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EXTRACTION OF *CITRUS SINENSIS* SEED OIL (SWEET ORANGE): PHYSICOCHEMICAL AND STATISTICAL ANALYSIS

Olu-Arotiowa, A.O.^{1,2}, Odeniyi, T.A.², Otolorin, J.A.² and Alade, A.O.^{1,3,4*}

¹ Department of Chemical Engineering, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

² Chemical Engineering Department, College of Science and Engineering, Landmark University, Omu-Aran, Kwara State

³ Bioenvironmental, Water and Engineering Research Group (BWERG), Ladoke Akintola University of Technology Ogbomoso, Nigeria

⁴ Science and Engineering Research Group, Ladoke Akintola University of Technology Ogbomoso, Nigeria

Abstract

The indiscriminate disposal of the Sweet Orange (*Citrus sinensis*), which is one of the fruits cultivated and consumed widely in Nigeria, constitutes environmental nuisance. The prospective of recycling this waste as resource attracted the interest of this study. Sweet Orange fruits were juiced to obtain the Sweet Orange Seeds (SOSs), which were washed dried and blended. SOSs oil (SOSO) was extracted using Soxhlet extraction method with n-hexane under varying conditions (time, solvent-solid ratio and temperature) and then characterized. The data obtained were subjected to Historical Data Design (HDD) under the Response Surface Method (RSM) of the Design Expert (7.1.0) software. The residual SOSs was subjected to proximate analysis. Maximum yield of SOSO obtained were 59%, 59% and 60% under the effects of increasing solvent-to-solid ratio (6:1–9:1), temperature (45–60 °C) and Time (160–180 minutes), respectively. Linear model with coefficient of determination (R^2) of 0.8305 was very suitable for the SOSO extraction. Acid value, saponification value, iodine value, refractive index, pH and relative density of the SOSO were 6.2 mg/KOH/g, 185 mg/KOH/g, 86.7 g/100 g, oil 1.48, 5.52, and 0.856 g/ml, respectively. Moisture content, fat, fibre, ash, crude protein and carbohydrate of the SOSs were 7.8%, 36.50%, 10.96%, 1.22%, 7.22% and 36.3 %, respectively. This study has shown that *Citrus sinensis* seed oil is a prospective bioresource for biotechnology applications.

Keywords: *Citrus sinensis*, Historical data design, Sweet orange seed, Oil

*Corresponding author e-mail: aalade@lautech.edu.ng

Introduction

Sweet orange (*C. sinensis*) is one of the important *Citrus* widely grown in the world (Abbateet *et al.*, 2012; Favela-Hernández *et al.*, 2016). It constitutes about 70% of the total annual production of *Citrus* species (Favela-Hernández *et al.*, 2016). The genus *Citrus* belongs to the Rutaceae family and its species

includes lemon (*C. limon*), citron (*C. medica*), sour orange (*C. aurantium*), grapefruit (*C. paradisi*) (grapefruit), mandarin or tangerine (*C. reticulata*), clementine (*C. clementine*) and sweet orange (*C. sinensis*) (Favela-Hernández *et al.*, 2016).

C. sinensis originated from the Asia Continent but it is distributed widely throughout the Pacific areas and Tropics of the world. The fruit consists of the pericarp which consists of the peel or rind, and the endocarp which consists of the pulp. This comprises eleven segments of juice filled with sour flavour, which turns sweet as the fruit ripens. The fruit is perennial and this facilitates its adaptation to a variety of climates in most regions (Ulloa *et al.*, 2012).

C. sinensis contains various chemical compounds such as Alkylamines, alkanes, carbohydrates, carbamates, carotenoids, coumarins, fatty acids, flavonoids, hydroxyamides, peptides, steroids, and volatile compounds, that are distributed within its fruits, peels, leaves, juice and roots (Saleem *et al.*, 2010; Kelebek *et al.*, 2011; Escudero-López, 2013; Aschoff, *et al.*, 2015; Favela-Hernández *et al.*, 2016). Similarly, *C. sinensis* contains some essential elements such as potassium, magnesium, calcium and sodium (Favela-Hernández *et al.*, 2016).

C. sinensis is consumed widely as a source of vitamin C, which is an important natural antioxidant in human health (Etebu and Nwauzoma, 2014). Extract obtained from *C. sinensis* has exhibited antioxidant activity (Favela-Hernández, *et al.*, 2016). *C. sinensis* is used traditionally for the treatment of various ailments such as angina, anxiety, bronchitis, constipation, cramps, cold, colic, cough, depression, diarrhea, hypertension, menstrual disorder, obesity, tuberculosis, and stress (Milind and Chaturvede, 2012).

C. sinensis is a one of the most widely cultivated fruit in many towns that spread across the Savanah and Rain Forest regions of Nigeria (Odbanjo and Sangodoyin, 2002). Nigeria's contribution to the global production of the *Citrus* fruits is relatively substantial. Nigeria produces about 10 % of

the world production of 36.0 metric tonnes (Odbanjo and Sangodoyin, 2002). The fruit is consumed directly, mostly, during the season. However, the emergence of the juice producing industries, which obtained natural juices, pulps, candies, and extracts from the fruits, has taken a fair share of its annual production.

There is growing concern for the environmental challenges emanating from rising volume and indiscriminate disposal of the *C. Senesis* wastes (Schieber *et al.*, 2001). The rinds, seeds and a times, the flesh constituting about 50% of the fruits are often discarded as waste by consumers and the processing industries (Hernández-Montoya *et al.*, 2009). These constitute environmental challenges in a typical society. Poor storage facilities and transportation account for the huge loss recorded annually. Various studies have reused wastes from orange as bio resource materials in the production of biogas (Ogunleye *et al.*, 2016), while other studies have examined the essential compounds and phytochemicals in the wastes of *C. sinensis* (Favela-Hernández *et al.*, 2016). The submission of Malacrida *et al.* (2012) that suggested that the seeds of *C. sinensis* is a promising source of oil has driven research towards extraction of oil from the seeds using various methods. Some studies have been advanced to the application of oil extraction from *C. sinensis* for biodiesel production (Sadiq, 2016). *This study investigated extraction of oil from sweet orange seeds and characterization of the proximate properties of the residual seed chaff. The data obtained were processed using the facilities embedded in Design Expert (7.1.0) software.*

Materials and Methods

Materials

Sweet orange (*C. sinensis*) were obtained

from the local market in Omu-Aran, Kwara State Nigeria. The *Citrus* variety was identified at the Teaching and Research Farm, Landmark University, Omu-Aran, Kwara State, Nigeria. All reagents used in the study were of analytical grade and high purity. They were sourced from reliable representatives of the major chemical suppliers in Nigeria.

