40 and Ca 6b-53.

Dissolved PFAD weighed at 20g was emptied into a round-bottom flask of 300ml. 40ml of ethanol and 10ml of 40% aqueous sodium hydroxide were added to the content of the flask and heated under reflux condenser for one hour. The mixture was rinsed into a litre separating funnel with 150ml of water. It was allowed to cool and later shaken vigorously with 300ml of laboratory grade ether as the separator was closed with a glass stopper. The separation was completed in few minutes. Soap solution formed was extracted twice with 250ml of ether, and washed the combined extracts with 20ml of water. The ethereal solution was transferred into a distilling flask and most of the ether was removed. Several other extractions of soap solution (a sixth time) were performed with 250ml portions of ether, washed and combined with the wash water previously used. The resultant mixture was transferred to the same distilling flask as before, and distilled to small bulk, and the extract was carefully rinsed into a 250ml separating funnel with 100ml of ether. 75ml of ether was poured into another separating funnel for the purpose of re-extraction of all the wash-liquors from the separator. This helped to prevent the loss of any unsaponified matter which dissolved in the soapy washings and of any portions of the upper layer which may have been emulsified in or accidentally run off with the lower layer. The extract was washed again with 5ml of wash liquor, using first water (twice), then 2N sodium hydroxide in 10% ethanol followed by two water washes. This extract

procedure was repeated twice more. Finally, the resulting mixture was washed with 0.5N hydrochloric acid to decompose any remaining soap, including traces of metal (calcium, magnesium, etc.) soap, and to remove the basic impurities often present in the PFAD. About 100ml of water was used to wash out the ethanol from the ether layer. As per precautionary method, any emulsion which appeared at the interface during washing was treated as part of the upper layer, hence it need hardly be mentioned that no lubricant of greasy nature is allowed on the separator taps. The ethereal extract was poured into a suitable weighing flask. Again, the first separator was rinsed with ether from the second. The mixture was distilled to remove water globules that were present, by evaporation of ethanol, and taken to read-off constant weight in a vacuum hot-plate desiccator. About 10ml of ethanol was added, neutralized to phenolphthalein end point, warmed to dissolve the unsaponifiable matter, and titrated with 1N sodium hydroxide, and phenolphthalein was used as indicator.

$$Unsaponifiable\ Matter = \frac{R - (B + F) . 100}{W}$$

where R is the weight of residue (g), F is the weight of fatty acid (g), B is the weight of blank (g), and W is the weight of sample (g).

2.2.4 Determination of Free Fatty Acid Composition

Fatty acids were quantified by gas chromatography using the 789 GC system (Agilent Technology, USA) and 5975C MSD with triple-axis detector (Agilent Technology, USA). HP-5MS capillary was used as column in GC-MS (5% - diphenyl, 95% - dimethylpolysiloxane; 30m length × 0.25mm ID × 0.25μm film thickness).

The temperature program was set up from 50℃ to 250℃ with 4℃/min, both the injector and detector temperature were 280℃, and helium gas was used as carrier gas. The injecting volume was $2\mu l$. Ionization energy EI of 70eV was used for mass spectroscopy detection with a source temperature of 150℃.

For the determination of FFA contained in the sample by gas chromatography, the PFAD samples were prepared by esterification according to the equation:

$$FAD + CH_3OH \xrightarrow{NaOH} Methylester + C_3H_8O_3$$

For GC-MS analysis, the sample containing fatty acids were derivatized by esterification to the more volatile methyl esters by the addition of methanol. 3g of PFAD was melted and heated to 55℃ in a water bath a mixture of $CH_3OH/NaOH$ was added (according to stoichiometry) and stirred for 10mins. After reaction, solution was centrifuged to separate layers. The top layer which contained the FAME sample was collected and diluted with cyclohexane (5ml) and prepared for GC analyses.

Fatty acid methyl ester (FAME) standards were prepared with analytical grade cyclohexane in concentration of solution ($4\mu g ml^{-1}$). For quantification of FAME in the sample, external standard was analyzed in triplicate. The method was subject to validation according to guidelines for validation of chromatographic methods. In this regard, response linearity of the FAs, sensitivity, limit of detection and quantification, recovery and precision of the analytical procedure were all calculated. Calibration curves were made as concentration dependence on the peak area of FAME. For the calculation of the peak area, the ChemStation Integrator software was used. The amount of each FAME was calculated from the concerned calibration curve and mathematically recalculated to wt%.

2.2.5 Determination of Acylglycerols and Slip Melting Point

Quantitative separation of mono-, di, and tri- acylglycerols in the PFAD and HNPFAD samples were determined following standard experimental principles of the American Oil Chemist Society (ACOS, 1990) Recommended Practice Cd 11c-93. The AOCS official methods Cc 3-25 was the experimental method applied to determine the slip melting point of both samples. Total fatty acid component present in the sample was the sum of FFA and acylglycerols.

2.3 Enzyme Hydrolysis of FAD

Enzyme hydrolysis of FAD was conducted in a reactor. The reactor consisted of a Pyrex glass beaker with volume capacity of 600ml, diameter of 9cm and height of 12.5cm, a magnetic stirrer used for agitation was in conjunction with hot plate equipment. This reactor was designed to function as continuous stirred tank reactor (CSTR). The reaction mixture at the beginning of each run constituted 50g melted FAD, distilled water (% v/w) and enzyme lipase (Aspergillus niger) (% w/w). The total weight of the reaction mixture was 150g. The reactor with its content was heated by a temperature-controlled hot plate at 65℃ under controlled stirring of 200 rpm. A piece of aluminium foil was used as a reactor cover to prevent the evaporation of the fluid during the progress of the experiment. Based on design of experiment protocol setup for enzymatic hydrolysis for different reaction of times, the reactions were stopped by filtering out the immobilized lipase with a double-layer cheese cloth. Water contained in the product mixture was separated by centrifugation at 3000 × g for about 2 min. The resulting mixture of lipid complex was contained and stored in a refrigerator for further analysis. The quantities of free fatty acid and vitamin E were determined, and the lipase was not recycled for further use. The quantity of FAD was kept constant.

2.4 Pre-concentration of Vitamin E

Products from enzymatic hydrolysis contained vitamin E (tocols), and extraneous substances such as FFA and acylglycerols. Neutralization of the product stream was carried out to separate and concentrate vitamin E in the product.

Neutralization of PFAD was carried out using a modified version of the method of Wang et al., (1998). Water in the product after reaction time was separated using a separating funnel based on difference in mixture densities. Lipid phase (30ml) was collected and then dissolved in 50 mL of ethanol and then neutralized with 0.5 N sodium hydroxide to the phenolphthalein end-point. Distilled water (50 mL) and 150mL hexane were then added and the sample was shaken for 1 min. The sample was subsequently allowed to stand for phase separation using a separation funnel as well. Hexane layer was separated in a separator funnel. The aqueous phase was re-extracted with an additional 100ml of hexane at least four times. Hexane extracts were then combined, washed at least three times with 150ml portions of distilled water to remove any residual sodium hydroxide and soap and centrifuged at 3000×g for 2min to separate residual water prior to the transfer sample into a 500 ml round-bottom flask, and further heated at 50℃ under vacuum to evaporate any water retained in the sample. The term hydrolysed-neutralized PFAD (HNPFAD) is used to described the lipid sample obtained from this process. The HPLC equipment was used to determine the concentration of tocopherols (vitamin E). Vitamin E concentration expressed in this study was the total concentration of all tocopherol isomers in percentage.

2.5 Extraction and Determination of Vitamin E (Tocopherol)

A known amount (0.05g) of sample (PFAD and HNPFAD) was dissolved in 2 ml of n-hexane and 5μl of the mixture was injected into a HPLC equipment. HPLC analyses for all samples

were performed at the Multi-Purpose Laboratory, located in the department of Chemistry of the Ahmadu Bello University (ABU), Zaria, Kaduna State, Nigeria. Prepared samples were analyzed using the HPLC (Agilent Technologies) at reverse phase mode. The HPLC (Agilent 1100 series) operated at Agilent isocratic condition with a stack configuration of: solvent cabinet, vacuum degasser, HP-1100 pump system, an autosampler, column compartment and fluorescence detector (G1321 A FLD). The column was a Hypersil ODS, 125 mm × 4 mm internal diameter with 5μm particles; a capillary length of 150 mm and 0.17 mm i.d. The mobile phase was a mixture of methanol and water (95:5, v/v) and eluted at a flow rate of 1.0ml/min. The analytical column was set at 290nm excitation wavelength and 325nm emission wavelength. The total separation time was 10mins. The tocopherols were identified by comparison of retention times standards of the α, γ and δ- tocopherols. Peak area was used for quantification. α-, γ- and δ- tocotrienols standards were unavailable and because of this, only tocopherol standards were used for analysis. The sum of concentrations of α, γ and δ-tocopherols was used as the total tocopherol content (vitamin E) of the FAD.