Methods

Sweet orange seed oil extraction

The rind of the sweet orange (SO) samples were carefully removed and the pulp were juiced in order to obtain the seeds present in them. The sweet orange seeds (SOSs) were rinsed, dried to constant weight, and milled to uniform particle size. The sweet orange seed oil (SOSO) was extracted via Soxhlet extraction (using n-hexane as the solvent) in a Soxhlet extractor apparatus under varying conditions (time, solvent-solid ratio and temperature) in order to study the effect of varying each condition on SOSO yield. Effect of solvent to solid ratio on the SOSO yield was carried out by varying the ratio from 6:1-9:1 at a fixed time (100 minutes) and temperature (50 °C). Effect of temperature on SOSO yield produced was carried out by varying the temperature from 50-60 °C at a fixed time (140 minutes) and solvent-to-solid ratio 8:1. Effect of time on SOSO yield was attained by varying the time from 60-100 minutes at fixed solvent-to-solid of 8:1 and temperature (45 °C). The extraction was repeated three times and the volume of SOSO was expressed as oil content (%) as calculated below, (Kyari, 2008). The solvent was recovered using a rotary evaporator, and the SOSO obtained was stored in a freezer for subsequent analysis.

$$\text{Oil Yield (\%)} = \frac{\text{weight of oil}}{\text{weight of sample}} \times 100 \quad (1)$$

Experimental design by historical method

Historical Data Design (HDD) under the Response Surface Methodology (RSM) was employed to examine the features of the data obtained from the extraction experiment. The independent variables were solvent-solid ratio (6:1-9:1), temperature (40-60 °C) and time (100-180 *min*) while the response was the SOSO yield. The regression analysis was carried out using statistical tools embedded in Design Expert software (version 7.1.0), Stat-Ease, Inc., Minneapolis, USA). Suitable model was selected, under the model summary statistics, based on highest coefficient of determination correlation coefficient (R^2) and relatively smallest standard deviation, provided the model is not aliased (Alade *et al.*, 2012; Mohammad *et al.*, 2014).

Characterization of the SOSO extracted

Determination of relative density of SOSO

The relative density of SOSO was determined using the standard density bottle. The weight of the bottle was first determined before being filled with distilled water, then decanted, and dried. The bottle was then refilled with SOSO. The relative density of SOSO was evaluated by the expression in Eqn [2].

$$\text{Relative density of oil} = \frac{W_2 - W_1}{W_3 - W_1} \times 100 \quad (2)$$

Where, W_1 = weight of empty bottle, W_2 = weight of equal volume of water, W_3 = weight of bottle and pure oil sample.

Determination of acid value of the oil

SOSO sample (2 g) was added to 50 ml of isopropyl alcohol in a conical flask. Then 3 drops of phenolphthalein indicator was added to the mixture, which was titrated with 0.1 M

NaOH from the burette. This was carried out in triplicates and the average titre value was used for the evaluation of the Acid Value (AOAC, 1988).

$$\text{Acid value} = \frac{5.61 \times \text{titre value}}{\text{Weight of sample}} \times 100 \quad (3)$$

Determination of saponification value of SOSO

Saponification value (S.V.), which is the weight of potassium hydroxide expressed in milligrams required to saponify 1 g of fat or oil, was determined by titration method using the AOAC (1988) technique. SOSO (2 g) was weighed into a 250 ml conical flask, mixed with 50 ml 0.5 M alcoholic KOH. This was refluxed for 30 min before adding 3 drops of phenolphthalein as indicator. The mixture was titrated against 0.5 M HCl until its pink coloration disappeared. A blank determination was carried out under the same condition and S.V. calculated.

$$\text{Saponification value} = (B - R) \times 28.05 \text{ weight of sample} \times 100 \quad (4)$$

Where, B = Blank titre and R = Real titre value.

Determination of peroxide value of SOSO

The peroxide value of SOSO was determined by titration method based on the AOAC (1988) technique. SOSO oil sample (2.0 g) was transferred into 250 ml flask where it was mixed with powdered potassium iodide (1 g) and a solvent mixture (2:1 of glacial acetic and trichloromethane). The solution was then placed in a water bath until complete dissolution. Thereafter, 20 ml of 50% potassium iodide was added and the sample was titrated with 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$. Regular starch solution was used as indicator. Blank experiment was conducted accordingly and peroxide value was evaluated according to equation 5.

$$\text{Peroxide value} = (R \times B) \left[\frac{\text{molarity of } \text{Na}_2\text{S}_2\text{O}_3}{\text{weight of sample}} \right] \quad (5)$$

where, R and B stand for oil and blank samples in term of titre values, respectively.

Determination of iodine value of SOSO

The Iodine value (IV) was determined by titration method using the (AOAC, 2000) technique. Iodine monochloride (8.0 g) dissolved in 200 ml glacial acetic acid and Iodine crystals (9.0 g) were dissolved in 300 ml of carbon tetrachloride (CCl_4) which were mixed to form Wij's solution which was made up to the mark with glacial acetic acid. SOSO sample (10 g) in a dry 250 ml conical flask was mixed with 10 ml of carbon tetrachloride and 20 ml of the Wij's solution prepared. The flask was stopped and kept in the dark cupboard for 30 minutes at room temperature after which 15 ml of 10% potassium iodide solution and 100 ml of distilled water were added. The mixture was titrated against 0.2 ml Sodium thiosulfate solution using starch as an indicator. A blank titration, without SOSO was conducted under the same conditions and iodine value of SOSO was evaluated according to Eqn (6).

$$\text{Peroxide value} = \frac{(A - B) \times N \times 12.69}{Q} \quad (6)$$

Where, A = volume of 0.1 in $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ solution used for the blank titration, B = Volume of 0.1 m of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ solution used for the Sample titration, Q = Weight in gram of the oil sample, Conversion factor = 12.69 and N = Normalty of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$.

Refractive index principle determination

Refractometer was used to determine the refractive index of the SOSO. The oil sample (2 drops) was placed on the prism of the instruments, which had been cleaned thoroughly. The thermometer reading was

recorded when the temperature of the oil was equilibrated with that of the thermo stated fluid. The knobs of the instrument were set and the fluid was demarcated by a sharp line which divides the field of view into equal halves. The reading was taken at this point when line coincided with the spot marks "X" in the field of the view at room temperature of 25 °C (Ibrahim and Yusuf, 2015).

$$\text{Refractive index } (R_i) = R + K(T_1 - T) \quad (7)$$

where, R_i = Refractive index reduced to standard temperature (25 °C). R = Reading obtained at temperature T , T = Standard temperature 25 °C, T_1 = Temperature at which reading was taken and K = Substituting factor (Constant) 0.000385 for Oils 0.000365

Proximate composition analysis of the seed residue powder

The SOSs flour (SOSFs) were subjected to proximate analysis (moisture content, ash content, crude protein, carbohydrate, fat, fiber and fat content) in order to determine its composition.

Moisture content

The SOSFs (2g) was weighed into a clean and dry petri dish. This was charged into an oven and allowed to dry 105 °C for about 5 to 6 hrs. to constant weight, before being cooled in the desiccator and weighed (AOAC, 1999). The percentage moisture content was estimated using Equation 8.

$$\text{Moisture content (\%)} = \frac{\text{weight of moisture}}{\text{weight of seed sample}} \times 100 \quad (8)$$

Ash content

The SOSFs (2g) was placed in a pre-heated crucible and was further heated in a muffle furnace at 600 °C for 4 hrs, until whitish-grey ash with constant weight was obtained

(AOAC, 1999, 2000). The crucible with its content was withdrawn and cooled in the desiccator and then weighed. The percentage ash content was estimated with the following equation

$$\text{Ash (\%)} = \frac{\text{weight of ash}}{\text{weight of seed sample}} \times 100 \quad (9)$$

Fat content

The SOSFs (5g) was placed inside a thimble and loaded into the main chamber of the Soxhlet extractor, which was placed onto the flask (on the heating mantle) containing the extraction solvent (n-hexane). This set-up was connected to the condenser. The flask was disconnected from the set-up at the expiration of the extraction time. It was placed in the oven at 65 °C for about 4 hrs. and then cooled in the desiccator before being weighed and the percentage fat was estimated according to Eqn. 10 (AOAC, 1999).

$$\text{Fat (\%)} = \frac{\text{weight of fat}}{\text{weight of seed sample}} \times 100 \quad (10)$$

Crude protein content

SOSFs (2g) was mixed with 25 mL of concentrated sulphuric acid, 0.5g of copper sulphate, 5g of sodium sulphate and a speck of selenium tablet in a Kjeldahl flask for digestion. Heat was applied, first, in the fume cupboard slowly to prevent frothing. The digestion process continued for 45 min until the solution became clear pale green. The mixture was cooled to room temperature and 100 mL of distilled water was added rapidly. The digestion flask was rinsed about 2-3 times and the rinsing was added to the bulk. The digested solution (10 mL) was added into the distillation apparatus and allowed to boil. Sodium hydroxide (10 mL) was added to prevent loss of ammonia and 50 mL of boric acid (2%) which contains screened methyl

red-methylene blue indicator was then distilled into it. The alkaline ammonium borate was formed and was titrated directly with 0.1 M hydrochloric acid. The titre values were recorded and the percentage crude protein was estimated according to Equation 11 (AOAC, 1995; 2000)

$$\text{Crude protein (\%)} = N \times 6.25 \quad (11)$$

where N = Nitrogen and 6.25 is the conversion factor

Crude fibre content

Defatted SOSFs (1.3 g) was mixed with 200 mL of preheated sulphuric acid (1.25%) in a flask and the mixture was boiled gently for about 30 min, while maintaining constant volume of acid by adding hot water. The mixture was then filtered and the residue was washed several times with hot water to neutral pH and then transferred into the beaker. Pre-heated sodium hydroxide (1.25%) (200 ml) was added and the mixture was boiled for 30 min, before being filtered with pre-weighed Whatman No 1 filter paper. The residue oven-dried at 600-650 °C for 4 hrs., until constant weight and then cooled in the desiccator before being re-weighed (AOAC, 2000). The percentage crude fibre was estimated using the Equation 12.

$$\text{Crude fibre (\%)} = \frac{\text{weight of crude fibre}}{\text{weight of seed sample}} \times 100 \quad (12)$$

Nitrogen free extract

The Nitrogen Free Extract (NFE) was determined based on the difference between the sum of percentages of all the nutrients already determined from 100 (Equation 13).

$$\text{Nitrogen (\%)} = 100 - [\text{moisture} + \text{ash} + \text{Fat} + \text{Crude protein} + \text{Crude fibre}] \quad (13)$$

Total carbohydrate content

The total carbohydrates content was determined by adding the sum of percentages of Nitrogen Free Extract (NFE) and Crude Fibre (CF) [Eqn 14].

$$\text{Carbohydrate (Nitrogen free extract)(\%)} = \text{Nitrogen (\%)} + \text{Crude fibre (\%)} \quad (14)$$

Results and Discussion

Extraction of oil on SOSO yield

The result obtained for effect of solvent to solid ratio on SOSO yield (Fig1a) indicated a steady increase of SOSO yield (55–59%) as the solvent to solid ratio also increases from 6:1-9:1 at constant temperature of 50°C and time of 100 minutes. The result obtained for the effect of steady time progresses (Fig.1b), showed that the oil yield increased from 55–60% as time increased from 160-180 minutes at constant temperature of 45 °C and 8:1 solvent-to-solid ratio. The result obtained for effect of temperature (Fig. 1c) showed that SOSO increased as the temperature increased from 54-59% increased from 45-60 °C at constant solvent-to-solid ratio of 8:1 and 140 minutes. Increase in temperature can enhance the capacity of solvents to dissolve the oil because the thermal energy can overcome cohesive and adhesive interaction.

Figure 1: Effect of (a) solvent to solid ratio, (b) temperature and (c) time on SOSO yield

The SOSO yields obtained from the citrus seeds samples (SOSs) in this study ranged from 45-60%. These values were higher than the oil yields (24.3-41.1% and 26.1-36.1%), investigated in Nigeria (Ajewole and Adeyeye, 1993), Tunisia (Saidani *et al.*, 2002), as well as 32.4-36.4% reported by Saleem *et al.* (1977) for oil yields of citrus (*C. sinensis*, *C. reticulata* and *C. paradisi*) seeds. The SOSO yields were comparable with 40.2-45.5% and 51.8% reported for Egyptian (Habib *et al.*, 1986) and Tunisian

sweet orange, respectively. Egbuonu and Osuji (2016) reported 54.49% SOSO from *C. sinensis* species obtained from the south-eastern part of Nigeria. Similarly, the range of SOSO yields in this study exceeded 15.0-24.0%, 17.0-21.0% and 20.0-25.0% found in cotton, soybean and olive, which are common conventional oil seed crops (Anwar *et al.*, 2008).