2.6 Tocopherols Quantification

An external calibration was performed prior to analyses of edible plant oil by injecting different concentrations on the column. Standard curves (peak area vs concentration) were calculated by linear regression analysis. Injections in triplicate were made at each concentration for both standards and samples. The calibration curves were constructed using standard solutions of α, β, γ and δ- tocopherols and used for quantification. The total tocopherol content is expressed as milligram per gram FAD (mg/g FAD).

2.7 Data Analysis

Triplicate samples were analysed and the mean values calculated for each physicochemical parameter determined. Mean and standard deviations physicochemical parameters of samples were calculated using MS excel. Identification of FFAs and vitamin E in PFAD and HNFAD samples were performed by retention time and comparison of product component of known FAME standard (palmitic, oleic, stearic, and linoleic acids) with GC-MS analysis.

2.8 Design of Experiment

For response surface design, the following table was the procedure for the experimental setup for central composite design. The design of experiment was adopted to study the responses which are percentage free fatty acid content and total vitamin E content of the hydrolysed sample. The independent variables that determined the experimental system were weight of water (H_2O), weight of lipase, and reaction time. Variables were coded at five levels: -α, -1, 0, 1, and α. The range of values that describe the magnitude of both actual and coded are shown in Table 2.1. In the central composite design, five start-points and six replicate runs were incorporated to provide for estimation of model curvature and allow for the estimation of pure error respectively Chu et al. (2003). All experiments within this setup were done in a random order to minimise the effect of unexplained variability in observed responses due to extrinsic factors. Statistical analyses for the optimization procedure was done using the Design Expert (Version 11) software. A quadratic polynomial was assumed for predicting the responses of the regression models.

Table 2.1 Experimental Protocol for Central Composite Design for enzymatic hydrolysis of palm fatty acid distillate

Independent Variable	**Unit**		**Coded values of variables**			
		$-\alpha$	-1	0	+1	$+\alpha$
			Actual values of variables			
Weight of H_2O	% (v/w)	16.36	30	50	70	83.64
Weight of Lipase	% (w/w)	0.61	3	6.5	10	12.39
Reaction time	Hr	0.9773	2	3.5	5	6.0227

Note: α value is equal to 1.682 in coded form.

3 Results and Discussion

3.1 Physicochemical Characteristics of Palm Fatty Acid Distillate

The physicochemical characteristics of PFAD and HNPFAD are shown in Table 3.1. PFAD was a yellow solid at room temperature with slip melting point (SMP) of 48.11℃. However, a differential scanning calorimetry analysis showed that PFAD was completely melted at 60℃.

Although lipase maximum activity was in the range of 70 - 80℃ (according to manufacture's specifications), hydrolysis was carried out at 65℃ in the study for optimum productivity of repeated and long-time uses of the lipase. The selected reaction temperature was reasonably high for lipase activity and to melt PFAD completely, yet, low enough to minimize degradation of vitamin E. SMP has a link with free fatty acid. PFAD contained high levels of FFA, especially palmitic acid and this explained the high SMP of PFAD. Levels of mono- and diacylglycerols in PFAD were 4.85% and 6.95% respectively, while triacylglycerol were almost undetectable. As a result of FFA removal by neutralization SMP value of HNPFAD was lower than the value of PFAD.

Table 3.1 Physicochemical Characteristics of PFAD and HNPFAD

		Sample	
Parameter	**Unit**	**PFAD**	**HNPFAD**
Slip melting point	℃	48.11 ± 0.66	38.62 ± 0.73
Free fatty acid	**%**	84.72 ± 0.08	*Not detected*
Monoacylglycerol	**%**	4.85 ± 0.12	72.15 ± 0.42
Diacylglycerol	**%**	6.95 ± 0.33	13.47.06 ± 1.51
Triacylglycerol	**%**	*Trace*	*Trace*
Total Fatty Component	**%**	96.52 ± 0.53	89.21 ± 1.93
Iodine Value	$g\ of\ I_2/100\ g\ of\ oil$	55.30 ± 0.21	43.83 ± 0.15
Saponification Value	$mg\ of\ KOH/100\ g\ of\ oil$	219.05 ± 0.03	142.65 ± 0.11
Unsaponifiable Matter	**%**	2.26 ± 0.11	10.35 ± 0.13
Total Vitamin E (as tocopherols)	**%**	0.39 ± 0.04	4.67 ± 0.08
Initial Concentration of Vitamin E	**%**	0.41	2.45

Note: values were reported as mean ± standard deviation.

Changes in the fatty components also affected the iodine value and the saponification value of the original PFAD sample. Iodine value is defined as the mass of iodine in grams absorbed by 100g of fat/oil. More so, it is a measure for the average number of double bonds – the extent of saturation – found in the fatty acids contained in the fat or oil (Chang et al., 2016). Double bonds within the fatty acid structure have halogen affinity, with any double bond consuming 1mol of halogen. The average number of double bonds can then be concluded from the halogen consumption (here: iodine). In Table 3.1, the mean iodine value (IV), which was empirically determined was registered as 55.30 $gI_2/100g$. This IV was consistent with the mean values reported by Chang et al. (2016). Saponification value SV describes the amount of alkali

(potassium hydroxide, KOH) required to saponify (neutralise) a definite quantity of fatty acids resulting from the complete hydrolysis of one gram of fat or oil as all fatty acids cleave one molecule of KOH. Indirectly, the SV is a measure of the average molecular weight of the triglycerides present in the fat or oil and therefore a characteristic number. In this study, the mean SV recorded in Table 3.1 was determined to be 219.05 mgKOH/g of oil and it is slightly higher than the mean SV reported by Chang et al. (2016). Elsewhere in the literature, the mean SV revealed from the studies made by Bonnie and Mohtar (2009) was higher than the value recorded in Table 3.1. Observed variation in SV was as a result of method in which the feedstock was processed, storage time of the feedstock and/or PFAD sample, and storage conditions. In the later, elevated temperatures and light contributed to the transformation of fatty acids to carbonyl compounds which reduces the FFA content in the fat and hence lower the SV. Both IV and SV were lower in NHPFAD when compared to PFAD due to the removal of FFA by hydrolysis followed by neutralization.

PFAD contained a small amount of unsaponifiable matter. The Unsaponifiable matter (USM) is defined as the oily (petroleum-ether soluble) matter which cannot be converted into soap after saponification (Abdulkadir and Jimoh, 2013). USM consist of some biological compounds such as phytosterols, vitamin E (tocopherols and tocotrienols), hydrocarbons like squalene etc. These group of compounds are available in trace amounts contained in vegetable oils, the feedstock to FADs. These substances are of pharmaceutical and nutritional importance and of high commercial value. In Table 3.1, the USM of the PFAD sample was recorded as 2.26%. The result was lower than those reported by Kifli (1983). An increase in the content of USM from 2.26% (contained in PFAD) to 10.35% after hydrolysis and neutralization was observed, and this in turn increased the vitamin E concentration from 0.39% in the original PFAD to 4.67%. Thus, these processes concentrated the vitamin E content of the oil by 11.97 times the concentration of vitamin E in the original PFAD.

Neutralization of PFAD increased mono- and diacylglycerol percentages with diacylglycerols as with diacylglycerols as the main fatty component. Percentage of triacylglycerol was almost undetected in HNPFAD sample. The net change of lipase hydrolysis in PFAD sample was to hydrolyse the di- and tri- acylglycerols into FFA and monoacylglycerol as the main fatty component in HNPFAD sample. Levels of total fatty component in HNPFAD sample was lower than that of PFAD because of the transformations in fatty acids and acylglycerols.

Free fatty acids FFAs are straight-chain carboxylic acids (either saturated or unsaturated) derived from the hydrolysis of fat or oil, or synthesised in vivo and found as three main esters – triglycerides, phospholipids, and cholesteryl esters. Its straight-chain falls under the aliphatic class of hydrocarbons (Moss et al., 1987). The mean FFA value empirically determined in this study was found to be 84.72%. This value was higher than the mean FFA value reported in the study made by Chang et al., (2016). Bonnie and Mohtah (2009), Moh et al., (1999) and Kifli (1983) carried out similar study as well. In their study it was observed that the mean value of FFA was also less than that also reported in this work (Table 3.1). This indicated that samples are subject to the following factors: processing techniques, such as hydrogenation and lipid modification through traditional plant breeding of parent stock, genetic transformation (Gunstone, 2002) aimed to improve oxidative or functional properties, the geographical and climatic conditions where the traditional plant (parent stock) was grown.