Development of model and model equation

The suitable model selected for SOSO yield

was linear (Table 1), with relatively highest R^2 of 0.8305 and corresponding relatively low standard deviation of 1.91. Other models with lower standard deviations and higher R^2 were disregarded because they were aliased, thus unsuitable for future applications (Montgomery, 2005). The R^2 value of 0.8305 implies that 83.05% of the variation in the SOSO yields can be attributed to the independent variables considered (Alade *et al.*, 2012). The model equations developed for the SOSO extraction process in terms of Coded and Actual Factors are stated in equations 15-16.

$$\text{Yield} = +56.75 - 0.47A + 1.37B + 4.00C \quad (15)$$

$$\text{Yield} = +41.55 - 0.32 * \frac{\text{Solvent}}{\text{solid}} \text{ratio} + 0.18 * \text{Temperature} + 0.067 * \text{Time} \quad (16)$$

where A is Solvent/solid ratio, B is Temperature and C is Time

Statistical analysis

The analysis of variance (ANOVA) embedded in the Design Expert software (7.1.0) was used to evaluate the statistical properties of the linear model developed for SOSO yield (Table 2). The F-value of 19.69 obtained indicates that the model is significant at p-value of 0.0043 which is less than 0.1 ($p < 0.1$). The model terms, particularly, temperature and time with p-value of 0.075 and 0.0017, respectively, are equally significant at $p < 0.1$. The statistical analysis suggested that the two independent factors have stronger influence on oil yield from SOS than the Solvent/solid ratio. Generally, temperature and time of extractions are important factors that influence rate of extraction of oil from biomass (Araromi *et al.*, 2017).

The Coefficient of Variance obtained for the model is 2.34% and value less than 10%

suggests high tendency of experimental reproducibility (Agarry, and Ogunleye, 2012). Similarly, Adequate Precision, which measured the signal-to-noise ratio, is 10.191, which is well above acceptable value of 4, thus indicating the suitability of the linear model developed in navigating the design space (Montgomery, 2005). The Predicted R^2 of 0.5475 is not as close to the Adjusted R^2 of 0.7579 as one might normally expect. This may indicate a large block effect, though the difference (0.2104) is very close to acceptable difference of 0.2 (Montgomery, 2005).

The residual of the response showed the extent of variation between the predicted values and the actual experimental values. The trend was illustrated on the plot of Actual Values versus Predicted Values (Fig. 2), which indicated slight scattering of the points from the normal diagonal. High value of R^2 is directly proportional to degree of closeness of the points to the diagonal and this justified the suitability of the model equation in representing the extraction process. Thus, R^2 -value of 0.8305 suggested about 17% variation between Actual Values versus Predicted Values.

Combined effect of factors on response

The combined effect of Solvent/Solid Ratio and Temperature on oil yield from SOS is shown in contour plot (Figure 3a) and three-dimensional (3D) (Figure 3b). The figures showed that the combined effect is almost completely because of the singular effect of temperature with having very small effect. The yield of SOSO increased, significantly, with increase in temperature whereas increase in solvent/solid ratio results in relatively very small decrease in yield of SOSO.

The contour plot (Figure 4a) and 3D plot (Figure 4b) indicates that Temperature and Time have significant combined effect on the yield of SOSO. The combined effect was

observed to be greater at higher values of the two factors, although the effect of time is higher than temperature. This signifies the fact that more oil can be obtained at a longer extraction time and this deduction is natural phenomenon. The trend observed (Figure 5a and Figure 5b) for this condition is very similar to those observed for combined effect of Solvent/Solid Ratio and Temperature on

the oil yield. It was observed that the combined effect is almost broadly due to the singular effect of Temperature with solvent/solid ratio having very slight effect. The yield of SOSO increased, significantly, with increase in Time while effect of Solvent/Solid Ratio on SOSO yield decreased with increase in Solvent/Solid Ratio.

Table 1: Model Summary Statistics

Source	Std. Dev.	R ²	Adjusted R ²	Predicted R ²	PRESS
Linear*	1.31	0.8305	0.7579	0.5475	32.19
2FI [^]	0.49	0.9798	0.9663	0.9358	4.57
Quadratic [^]	0.60	0.9846	0.9486		+
Cubic [^]	0.000	1.0000	1.0000		+

*Selected, [^] Aliased

Figure 2: plot of Actual Values versus Predicted Values

Figure 3: Combined Effect of Solvent/Solid Ratio and Temperature at constant Time

Figure 4: Combined Effect of Temperature and Time at Constant Solvent/Solid Ratio

Figure 5: Combined Effect of Solvent/Solid Ratio and Time at Constant Temperature

Optimization

The limit for optimizing the independent factors (Solvent/solid ratio, Temperature and Time) were 'in range' while 'maximise' was set for SOSO yield. The operation condition with highest desirability was selected as the optimum (Table 3). This was not validated as usually practiced in previous studies, since the

primary data were from historical source. The optimum theoretical value obtained from the software for SOSO yield was 60.0013% based on maximum condition of 7.75:1, 49.13 °C and 179.11 min for Solvent/solid ratio, Temperature and Time, respectively (Figure 6).

Table 3: Constraints for Theoretical Optimisation

Name	Lower Goal	Upper Limit	Lower Limit
Solvent/solid ratio	is in range	6	9
Temperature	is in range	45	60
Time	is in range	60	180
Yield	maximize	50	60

Figure 6: Graphical representation of optimized conditions generated by the software

Physiochemical properties of sweet orange seed oil

Acid value is a measure of the amount of carboxylic acid group in the substance, which gives idea about the tendency of the oil to spoil age and shelf life and thus its quality (Ekpa and Ekpe, 1995). Similarly, acid value is a measure of the total acidity

of the lipid including all the constituent fatty acid that make up the glyceride molecule (Dahuwa *et al.*, 2014). Acid value of oil further indicates its suitability as edible or non-edible and acid value below 5 mgKOH/g oil is classified as edible, while the oil is non-edible when the value is above 5mg KOH/g oil (Table 4) (Bello *et al.*, 2011).

Table 4: Physiochemical Properties of Sweet Orange Seed Oil

Parameters	This study	Anwar <i>et al.</i> , 2008	Sadiq (2016)
Acid value	6.2 mg/KOH/g oil	0.50 mg/KOH/g oil	8.2 mg NaOH/g oil
Saponification value	185 mg/KOH/g oil	189 mg/KOH/g oil	193.25mg KOH/g oil
Iodine value	86.7 g I/100g oil	99.85 g I/100g oil	56.40(g I/100 g)
Refractive index	1.48	1.4645	NR
pH	5.52	NR	NR
Relative density	0.856 g/ml		0.920 g/ml at 17 °C
Peroxide value	6.7 meq/kg oil	1.67 meq/kg oil	6.7 meq/kg oil

NR- Not Reported

The acid value of SOSO in this study was 6.2 mg/KOH/g and therefore can be classified as non-edible oil. The acid value of sweet orange seed oil is specified to be 9.89 mg/KOH/g. The acid value (6.2 mg/KOH/g) of SOSO is less than 8.2 mg/KOH/g oil reported by Sadiq (2016) but higher than 0.2-1.2, 0.5 and < 2.0 mg/KOH/g oil found in *C. Sinensis* species of Egyptian, Pakistani and Brazilian origin (Anwar *et al.*, 2008). Generally, higher acid value of oils is not desirable due to high tendencies of undergoing higher hydrolytic deterioration which usually lead to generation of unpleasant savour and stench.

The saponification value of a particular oil portends its suitability in soap making and may be a measure to detect the extent of adulteration of the oil. Saponification value is used, generally, to evaluate molecular weight of fatty acids present in the triacyl glycerols in the oil sample. Lower saponification value indicates the presence of high content of low molecular weight triacyl glycerols (Kittiphoom and Sutasinee, 2013). The Saponification value of the SOSO was 185 mg/KOH/g oil and it is higher than the value (171.9–184.9) reported by Saleem *et al.* (1977), but less than the value 193.25mg KOH/g oil reported by Saidq (2016) for Nigerian *C. Sinensis*). The Saponification value of SOSO were within the ranges (189.6-196.8, and 186.0-196.0 mg KOH/g oil

reported for Egyptian and Nigerian citrus seed oil (Habib *et al.* , 1986; Ajewole and Adeyeye, 1993). The SOSO saponification value compared with values 187-195, 189-198, 184-196, 185-198, 188-195 and 179-195 mg KOH/g oil of oils obtained from oil seed crops such as corn, cottonseed, olive, pumpkin, soybean and rice bran (Rossell, 1991).

Iodine value measures the degree of unsaturation of oil and higher iodine value indicates the presence of higher concentrations of C=C bond in the oil, thus indicating a higher degree of unsaturation (Nathan, 2013). Iodine value is used also to assess the quality and stability of typical oil in order to determine its suitability industrial applications (Okoye *et al.*, 2011). The iodine value number of SOSO in this study was 86.7gofl/100gofoil which is less than the value (99.85 g I/100 g oil) reported for Pakistani varieties of *C. sinensis* (Anwar *et al.*, 2008).

Similar trend was observed for the ranges (100-114, 104.2-105.0 and 91.5-102.5 g I/100 g oil) reported for Nigerian, Brazilian and Egyptian citrus seed oils, respectively, (Ajewole and Adeyeye, 1993; Reda *et al.*, 2005; El-Adawy 1999). The SOSO iodine value was lower than the values (99-119, 92-125, 90-110 and 104-120 g I/100 g oil) reported for seed oils such as

cotton, mustard, kapok, and sesame, respectively (Rossell, 1991). While SOSO iodine value fell within the value (75–94) obtained for olive (Rossell, 1991), it is higher than the value (56.40g I/100 g) reported by Sadiq (2016) for *C. sinensis* variety in Nigeria. The relatively lower SOSO iodine value obtained in this study may suggest the high resistance of the oil against the possibility of going rancid.

The refractive index of typical oil depicts its degree of purity. The refractive index of SOSO in this study was 1.48 at 40 °C, which is relatively higher than the values (1.4650-1.4681 and 1.4672-1.4684 reported by Habib *et al.* (1986) and El-Adawy *et al.* (1999) for Egyptian citrus seed oils, respectively and 1.4639 to 1.4670, reported by Anwar for Pakistani citrus seed oils, respectively. The refractive index of SOSO in this study was found to be higher, slightly, than the ranges of refractive index (1.458-1.466, 1.461-1.469, 1.460-1.465, 1.462-1.465, 1.460-1.466, 1.467-1.470 and 1.467-1.469) reported for cottonseed, mustard seed, groundnut, almond kernel, kapok seed, soybean and sunflower oils, respectively (Rossell, 1991). The refractive index of SOSO falls outside the range (1.445-1.470) of recommended refractive index for edible vegetable oil (Ibrahim and Yusuf, 2015).

The low pH level of typical oil indicates the presence of a reasonable amount of fatty acids in the oil and it is a good indicator of suitability of the oil in industrial process, particularly in food processing industries. The pH level of SOSO was 5.52 which are relatively not acidic as can be related to its high acid value (6.2 mg/KOH/g oil).

The relative density of typical oil is as important as its refractive index, because the degree of variation of these two properties

from their standard or true value indicate the level of purity of the oil sample (Anwar, 2008). The relative density of SOSO was 0.856g/ml at 17 °C and this is lower than the densities 0.913-0.933 g/ml, 0.920 (mg/ml @ 25 °C and 0.920 g/ml at 17 °C, reported by Habib *et al.* (1986), Anwar *et al.* (2008) and Sadiq (2016), for Nigerian, Brazilian and Egyptian citrus seed oils, respectively, but within the range 0.884-0.962, reported by El-Adawy *et al.* (1999) for Egyptian citrus seed oils.

Peroxide value is an important parameter used for detecting the tendency of oil to go rancid and high peroxide value is related to high rancidity rate (Kittiphoom and Sutasinee, 2013; Ibrahim and Yusuf, 2015). The Peroxide value of SOSO in this was 6.7 meq/kg oil which is similar to the value (6.7 meq/kg oil) reported by Sadiq (2016) for Nigerian citrus seed oils, but higher than 5.8 meq/kg, and 1.67 meq/Kg reported by Ibrahim and Yusuf (2015) and Dugo and Bonaccorsi (2013) for other species of Nigerian sweet orange seed oils, as well as 1.90-2.0 meq/Kg and 2.21 meq/Kg reported by Reda *et al.* (2005) and Anwar *et al.* (2008) for Brazilian and Pakistani sweet orange seed oils, respectively.