Fatty acid composition of PFAD sample was determined using gas chromatography equipment in conjunction with a mass spectroscopy detector, after preparing sample methyl esters by a method known as derivatisation. Based on the comparison of the retention times measured by the analysis of the analytical standard and by that of the sample, it was possible to identify the fatty acids contained in the PFAD sample. Figure 3.1 shows the chromatogram of the derivatised fatty acid in relation to peak value (abundance) and retention time (min).

Table 3.2 is an array of compounds the composition of FFA profile with their respective value. From the PFAD sample, FFA profile included lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), two isomers of oleic acid (C18:1; n9 and C18:1; n11), two isomers of linoleic acid (C18:2; n9 and C18:2; n12), eicosenoic acid (C20:1; n11), arachidic (or eicosanoic) acid (C20:0) and docosanoic acid (C22.0). These fatty acids were grouped into two distinct classes, the saturated and the unsaturated fatty acids respectively, and also the unsaturated class was further broken down to give two subclasses – monounsaturated and polyunsaturated fatty acids respectively as shown in Table 4.2.

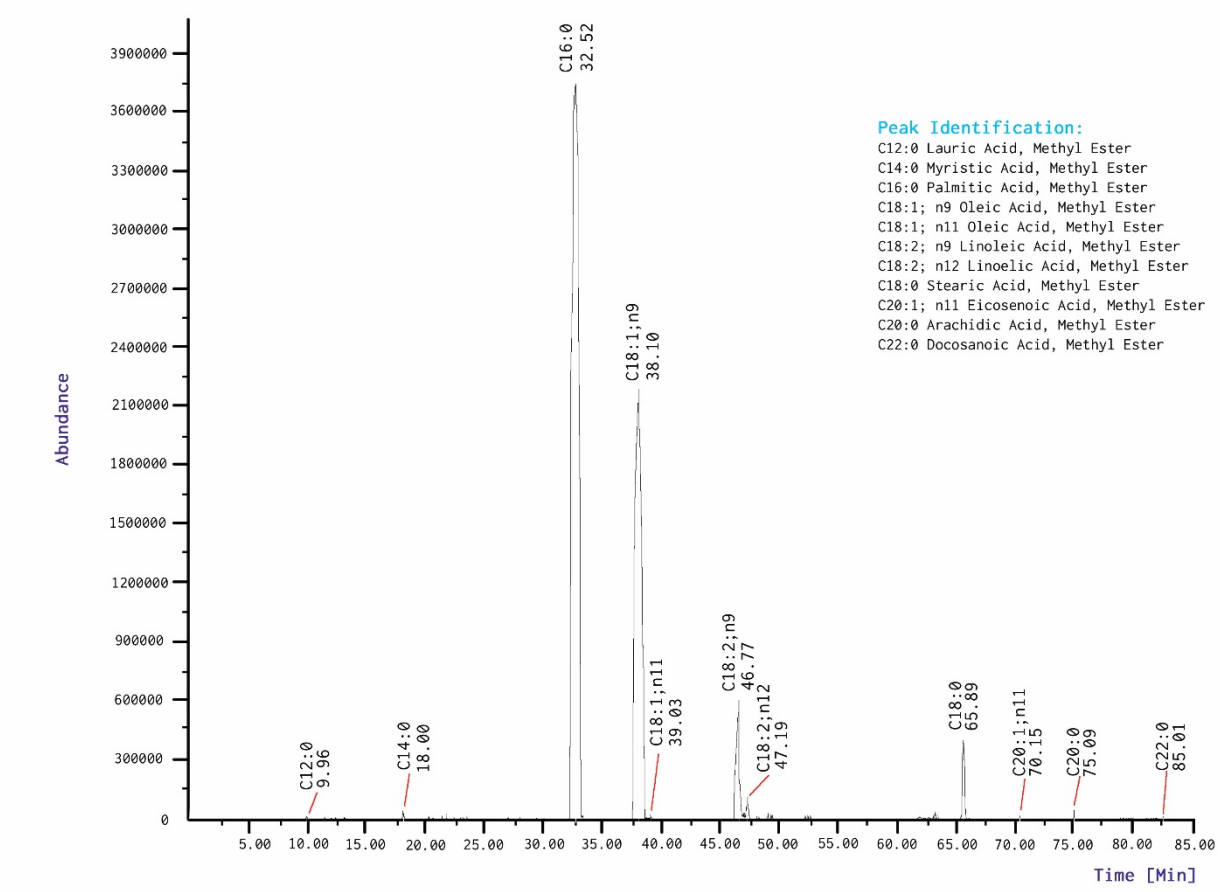

Figure 3.1 GC-MS data for derivatised PFAD

Palmitic acid (C16:0) was found to be the dominant fatty acid in the PFAD sample with the longest peak (Fig. 4.1) and largest percentage value of 46.63. It belongs to the saturated class

of fatty acids. Next is the oleic acid (C18:1; n9), under the unsaturated fatty acids and belonging to the monounsaturated fatty acid subclass was found to be the fatty acid valued at 32.30%. The values of palmitic acid and oleic acid in Table 3.2 were found comparable with values from different reported studies ranged between 35 – 60% for palmitic acid and between 15 – 46% for oleic acid (Chang et al., 2016; Bonnie and Mohtah, 2009; Moh et al., 1999 and Bostinestiean et al., 2012).

All major fatty acids have been identified by many authors and there are inherent differences in individual contents of fatty acids when compared to the bibliographic references. These inconsistencies are ascribed to the nature of cultivars used, the cultivation or environmental factor, and process conditions of refinement of feedstock.

Table 3.2 Mass spectroscopy of identified compounds from derivatised PFAD

No.	Chemical Formula	Fatty Acid	Group	Molecular Weight (g/mol)	Retention Time (min)	Area	Area (%)
1	C12:0	Lauric Acid	SAFA	200.32	9.96	128830.875	0.05
2	C14:0	Myristic Acid	SAFA	228.37	18.00	669920.550	0.26
3	C16:0	Palmitic Acid	SAFA	256.43	32.52	119890012.300	46.53
4	C18:1; n9	Oleic Acid	MUSFA	282.46	38.10	83224745.250	32.56
5	C18:1; n11	Oleic Acid	MUSFA	282.46	39.03	927583.300	0.36
6	C18:2; n9	Linoleic Acid	PUSFA	280.45	46.77	21540522.300	8.36
7	C18:2; n12	Linoleic Acid	PUSFA	280.45	47.19	4328717.400	1.68
8	C18:0	Stearic Acid	SUFA	284.48	65.89	25225085.330	9.79
9	C20:1; n11	Eicosenoic Acid	MUSFA	302.51	70.15	77298.525	0.03
10	C20:0	Arachidic (Eicosanoic) Acid	SAFA	312.53	75.09	618388.200	0.24
11	C22:0	Docosanoic Acid	SAFA	340.58	85.01	360726.40	0.14

Regarding groups and ratios between the different classes of fatty acid, it can be found in Figure 3.2 that the saturated fatty acid (SFA) had the combined value of 57.01% and the unsaturated fatty acid (USFA) had an aggregated value of 42.99% of which the monounsaturated fatty acid (MUSFA) subclass was dominant.

The ratio of saturated to unsaturated fatty acids (SFA:USFA) was calculated as 1.33. Ratio of this sort represented the relation between two major fatty acid groups and value 1.33 indicated that a high proportion of fatty acid profile of the PFAD sample was composed of SFA when compared with USFA as seen in Fig 3.2.

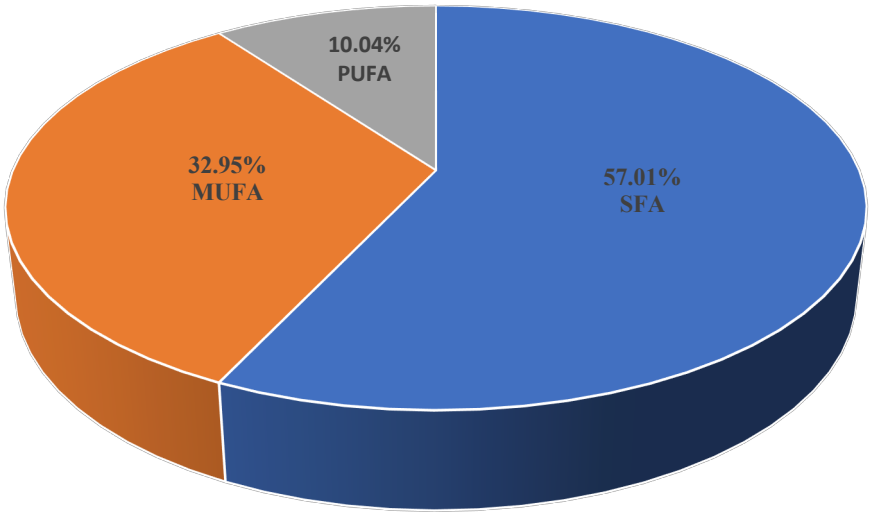

Figure 3.2 PFAD fatty acid groups

3.2 Effect of Process Variables on Enzymatic Hydrolysis of PFAD

Effects of process variables were studied in line with optimization results. To study the effect of one variable, other variables are held constant.