Similar trend was observed for the peroxide value (5.80 and for 3.12 meq/kg) of melon and pawpaw seed oils (Ibrahim and Yusuf, (2015). Generally, the peroxide values between 20 and 40 meq/kg result in rancid taste while values less than 10 mg/g oil indicate freshness the oil, thus oils characterised with high peroxide values are vulnerable to rancidity (Thomas, 2002; Ibrahim and Yusuf, 2015). Therefore the low peroxide value (6.7 meq/kg) of SOSO indicates that it is stable and its quality could not deteriorate with time.

Proximate analysis of the sweet orange seed flour

Proximate analysis of SOSs residue Table 5) showed the presence of 7.8% moisture content and this is higher than 6.55% reported by Oikeh *et al.*, (2013), but less than 8.32% observed by Egbuonu and Osuji (2016) for the variety of *C. sinensis* found in Nigeria. Compared with other oil seeds, moisture

content of SOSs was lesser than 9.20% and 9.89% reported for African breadfruit and soybean, respectively (Samaila *et al.*, 2006), but higher than 3.815 obtained in water melon seed (Egbuonu, 2015). Higher moisture content of agricultural seeds prone them to microbial growth which accelerate their spoilage and consequently reduce their shelf life (Ponnusha *et al.*, 2011; Oikeh *et al.*, 2013).

Table 5: The Proximate Content (%) of the Sweet Orange Seed Flour

Parameters	This study	Oikeh <i>et al.</i> , 2013	Egbuonu and Osuji (2016)
Moisture content (%)	7.8	6.25	8.32
Fat (%)	36.50	12.00	11.08
Fibre (%)	10.96	5.30	2.98
Ash (%)	1.22	2.00	3.02
Crude protein (%)	7.22	6.13	6.77
Carbohydrate (%)	36.3	68.32	67.83

The crude fat content of the SOSs was 36.50% which was higher than 12.04% and 11.08% reported by Oikeh *et al.* (2013) as well as Egbuonu and Osuji (2016) for Nigerian *C. sinensis*. Fat are essential ingredients used in the food and cosmetic industries (Mathew *et al.*, 2014). When compared with conventional and non-conventional oilseed crops, the fat content of the SOSs residue was higher than the contents found in cherry (13%), pawpaw (26.01%) and guava (19.01%) seeds (Mathew *et al.*, 2014). It compares well with bitter melon (34 %) but less than the amount found in water melon (44%) seeds. The SOSs such as the bitter melon and water melon seeds can be considered as good source of oil due to its high fat content (> 30%) (Mathew *et al.*, 2014). Similarly, SOSs can be sourced as cheap and renewable source of dietary fat (Egbuonu and Osuji, 2016).

The crude fibre of SOSs in this study was 10.96% which was higher than 5.3 and 2.98%

reported by Oikeh *et al.* (2013) as well as Egbuono and Osuji (2016), respectively, for *C. sinensis* in Nigeria. The value (6.90%) reported for Pakistani variety (Anwar *et al.*, 2008) is lesser than the value reported in this study. Similarly, the crude fiber obtained for African breadfruit (1.55%) and soybean (6.46%) (Samaila *et al.*, 2016) as well as grapefruit (8.50%) (Anwar *et al.*, 2008) are lesser than contains in SOSs, although it compared well with cherry (10%), but lesser than the content found in watermelon (14.02%), pawpaw (14.01%), guava (12%) and bitter lemon (Mathew *et al.*, 2014). The SOSs residue can be considered as cheap and renewable source of crude fibre which is a good raw material for food enrichment, particularly poultry and fish meal (Figuerola *et al.*, 2005).

The ash content of the SOSs in this study was 1.22% compared to 2.0% and 3.02% reported by Oikeh *et al.* (2013), respectively. It is lesser

than the value (4.6-5.6%) reported for different citrus species in Pakistan (Anwar *et al.*, 2008), as well as 3.1-3.4% and 2.2-3.5% reported by El-Adawy *et al.* (1986) and Habib *et al.* (1999), respectively for Egyptian citrus seeds. The ash content of SOSs is lower, significantly, than the cherry (2.0%), guava (3.0%), pawpaw (4.0%), bitter melon (4.0%) and water melon (6.0%) seeds reported by Mathew *et al.* (2014). Lesser ash content of SOSs residue, in this study, indicates the presence of lesser amount of minerals in the seed and may not be a good source for mineral enrichment in food and feed formulations.

The crude protein of SOSs is 7.22% which is higher than 6.13 and 6.77 % reported by Oikeh *et al.* (2013) and Egbuono *et al.* (2016), respectively, for Nigerian *C. sinensis* seeds. Similar trend was observed for *C. sinensis* seeds with 3.1-3.2% and 5.56% reported by Akpata and Akubor (1999) as well as Anwar *et al.* (2008). SOSs crude protein was lesser than 13.8-17.4% and 15.9-19.9% observed for Egyptian *C. sinensis* seeds (El-Adawy *et al.*, 1986; Habib *et al.*, 1999). Similar trend was observed for water melon, pawpaw, guava and bitter lemon seed which have 21.50%, 8.75%, 8.05% and 19.5% crude protein, respectively, but it compared well with protein content (7.0%) observed in cherry (Mathew *et al.*, 2014). The SOSs can be sourced as plant protein which is suitable for animal feed.

The carbohydrate contents (68.32% and 67.83%) of *C. sinensis* seeds characterised by Oikeh *et al.* (2013) as well as Egbuono and Osuji (2016), respectively, were exceedingly higher than 36.3% obtained for SOSs in this study. The carbohydrate content of SOSs (62.33%, 36.25% and 53.95%) are higher than fat contents (13%, 26% and 19%) of cherry, pawpaw, and guava seeds (Mathew *et al.*, 2014). The fat and carbohydrate content of SOSs compared favourably with each other

and this is very rare, thus this suggests SOSs as good bio resources for biotechnology application.

Conclusion

The oil content and proximate characteristics of sweet orange seeds (SOSs) were investigated in this study with the aim of determining their resourcefulness as renewable bio resources for industrial applications. The percentage oil yield is economical and its characteristics compared well with SOSs from other regions of the world. Similarly, the SOSs oil characteristics are better than those obtained from conventional and non-conventional seeds. The extraction process data was well fitted to linear models with high correlation coefficient ($R^2 = 0.8305$). The proximate analysis of SOSs residue indicates high percentage of fat (36.50%), protein (7.22%) crude fibre (10.96%) and carbohydrate (36.8%). This indicates that the waste sweet orange (*C. sinensis*) seeds is a reservoir of biomaterials that can be adopted for biotechnology applications.

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Disclosure of any conflict of interest

Authors declare that there is no conflict of interest

References

- Abbate, L., Tusa, N., del Bosco, S. F., Strano, T., Renda, A. and Ruberto, G. 2012. Genetic improvement of Citrus fruits: New somatic hybrids from *Citrus sinensis* (L.) Osb. and Citrus limon (L.). *Burm. Food Research. International* 48, 284-290.
- Agarry, S. E. and Ogunleye, O. O. 2012. Box-Behnken Designs Application to Study Enhanced Bioremediation of Soil Artificially Contaminated with Spent Engine Oil Using Biostimulation Strategy. *International Journal of Energy and Environmental Engineering*. Germany 3, 31-43.
- Ajewole, K. and Adeyeye, A. 1993. Characterization of Nigerian citrus seed oils. *Food Chemistry* 47, 77-78
- Akpata, M. I., and Akubor, P. I. 1999. Chemical composition and selected functional properties of sweet orange (*Citrus sinensis*) seed flour. *Plant Foods and Human Nutrition* 54, 353-362
- Alade, A. O., Amuda, O. S., Ogunleye, O. O. and Okoya, A. A. 2012. Evaluation of Interaction of Carbonization Temperatures and Concentrations on the Adsorption Capacities and Removal Efficiencies of Activated Carbons Using Response Surface Methodology (RSM). *Journal of Bioremed Biodegrad* 3, 134. doi: 10.4172/2155-6199.1000134
- AOCS. 1997. Official and recommended practices of the American Oil Chemists Society, 5th edn. AOCS Press, Champaign
- Anwar, F., Naseer, R. Bhanger, M. I., Ashraf, S., Talpur, F. N. and Aladedunye, F. A. 2008. Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. *Journal of American Oil Chemistry Society* 85, 321-330.
- AOAC. 1988. Official methods of analysis of the association of official analytical chemists. Analytical Chemist. (3rd Ed.) Washington. D.C.
- AOAC. 1990. The Official Methods of Analysis of the Association of Official Analytical Chemists (15th Ed.). Arlington, Virginia: The Association of Official Analytical Chemists.
- AOAC. 2000. Official Methods of Analysis of the association of official analytical chemists. Analytical Chemist. 17 Edition, Washington. D.C.
- AOAC. 1995. Official methods of analysis of the association of official analytical chemists. Horwitz, W. (EN.), Benjamin Franklin Station, Washington D.C.
- AOAC. 1999. Official Methods of Analysis of the Association of Official Analytical Chemist. 16th Edn., Washington D.C.
- AOAC. 2000. Official Methods of Analysis of the Association of Official Analytical Chemists. 17th Edn., Washington D.C.
- Araromi, D. O., Alade, A. O., Bello, M. O., Bakare, T., Akinwande, B. A., Jameel, A. T. and Adegbola, S. A. 2017. Optimization of Oil Extraction from Pitanga (*Eugenia Uniflora* L) Leaves Using Simplex Centroid Design (SCD), *Separation Science and Technology*, DOI: 10.1080/01496395.2017.1287199
- Aschoff, J. K., Kaufmann, S., Kalkan, O., Neidhart, S., Carle, R. and Schweiggert, R. M. 2015. In Vitro Bioaccessibility of Carotenoids, Flavonoids, and Vitamin C from Differently Processed Oranges and Orange Juices (*Citrus sinensis* (L.) Osbeck). *Journal of Agriculture and Food Chemistry* 63, 578-587
- Bello, M. O., Akindele, T. L., Adeoye, D. O. and Oladele, A. 2011. Physicochemical Properties and Fatty Acids Profile of Seed Oil of *Telfairia occidentalis*. *International Journal of Basic and Applied Sciences*. IJBAS-IJENS 11(69), 110306-4747
- Dahuwa, K., Ejilah, I. R. and Dandakouta, H. 2014. Marula (*sclerocarya birrea*) oil as an alternative feed stock for biodiesel production in Nigeria. Proceedings of the 27th AGM and International Conference of the Nigerian Institution for Mechanical Engineers pp. 159-167
- Dugo, G. and Bonaccorsi, I. 2013. (Eds.). *Citrus Bergamia: Bergamot and its derivatives*. CRC Press.