3.2.1 Reaction Time

Figure 3.3 shows the changes in FFA content and the concentration of vitamin E extracted during course of enzymatic hydrolysis of PFAD with 70% v/w of water, by Aspergillus niger lipase at 65℃. The lipase concentration of 10% w/w hydrolyzed the acylglycerols in the PFAD rapidly for the first 6hrs and FFA in the reaction mixture increased from an initial content of 88.72% to 93%. The hydrolysis reaction achieved its equilibrium after 5hrs of reaction time and the FFA levels in the resulting mixture reached a maximum of about 93.31%. similar pattern of changes was observed in the concentrations of vitamin E extracted. Initial concentration of vitamin E in the PFAD was 0.41%, and neutralization of PFAD concentrated to 2.45%. Hydrolysis of the acylglycerols in the PFAD further increased the concentration of vitamin E extracted from the hydrolyzed PFAD from 2.45 % to 6.99% after 5hr of reaction time. Reaction was carried out at temperature of 65 $\pm$ 1 ℃. It was observed that at increased reaction time beyond 5hr, the concentrations of vitamin E began to decrease, shrinking back gradually. This may be caused by the oxidation of some vitamer within the vitamin E complex (or a portion of the vitamin E) released due to prolonged heating time. On the other hand, FFA released began to drop very sluggishly at prolonged time (>5hr). This may be attributed to the limitation of active sites on the PFAD available for lipase to release more FFAs during hydrolysis.

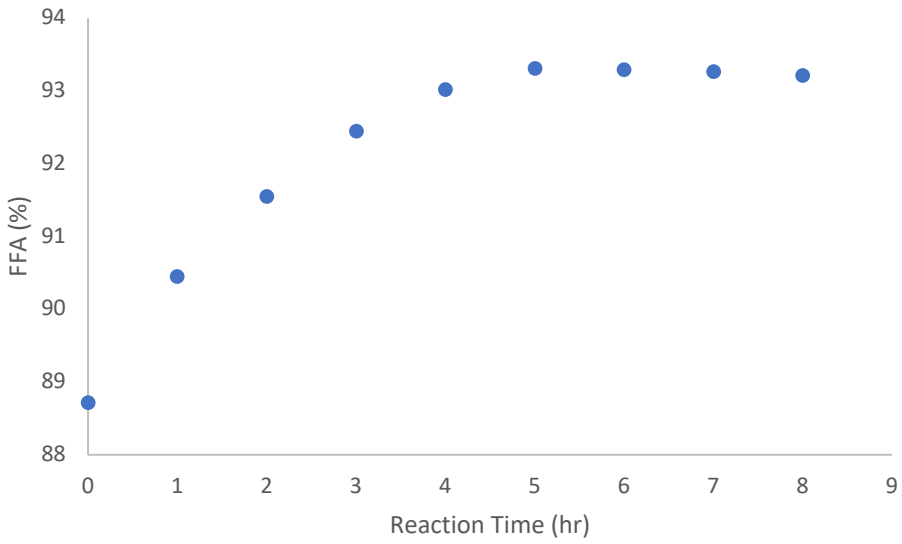

Figure 3.3 Effect of changes in FFA content as a function of reaction time

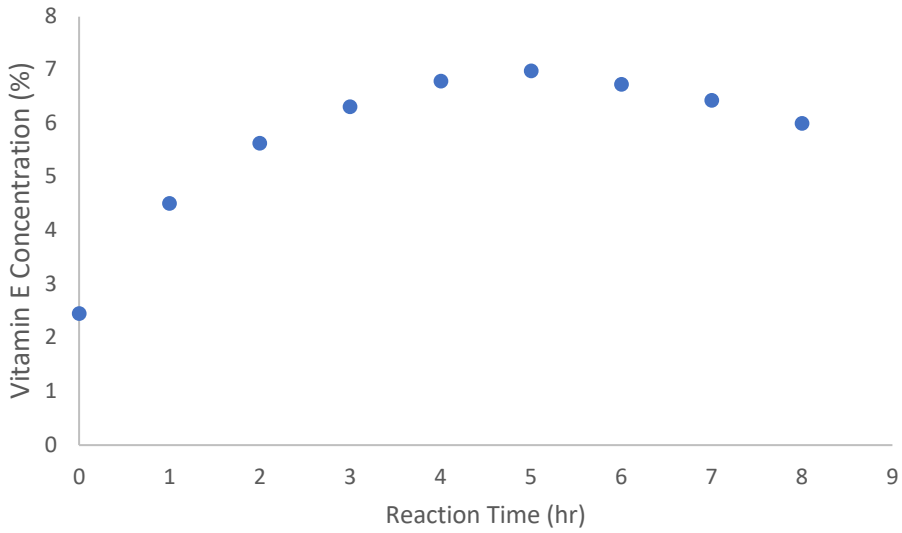

Figure 3.4 Effect of changes in FFA content as a function of reaction time

3.2.2 Effect of Water Content

Table 3.2 reveals the changes in FFA content and concentration of vitamin E extracted from the reaction mixtures with different water contents. Following optimization result, the system

was set up at reaction time of 5hr at 65±1 ℃ with lipase concentration of 10% w/w. PFAD was hydrolysed most effectively at water content of 70% v/w, and at this point FFA content in the resulting mixture was recorded as 93.94%, and vitamin E extracted from PFAD was 6.69%. In the system, lipase catalysis, hydrolysis and esterification were reactions that occurred simultaneously. Large amount of water was necessary to shift the equilibrium favouring hydrolysis reaction. However, the degree of hydrolysis decreased with more than 70% v/w of water. This phenomenon is attributed to low enzyme concentration in relation to available water phase as a result of large amount of water. Similar trend was observed in the study made by Chu et al. (2002). Levels of vitamin E extract also decreased significantly from 6.96% to 4.88%, when water content increased from 70 % v/w to 80% v/w.

Table 3.2 Changes in percentages of free fatty acid (FFA) and vitamin E extracted as a function of water content in the reaction mixture.

Water Content (%v/w)	**FFA Content (%)**	**Vitamin E Content (%)**
20	89.32 ± 0.27	2.89 ± 0.13
30	89.94 ± 0.28	3.31 ± 0.05
40	90.81 ± 0.15	3.95 ± 0.36
50	92.42 ± 0.11	4.56 ± 0.09
60	92.87 ± 0.13	5.67 ± 0.41
70	93.94 ± 0.28	6.96 ± 0.25
80	90.96 ± 0.09	4.88 ± 0.18

3.2.3 Effect of Lipase Concentration

Table 3.3 shows the FFA content of PFAD and concentration of vitamin E, as a function of lipase concentration in the reaction mixture. The system parameters were set at a temperature of 65 ± 1 ℃, with 70% v/w water content and reaction time of 5hr. FFA content liberated and vitamin E extracted increased steadily as the amount of lipase used for hydrolysis increased. In series of hydrolysis performed, the peak values of FFA content liberated and vitamin E extracted were 93.48% and 7.04% respectively at lipase concentration of 10.5%, and beyond this, hydrolysis extent did not increase much even though more lipase was used. This could be caused by unavailability of substrate, or the number of units of substrates per unit of lipase was relatively low when the concentration of lipase increased. Similar changes were observed in vitamin E concentration extracted from the samples. Vitamin E initially increased significantly with increase of FFA levels in the PFAD when lipase concentration in the reactant increased from 0.5 to 10.5% w/w. Further increase in lipase concentration to 13.5% did not significantly increase the vitamin E concentration extracted from the samples.

Table 3.3 Changes in percentages of free fatty acid (FFA) and vitamin E extracted as a function of lipase concentration in the reaction mixture.

Lipase Concentration (%)	FFA liberated (%)	Vitamin E extracted (%)
0.5	88.95 ± 0.15	3.42 ± 0.01
2.5	90.14 ± 0.44	4.88 ± 0.02
4.5	91.32 ± 0.23	5.83 ± 0.08
6.5	92.01 ± 0.12	6.41 ± 0.01
8.5	92.98 ± 0.01	6.95 ± 0.16
10.5	93.48 ± 0.03	7.04 ± 0.11
12.5	92.67 ± 0.01	6.79 ± 0.05
14.5	90.51 ± 0.03	4.02 ± 0.14

3.2.4 Effect of Temperature

The effects of reaction temperature on hydrolysis of PFAD and vitamin E concentration are shown in Table 3.4. Data from the optimization result (water content of 70% w/w, reaction time of 5hr, and lipase concentration of 10% w/w) was maintained to study this effect on extent of PFAD hydrolysis. FFA levels gradually increased when the reaction temperature increased to 65℃. Vitamin E extracted also increased significantly from an initial value of 4.35% at 50℃ to 7.09% at 65℃. Between 65℃ to 70℃, the FFA content attained high values (93.88% and 93.04% respectively) and these temperature values signified the optimum temperature for the lipase hydrolytic activity. Increase in temperature up to 70℃ may be responsible for the increase the FFA content and vitamin E content extracted, both in the product, by shifting the equilibrium of the reaction to favour a faster hydrolysis. However, vitamin E concentration extracted from the PFAD decreased significantly when was temperature increased. High

temperatures (>70℃) might have oxidized the tocols thereby reducing the output of the reaction and recovery. Furthermore, elevated temperature may have inactivated the lipase which yielded reduced contents of FFA and vitamin E even though the lipase has been immobilized.

Table 3.3 Changes in percentages of free fatty acid (FFA) and vitamin E extracted as a function of reaction temperature.

Temperature ℃	**% FFA**	**% Vitamin E**
50	88.25 ± 0.81	4.35 ± 0.03
55	90.10 ± 0.66	5.56 ± 0.02
60	91.82 ± 0.10	6.08 ± 0.54
65	93.88 ± 0.50	7.09 ± 0.33
70	93.04 ±0.01	6.87 ± 0.25
75	90.33 ± 0.22	5.49 ± 0.61
80	89.01 ± 0.41	5.33 ± 0.50
85	88.09 ± 0.02	4.32 ± 0.22

3.3 Optimization of Enzymatic Hydrolysis Process

Response surface methodology (RSM) was the technique applied to optimize the production of tocopherol concentrates from PFAD from the enzymatic hydrolysis process. Several statistical tools within the RSM were systematically utilized to evaluate the effects of process variables and the interactions among them. Out of many designs available in the RSM, the central composite design (CCD) was particularly used to optimize the enzymatic hydrolysis process, and responses were predicted using the optimization functions of the Design Expert (version 10) software.

For detection of tocopherol, the optimization of enzymatic hydrolysis process set-up is shown in Table 2.1 and the experimental array is displayed in the next section of this work (in terms of coded values).

3.3.1 Experimental Data Analysis

Experimental data analysis makes use of statistical methods and tools for data evaluation and useful to give overall report trend. It also tells how well a range of data points fit a regression model. Several tests and calculations were necessary to prove how adequate a model is in describing the system.

Analysis of experimental data is appropriate when the fit summary, model and analysis of variance (ANOVA) are statistically significant and adequate in describing the extent to which the response depends on factor variable. Optimization of process parameters was made viable from the response surface plot gotten from analysis of data.

Table 3.4 Model Fit Summary

Source	Sequential p-value	Adjusted R-Squared	Predicted R-Squared	Remark
Linear	0.0245	0.3288	0.1547	
2FI	0.5115	0.3038	-0.3657	
Quadratic	**0.0001**	**0.8755**	**0.4980**	**Suggested**
Cubic	< 0.0001	0.9990	0.9298	Aliased

3.3.2 Analysis of Variance

Analysis of variance (ANOVA) is a technique that use test-based variance ratios to determine whether or not significant differences exist among means of several groups of observations,

where each group follows a normal distribution. ANOVA also extends to t-tests used to determine whether or not two means differ in the case where there are three or more means.

The analysis of variance for response surface quadratic model for response 4 (total tocopherol content; as vitamin E) was computed as shown in Table 3.5, as it illustrates partial sum of squares of the third type. Results were analysed and compared to know which factor or factors are significant model terms.

Table 3.5 Analysis of Variance (ANOVA) for Response Surface Quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remark
Model	34.17	9	3.80	15.85	< 0.0001	***Significant***
A-H2O weight	*1.77*	*1*	*1.77*	*7.39*	*0.0216*	
B-Lipase weight	*4.67*	*1*	*4.67*	*19.49*	*0.0013*	
C-Reaction time	*9.46*	*1*	*9.46*	*39.49*	*< 0.0001*	
AB	*0.028*	*1*	*0.028*	*0.12*	*0.7412*	
AC	*9.113E-003*	*1*	*9.113E-003*	*0.038*	*0.8493*	
BC	*3.21*	*1*	*3.21*	*13.42*	*0.0044*	
A^2	*9.29*	*1*	*9.29*	*38.79*	*< 0.0001*	
B^2	*4.33*	*1*	*4.33*	*18.09*	*0.0017*	
C^2	*4.17*	*1*	*4.17*	*17.40*	*0.0019*	
Residual	2.40	10	0.24			
Lack of Fit	*2.40*	*5*	*0.48*			
Pure Error	*0.000*	*5*	*0.000*			
Cor Total	36.57	19				

The model F-value of 15.85 implied the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Furthermore, the values of "Prob > F" less than 0.0500 exposed the significant model parameters. In this case parameters A, B, C, BC, A^2, B^2 and C^2 are significant model terms (Table 3.5). Still on parameter significance to the model

build-up, inter-parameter model term *BC* and the intra-parameter model terms (also regarded as squared parameters) - A^2, B^2 and C^2 were parameters that fully project the power of the model. In the ANOVA above, *p*-values greater than 0.1000 indicated model terms were not significant, hence other interactive factors such as *AC* and *AB* fall into this category.

Lack of fit is the variation of the data around the fitted model. If the model does not fit the actual response behaviour, this will be significant thus the model would not be used as a predictor of the response. On the other hand, pure error sum of squares is a sum of squares calculated for residuals of repeated runs only; used as a measure of experimental error. Table 3.5 displays the values of the sum of squares and the mean square of the pure error as 0.000 respectively. There is no *p-value* and *f-value* computed for model lack of fit and pure error tests. This is because there is no variation in the response values between replicated runs (design points).

Table 3.6 Correlation Functions

Standard Deviation	0.49
Mean	5.13
C.V. %	9.54
PRESS	18.36
-2 Log Likelihood	14.31
Coefficient of Determination (R-Squared)	0.9345
Adjusted R-Squared	0.8755
Predicted R-Squared	0.4980
Adequacy Precision	10.893
BIC	44.27
AICc	58.76

The R-Squared and the Adjusted R-Squared correlation functions were used in this study to check the model adequacy. The coefficient of Determination (R-Squared) statistically expresses the explanatory power of the variables in the model. In Table 3.6, the Coefficient of

Determination (R-Squared) of 0.9345 indicated that more than 93% of the experimental data explained the independent variables and thus described the model more explicitly. Adjusted R-Squared topples the R-Squared and adjusts the number of variables included in the response surface quadratic model, leading to higher accuracy and precision in describing the model. An Adjusted R-Squared of 0.8755 indicates that an addition of a new model term (or some more model terms) will improve the model by about 87% more likely than would be expected by chance. The Predicted R-Squared of 0.4980 was not close to the Adjusted R-Squared of 0.8755. The difference was more than 0.2 and this indicated large block effect in the response surface quadratic model. Adequacy Precision measures the signal to noise ratio. A ratio greater than 4 is desirable, therefore from Table 3.6, the ratio of 10.893 indicated an adequate signal and hence, this quadratic model could be used to navigate design space.

Table 3.7 Model Coefficients

Factor	Coefficient Estimate	Df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	6.42	1	0.20	5.98	6.87	
A-H_2O weight	0.36	1	0.13	0.065	0.66	1.00
B-Lipase weight	0.58	1	0.13	0.29	0.88	1.00
C-Reaction time	0.83	1	0.13	0.54	1.13	1.00
AB	0.059	1	0.17	-0.33	0.44	1.00
AC	0.034	1	0.17	-0.35	0.42	1.00
BC	0.63	1	0.17	0.25	1.02	1.00
A^2	-0.80	1	0.13	-1.09	-0.52	1.02
B^2	-0.55	1	0.13	-0.84	-0.26	1.02
C^2	-0.54	1	0.13	-0.83	-0.25	1.02

CI, Confidence Interval; VIF, Variance Inflation Factor; df, Degree of Freedom

Proper evaluation of experimental data consists of using statistical methods and tools that offer freedom to evaluate complex hypothesis (Deluba and Olive, 1996). In this study, regression

analysis and multivariate statistics were applied to describe the effects of variables on the yield of tocopherol. As already known, water weight (A), lipase weight (B) and reaction time (C) were the selected independent variables, hence depicting a multivariate system in which multivariate multiple regression analyses (results shown in Tables 3.5 and 3.6) were applied to predict the statistical relationship between the response variable (total tocopherol, as vitamin E). The response surface model has been developed to have the coexistence of both the interacting and non-interacting model variables along with their respective coefficients. Considering the results from both Tables 3.5 and 3.6, coefficients of model terms were estimated and summarized in Table 3.7 in terms of confidence interval, degree of freedom, and variance inflation factor. Therefore, the final equation in terms of coded factors is as follows:

$$\textbf{Total Tocopherol (as Vit. E)} = 6.42 + 0.36A + 0.58B + 0.83C + 0.059AB + 0.034AC + 0.63BC + 0.80A^2 - 0.55B^2 - 0.54C^2 \quad (3.1)$$

The equation in terms of coded factors could be used to make predictions about the response for given levels for each factor. High levels were coded as +1 and the low levels of the factors were coded as -1. The coded equation was useful for identifying the relative impact of the factors by comparing the factor coefficients. Furthermore, in Eqs. 3.1**,** the coefficients were scaled to accommodate the units of each factor and the intercept was not at the centre of the design space.