- Egbonu, A. C. C. 2015. Comparative investigation of the proximate and functional properties of watermelon (*Citrus lanatus*) rind and seed. *Research Journal of Environmental Toxicology* 9(3), 160-167
- Egbonu, A. C. C. and Osuji, C. A. 2016. Proximate Composition and Antibacterial Activities of *Citrus sinensis* (Sweet Orange) Peel and Seed Extracts. *European Journal of Medicinal Plants* 12(3), 1-7
- Ekpa, O. D. and Ekpe, U. J. 1995. The effects of coconut oil concentration and air exposure to the total acidity of palm oil. *Global Journal of Pure and Applied Sciences* 11, 51-58.
- El-Adawy, T. A., Rehman, E. H., El-Bedawy, A. A. and Gafar, A. M. 1999. Properties of some citrus seeds. Part 3. Evaluation as a new source of potential oil. *Nahrung* 43, 385-391
- Escudero-López, B., Cerrillo, I., Herrero-Martín, G., Hornero-Méndez, D., Gil-Izquierdo, A., Medina, S., Ferreres, F., Berná, G., Martín, F. and Fernández-Pachón, M. S. 2013. Fermented orange juice: Source of higher carotenoid and flavanone contents. *Journal of Agriculture and Food Chemistry* 61, 8773-8782.
- Etebu, E. and Nwauzoma, A. B. 2014. A review on sweet orange (*Citrus Sinensis* Osbeck): Health, diseases, and management. *American Journal of Research* 2, 33-70.
- Favela-Hernández, J. M. J., Santiago, O. G., Ramírez-Cabrera, M. A., Esquivel Ferriño, P. C. and Camacho-Corona, M. R. 2016. Chemistry and Pharmacology of *Citrus sinensis*. *Molecules* 21(247), 1-24
- Figuerola, F., Hurtado, M. L., Estevez, A.M., Chiffelle, I. and Asenjo, F. 2005. Fibre concentrates from apple pomace and citrus peel as potential fibre sources for food enrichment. *Food Chemistry* 9, 395-401.
- Habib, M. A., Hammam, M. A., Sakr, A. A. and Ashoush, Y. A. 1986. Chemical evaluation of Egyptian citrus seeds as potential sources of vegetable oils. *Journal of American Oil Chemistry Society* 3, 1192-1197
- Hernández-Montoya V., Montes-Morán M. A. and Elizalde-González, M. P. 2009. Study of the thermal degradation of citrus seeds. *Biomass Bioenergy* 33, 95-99.
- Ibrahim, I. A. A. and Yusuf, A. J. 2015. Extraction and physicochemical analysis of *Citrus sinensis* seed oil (sweet orange). *European Journal of Experimental Biology* 5(7), 77-81
- Kelebek, H. and Selli, S. 2011. Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (*Citrus sinensis* L. Osbeck) orange juice. *Journal of Science, Food and Agriculture* 91, 1855-1862.
- Kittiphoom, S. and Sutasinee, S. 2013. Mango seed kernel oil and its physicochemical properties. *International Food Research Journal* 20(3), 1145-1149.
- Kyari, M. Z. 2008. Extraction and characterization of seed oils. *International Agrophysics* 22, 139-142
- Malacrida, C. R., Kimura, M. and Jorge, N. 2012. Phytochemicals and antioxidant activity of citrus seed oils. *Food Science and Technology Research* 18, 399-404.
- Mathew, T. J., Ndamitso, M. M., Otori, A. A., Shaba, E.Y., Inobene, A. and Adamu, A. 2014. Proximate and Mineral Composition of Seeds of some Conventional and Non-Conventional Fruits in Niger State Nigeria. *Academic Research International* 5(2), 113-117
- Milind, P. and Chaturvede, D. 2012. Orange: Range of benefits. *International Research Journal of Pharmacy* 3, 59-63.
- Mohammad, R., Issa, M., Shahrokh, N., Mahdi, J. and Leila, S. 2014. Physicochemical characteristics of citrus seed oils from Kerman, Iran. *Journal of Lipids* Article ID 174954
- Montgomery, D. C. 2005. Design and analysis of experiments (6th Ed). Singapore (SG): John Wiley and Sons. Pg. 101
- Nathan, W. M. 2013. Development of hemp oil based bioresins for biocomposites. A Dissertation

submitted for the award of Doctor of philosophy. Centre of excellence in engineered fibre composites. Faculty of engineering and surveying University of Queensland, Australia.

Osibanjo, O. O. and Sangodoyin, A. Y. 2002. An improved understanding of current agricultural and industrial waste management techniques in southwestern Nigeria using field evidence. *Journal of Urban Environmental Research* 3(1), 67-75

Ogunleye, O. O., Aworanti, O. A., Agarry, S. E. and Aremu, M. O. 2016. Enhancement of animal waste biomethanation using fruit waste as co-substrate and chicken rumen as inoculums. *Energy sources, Part A: Recovery, Utilization, and Environmental Effects* 38(2), 1653-1660.

Oikeh, E. I., Oriakhi, K. and Omoregie, E. S. 2013. Proximate Analysis and Phytochemical Screening of *Citrus sinensis* Fruit Wastes. *The Bioscientist* 1(2), 164-170

Okoye, C. O. B., Ibeto, C. N. and Ihedioha, J. N. 2011. Preliminary studies on the characterization of orange and pawpaw seed oils. *American Journal of Food Technology* 6, 422-426.

Ponnusha, B. S., Subramaniam, S., Pasupathi, P., Subramaniam, B. and Virumandy, R. 2011. Antioxidant and Antimicrobial properties of Glycine Max: A review. *International Journal of Current Biological and Medical Science* 1(2): 49-62.

Reda, S. Y., Sauer, E. L., Batista, A. E. C., Barana, A. C., Schnitzel, E. and Carneiro, P. I. B. 2005. Characterization of rang purlime (*Citrus limonia Osbeck*) and "Sicilian" lemon (*Citrus limon*) seed oils, an agro-industrial waste. *Cience Technology Aliment* 25, 672-676.

Rossell, J. B. 1991. Vegetable oil and fats. In: Rossell JB, Pritchard JLR (Eds) Analysis of oil seeds, fats and fatty foods. *Applied Sciences*, New York, pp.261-319

Sadiq, A. S. 2016. Assessment of Nigeria sweet orange seed oil for biodiesel production. *Nigerian Journal of Tropical Engineering* 9(1 & 2), 1-9

Saidani, M., Dhifi, W. and Marzouk, B. 2004. Lipid evaluation of some Tunisian citrus seeds. *Journal of Food Lipids* 11, 242-250

Saleem, M., Sarwar, M., Khan, S. A. and Bhatti, M. K. 1977. Fatty acids of indigenous resources for possible industrial applications. *Pakistan Journal of Science and Industrial Research*. 20:305-306

Saleem, M., Farooq, A., Ahmad, S., Shafiq, N., Riaz, N., Jabbar, A., Arshad, M., and Malik, A. 2010. Chemical constituents of *Citrus sinensis* var. Shukri from Pakistan. *Journal of Asian Natural Products Research* 12, 702-706.

Samaila, J., Nwabueze, T. U., Usaman, M. A., Ojo, S., Nwokocha, L., Yusuf, J. and Ibrahim, A. B. 2016. Comparative study of the proximate and mineral compositions of extruded African Breadfruit (*Trecullaia Africana*) mix with some commercial pasta products. *Journal of Scientific Research and Reports* 9(4), 1-9

Schieber, A., Stintzing, F. and Carle, R. 2001. Byproducts of plant food processing as a source of functional compounds: recent developments. *Trends Food Science and Technology* 12, 401-413.

Thomas, A. 2002. Fats and fatty oils, Uilmanns Encyclopedia of industrial chemistry. Weinheim Willey publication-VCH.

Ulloa, F. V., García, S. S., Estrada, M. C., López, D. L. P., Cruz, M. C. R. and García, P. S. 2012. Interpretation methods of nutrient diagnosis in orange cv. Valencia (*Citrus sinensis* L. Osbeck). *Terra Latinoam* 30, 139-145.