3.3.3 Response Surface Plots

In this study, process parameters were optimized through response surface quadratic model (RSQM), and contour plots in form of 2D and 3D were used to describe the model. These plots

show the interactions between two variables in the optimized phase thereby providing several solutions by the combination of parameters that could yield the best optimum results for this type of enzymatic hydrolysis of PFAD process. Figures 3.3 to 3.5 are the pictorial representations of two-factor interactions describing the RSQM. Here, the gridded contour plots were generated to interpret the response variable (total tocopherols) in a rectangular coordinate system. The plots show a 3-D surface in 2-D with contour lines delineating changes in the 3-D space. The contour lines represent lines of equal response value and the profile was visualized as response contours of two-factors at a time scenario. The remaining predictors contributing least to the response are fixed at a given point. For instance, in Fig. 3.3, lipase weight and water weight were fixed at one base to extract result from the prediction profiler. Similarly, Figures 3.4 and 3.5 were depicted in the same manner.

In Figures 3.6 to 3.8, the gridded surface plots are representations of the surface plots in actual 3-D space. The response surface model with the incorporation of the second order effects of non-linear relationship was the optimisation technique to determine the yield of tocopherols during the enzymatic hydrolysis of PFAD, therefore the plots determined the optimum operating conditions reaching maximum from the best-fitted model with a map of contour lines, followed a direction of movement along the path of maximum response.

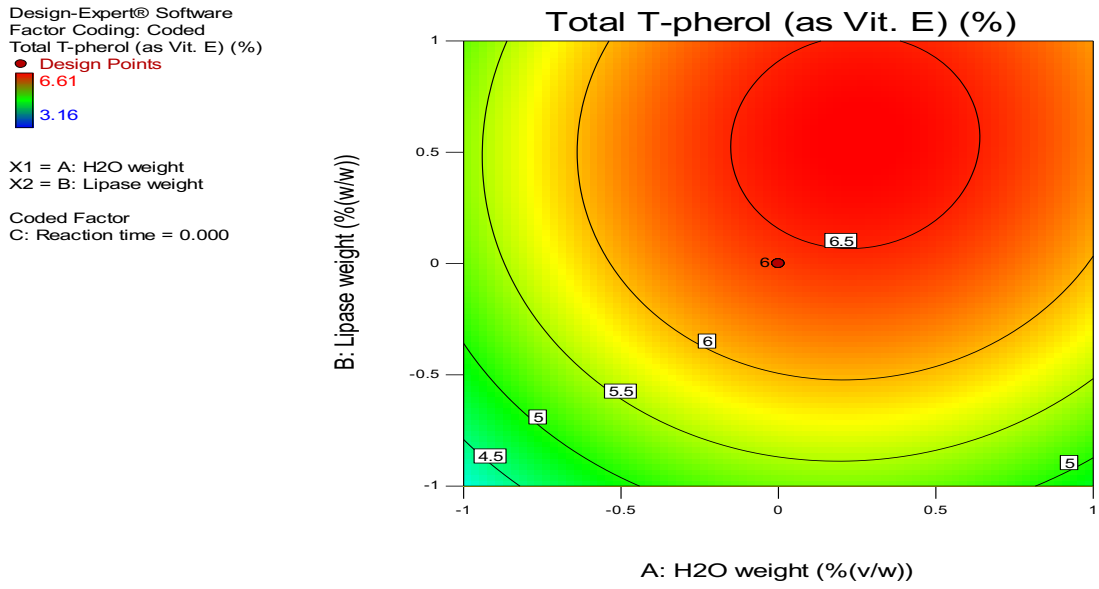

Figure 3.3. 2D Contour plot of the interaction of lipase weight (%w/w) & H_2O weight (%v/w).

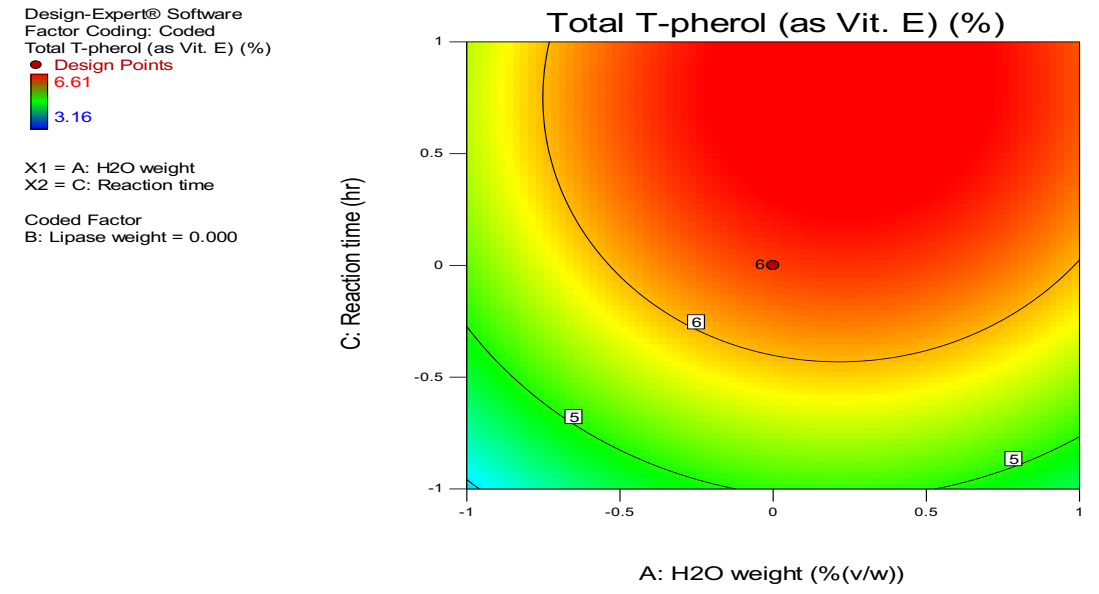

Figure 3.4. 2D Contour plot of the interaction of reaction time (hr) & H_2O weight (%v/w)

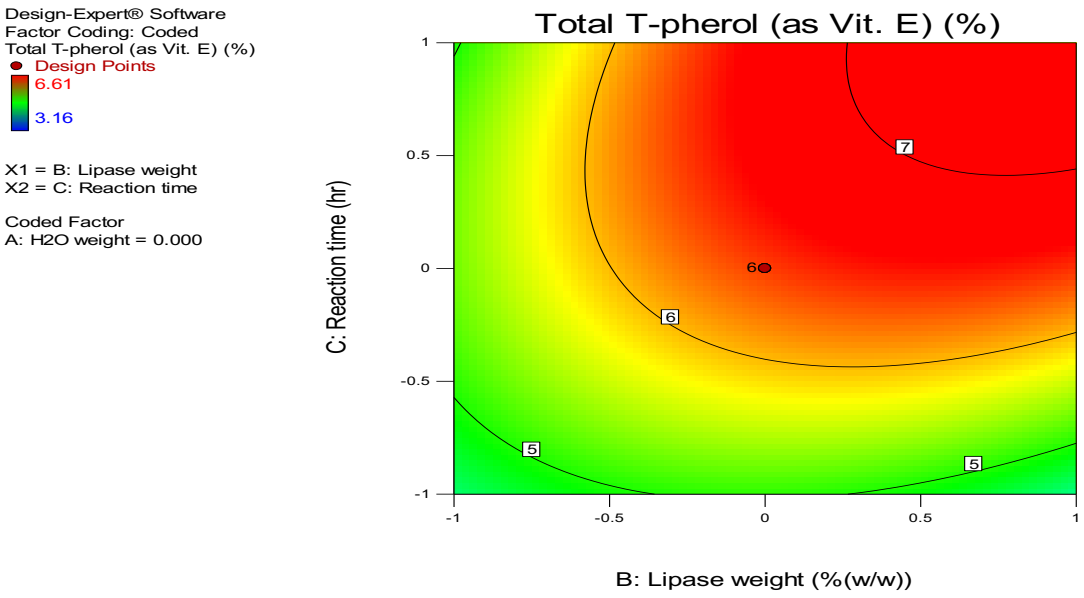

Figure 3.5. 2D Contour plot of the interaction of reaction time (hr) & lipase weight (%w/w)

The response surface model with the incorporation of the second order effects of non-linear relationships was the technique used to determine the best possible combinations of variables to predict the yield of tocopherols in this study. The grided surface plots shown in Figures 3.6 to 3.8 are the representations of the surface plot in 3-D space. According to Soumya (2017), the plots determining the optimum operating conditions reaching maximum from the best-fitted model with a map of contour lines, follow a direction of movement along the path of maximum response. Hence, for the response surface plots, responses are derived from the peak of the contour surfaces with respect to two underlying variables at a particular instaance as shown in Fig. 3.6, 3.7 and 3.8 respectively.

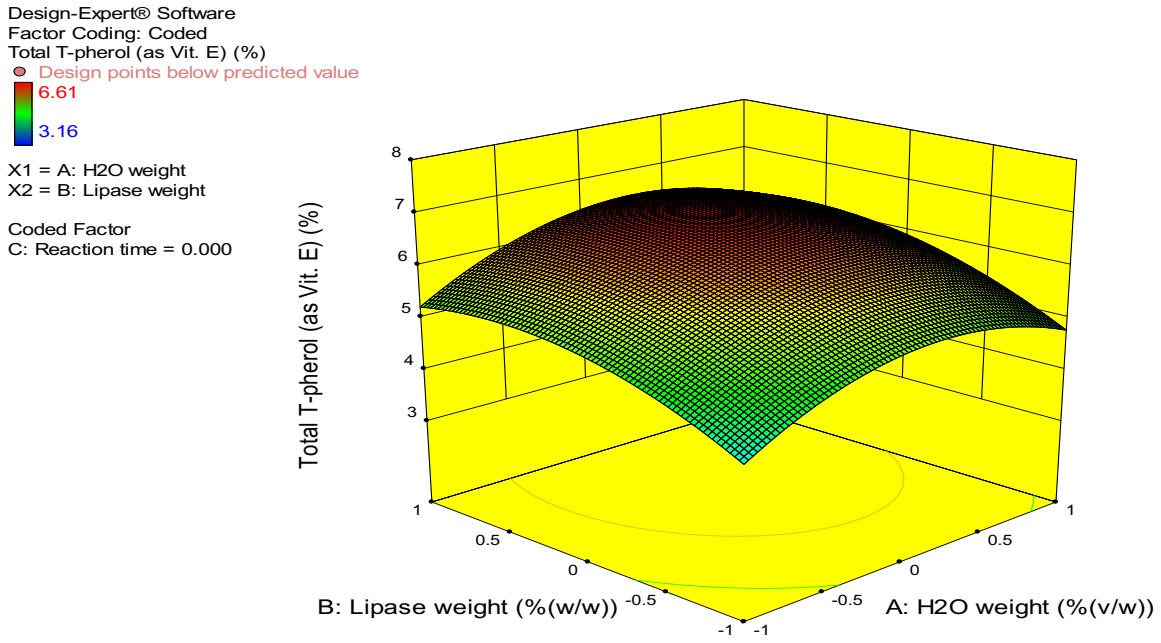

Figure 3.6. 3D Response surface plot of the interaction of reaction time (hr) & lipase weight (%w/w)

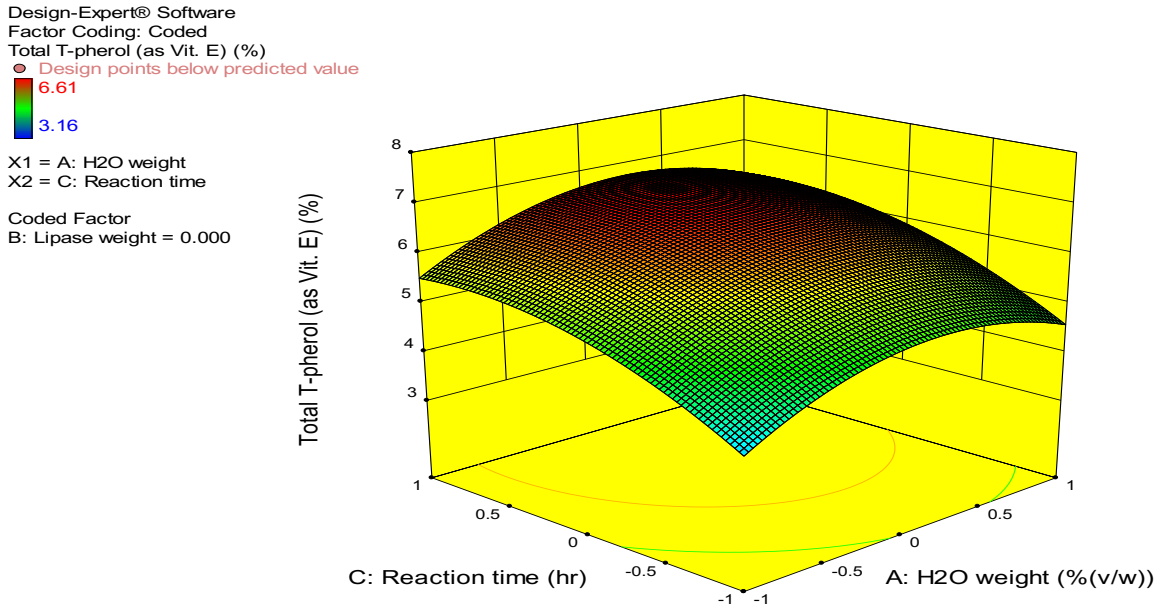

Figure 3.7. 3D Response surface plot of the interaction of lipase weight (%w/w) & H_2O weight (%v/w)

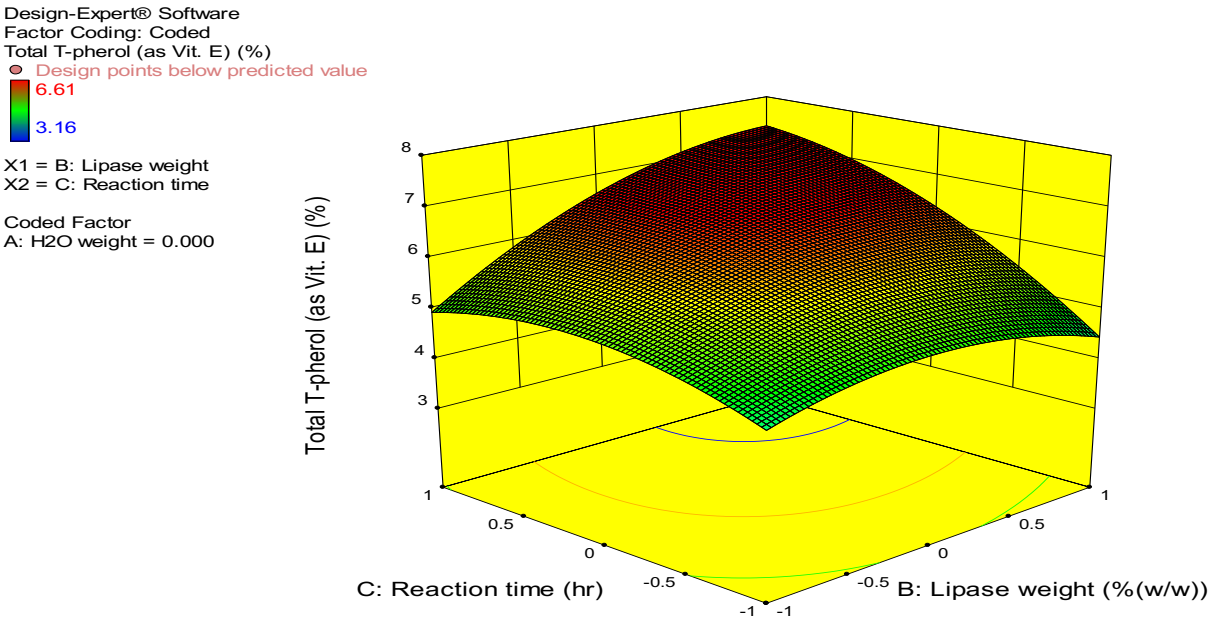

Figure 3.8. 3D Response surface plot of the interaction of reaction time (hr) & H2O weight (%v/w)

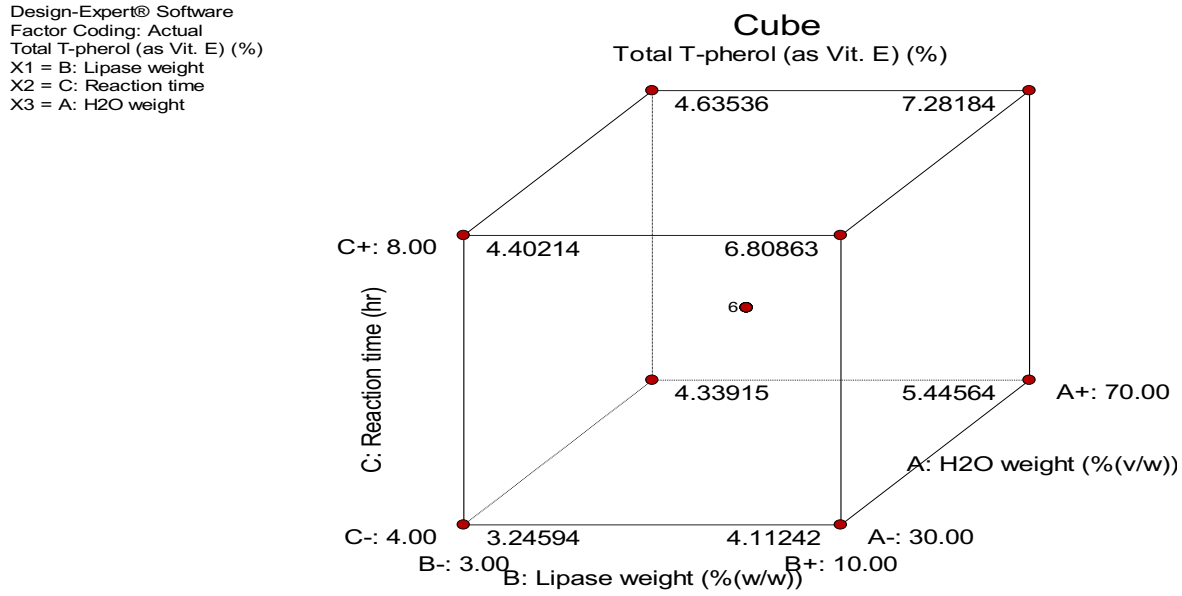

Figure 3.9. Cubic representation of all factor interactions on total tocopherol (as vitamin E) content

A combined form of interaction of all three parameters can be seen in a cubic representation as shown in Figure 3.9.

3.3.4 Optimization Result

About a hundred different optimized numerical solutions were suggested by the Design Expert Software. Thus, H_2O weight of 60.604 %(v/w), lipase weight of 7.130 %(w/w) and reaction time of 2.713 hrs gave the best (at optimized state) experimental protocol for the enzymatic hydrolysis PFAD in terms of yield of total tocopherol (as vitamin E) with a desirability of unity.

4 Conclusion

The application of RSM proved a valuable tool in the extraction of tocopherols, a substance with vitamin E activity and also the concentration of FFAs respectively from PFAD. Experimental design enables the identification of important process parameters and hence their effects by statically describing the interactions of the select variables. The hydrolytic action on PFAD for the extraction of tocopherols and FFA using commercially immobilised lipase (Aspergillus niger) proved to be an efficient, and cost saving method of producing bio-active compounds from the by-product of deodorization stage of vegetable oil processing.

Authors' Contributions

Author	Contribution
Tochukwu Kevin Dibia	Provided research funding, study materials, reagents, and analyses of data; manuscript composition, document review and manuscript edition.
Philomena Kanwulia Igbokwe	Coordinated research activities and supervised the research.
Christian Oluchukwu Asadu	Co-supervised manuscript composition, co-reviewed and co-edited the manuscript.

Acknowledgements

The authors wish to thank the staff of the Multipurpose Laboratory, Ahmadu Bello University, Zaria, Kaduna State, Nigeria for their immense contributions to data acquisition through guided experimental procedures and approaches.

Conflict of Interest

The authors categorically state that there is no conflict of interest whatsoever.

References

Abdulkadir A.G. and Jimoh W.L.O (2013). Comparative Analysis of Physicochemical Properties of Extracted and Collected Palm Oil Tallow. ChemSearch Journal 4(2): 44-54

AOCS Firestone, D. (6th Edition), Official Methods of Analysis, Association of Analytical Chemistry, Inc. Arlington, USA (2013).

Bonnie Tay Yen Ping and Mohtar Yusof (2009). Characteristics and Properties of Fatty Acid Distillate from Palm Oil. Oil Palm Bulletin. 59; pg. 5-11.

Botinestean Cristina, Hadaruga G. Nicoleta, Hadaruga I. Daniel, Jianu Ionel (2012). Fatty Acid Composition by Gas Chromatograhy-Mass Spectrometry (GC-MS) and Most Important Physcial Chemicals Parameters of Tomatoes Seed Oil. Journal of Agroalimentary Process and Technologies, 18 (1). Pg. 89-94

Camilla Chua Soo Leng, Badlish Sham Baharin, Yaakob Che Man and Chin Ping Tan (2008). Optimisation of the enzymatic hydrolysis for the concentration of squalene in palm fatty acid distillate. Journal of the Science of Food and Agriculture; 88, 1512-1517

Chu B.S., Baharin B.S. and Quek S.Y. (2002). Factors Affecting Pre-concentration of Tocopherols and Tocotrienols from Palm Fatty Acid Distillate by Lipase-Catalyzed Hydrolysis. Food Chemistry, 79; pg. 55-59.

Cibelem Iribarrem Benites, Bruno Colling Klein and Soely Maria Pissini Machado Reis (2014). Neutralization of soybean oil deodorizer distillate for vitamin E. Hindawi Publishing Corporation. International Journal of Chemical Engineering, Vol. 2014, Article ID 2742846, 7 pages. http://dx.doi.org/10.1155/2014/742846.

de Greyt W. F. J. (2012). Current and future technologies for the sustainable and cost-efficient production of high-quality food oils. European Journal of Lipid Science and Technology, vol. 114, pp. 1126–1139).

Duleba J. Anthony and Olive L. David (1996). Regression analysis and multivariate analysis: Seminars in Reproductive Medicine, 14(02), 139-153. Doi: 10.1055/s-2007-1016322

Hickman K.C.D. 1944. Purification of sludges, scums and the like to prepare relatively purified tocopherols. European Patent Application EP 0 992 499 A2

Jana Orsavova, Ladislava Misurcova, Jarmila Vavra Ambrozova, Robert Vicha and Jiri Mlcek (2015). Fatty acid composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. International Journal of Molecular Science; 16, 12871-128890. doi: 10.3390/ijms160612871

Jones P.J.H., Ntanios F.Y., Raeini-Sarjaz M., and Vastone C.A. (1999). Cholesterol lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipoidemic men. American Journal of Clinical Nutrition; 69: 114-50

Kifli H. (1983). Survey on Characteristics of Malaysian Palm Fatty Acid Distillate (PFAD) and Palm Acid Oil (Soap Stock). PORIM Report PO (59)83.

Lester E. Smith (1928). The Determination of Unsaponifiable Matter in Oils and Fats. Royal Society of Chemistry. Analyst, 53, 632-641.

Mikko Makela (2017). Experimental design and response surface methodology in energy applications: A tutorial review. Energy Conservation and Management; 151; 630-640

Moh M.H., Man Y.C., Van de Voort F.R., and Abdullah W.J.W. (1999). Determination of Peroxide Value in Thermally Oxidized Crude Palm Oil by Near Infrared Spectroscopy. Journal of American Oil Chemist Society. 76, pg. 19-23

Moss G.P., Smith P.A.S., and Tavernier D. (1987). IUPAC Compendium of Chemical Terminology: Pure and Applied Chemistry (Second Edition). International Union of Pure and Applied Chemistry. Pg. 1307 – 1375. doi: 10.1351/pac199567081307. ISBN: 978-0521-1150-6.

Sierksma A., Weststrate J.A., and Meijer G.W. (1999). Spreads enriched with plant sterols, either esterified 4,4-dimethylsterols or free 4-desmethylsterols, and plasma total- and LDL-cholesterol concentrations. British Journal of Nutrition; 82: 273-82.

Siew-Young Quek, Boon-Seang Chu and Badlishah Sham Baharin (2017) Commercial Extraction of Vitamin E from Food Sources. Encyclopedia of Vitamin E (eds V.R. Preedy and R.R. Watson

Soumya Raja (2017). Response surface methodology, analysis and interpretation. Data and Analytics Practice of KPMG

Tanaka Y, Hirano J and Funada T, Concentration of docosahexaenoic acid in glyceride by hydrolysis of fish oil with Candida cylindracea lipase**.** J Am Oil Chem Soc 69:1210–1214.