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OPTIMIZATION OF OIL EXTRACTION FROM SOYBEAN USING AZEOTROPIC TERNARY SOLVENT MIXTURES AND CAKE ANALYSIS

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ABSTRACT

Soybean (*Glycine max*) sample was subjected to solvent extraction with an azeotropic ternary solvent mixture (5-10% water, 5-10% ethanol, and 80-85% ethyl acetate) optimised based on D-optimal Design (DOD) under the Mixture Methodology of the Design Expert (7.0.1). The azeotropic solvent mixture developed was 9.17%, 6.67%, and 84.17% of water, ethanol, and ethyl acetate, respectively, with a 15.56 % yield of soy oil. The extraction suited a Quadratic model and the Analysis of Variance (ANOVA) indicate a Correlation Coefficient (R^2) of 0.9921. The Refractive Index, Fatty acid, as well as Acid, Saponification, Iodine and Peroxide values of the Soy oil, are 1.454, 8.39, 16,3, 56.12, 15.17 and 27.00, respectively. Moisture, ash, fibre, lipid, crude protein and carbohydrate contents of the defatted soybean cake are 16.75, 4.85, 5.00, 2.60, 31.54 and 39.86 %, respectively. The optimised solvent mixtures demonstrated suitable performance for the safe extraction of oil from soybean

1.Introduction

Soybean is a leguminous plant seed, mainly cultivated for its oil and protein content (Lawson *et al.*, 2010). Oil from soybean have been obtained through various processes and techniques such as mechanical pressing, solvent extraction, supercritical fluid extraction (Koubaaa *et al.*, 2016; Rai *et al.*, 2016), compressed fluid extraction (Coelho *et al.*, 2016), microwave and ultrasound-assisted extraction (Araujo *et al.*, 2013) have been proposed for effective oil extraction from oilseeds. The solvent extraction process usually leads to lower turbidity and higher yield oil, compared to the mechanical process (Sulaiman et al., 2013), although the residual solvent after the extraction has attracted attention (Agu 2014).

Common solvents used for the extraction process include hexane and its isomers, petroleum ether, ethanol, butanol, and other organic solvents (Araromi *et al.*, 2017). Long and Abdelkader (2011) employed five solvents to extract lipids from *Nannochloropsis* microalgae. The solvent ratios were combined as hexane/cyclohexane (1:1), cyclohexane/2-propanol (2:1), hexane/2propanol (3:1), and cyclohexane/1- butanol (9:1), to be equivalent azeotropic mixture ratio, based on mole fractions established in the Azeotrope databank (Ponton, 2001). Scanty information exists in the literature on the use of an azeotropic mixture for

oil extraction for oilseeds particularly, soybean. This study is set to combine three solvents (water, ethanol, and ethyl acetate) at and near azeotropic conditions and then investigate the efficiency of the mixture in extracting oil from the soybean sample.

Various studies have been conducted on the optimization of the solvent extraction of edible and non-edible oils from various oilseeds. Sayyar *et al.,* (2009) optimized five factors for the extraction of oil from Jatropha seeds using n-hexane and petroleum ether. Mampouya *et al.*, (2013), optimised the Soxhlet extraction of oil from Safou Pulp using trichloroethane, chloroform, hexane, and petroleum ether as a solvent. Bokhari *et al.,* (2014) optimised selected parameters that affect the solvent extraction of crude rubber seed oil using response surface methodology (RSM). Dagostin *et al.,* (2015) studied the application of a mixture of alkyl esters and ethanol for Soybean (SB) oil extraction, while Glendara and Joenes (2015) optimised time and temperature that influenced the liquid extraction of SB oil using a binary mixture of ethanol and hexane. Adeyanju *et al.*, (2016) employed RSM to optimise the operating condition for the extraction of coconut oil. Our extensive literature search indicated that no work related to the use of an azeotropic solvent mixture for the extraction of soy oil from soybean has been conducted. This study specifically, employed D-Optimal Design under the Mixture Methodology of the Design Expert (7.0.1) to optimize the mixture of water, ethanol, and ethyl acetate solvents for the effective extraction of soy oil from soybean.

2.Materials and Method

2.1 Materials and Sample Preparation

The Soybean (SB) (*Glycine Max (L*) sample was obtained in the Market and authenticated at Forestry Research Institute of Nigeria (FRIN) Jericho, Ibadan, Nigeria. The SB sample was cleaned of chaff and other foreign materials and later oven-dried at 50 °C for 24 h to constant moisture content. The dried SB sample was dry-blended and sieved to uniform particle size (0.2 mm) (Araromi *et al.,* 2017). All the reagents used in this study were of analytical grade and obtained from designated suppliers.

2.2 Solvent Extraction

2.2.1. Soxhlet extraction of oil from SB

The Soxhlet apparatus was set up accordingly with much care to avoid leakages, then 300 mL of the solvent (water, ethyl acetate, and ethanol) mixture was mixed with 50 g of the SB sample and then heated to the azeotrope temperature (69.9 °C) of the mixed solvents for 4 h. The resulting solution was evaporated in a rotary evaporator to obtain pure Soy oil (Araromi *et al.,* 2017), which was cooled in a desiccator and then weighed. The oil yield was determined from Eqn. 1, based on the average of the three replicates of the experiment (Lawson *et al.,* 2010).

$$oil\ yield = \frac{weight\ of\ extracted\ oil}{weight\ of\ soybean\ fed\ in}\ X\ 100 \quad (1)$$

2.2.2. Optimization of ternary solvent mixture for extraction of Soy oil

The three solvent (water, ethanol, and ethyl acetate) mixture was optimized using D-optimal Design under the Mixture Methodology of the Design Expert (7.0.1) software. The component levels (5-10% water, 5-10% ethanol, and 80-85% ethyl acetate), (Table 1), based on mole fractions in the Azeotrope databank (Ponton, 2001) were fed into the software. The experimental runs generated by the software were subjected to the Soxhlet extraction process and the corresponding oil yield was documented. The data obtained were subjected to Analysis of Variance (ANOVA) using statistical tools embedded in the software. Three-dimensional plots and their respective contour plots were obtained based on the effects of the levels of the three components. The effects of the interaction of the three components on the response were studied. The significance of the model equations and their terms were evaluated using statistical tools such as coefficient of determination (R^2), Fisher value (F-value), probability (P-value), and residual (Mohammad *et al.,* 2014).

Table 1. Selected Factors and their Levels for the Central Composition Design (CCD)

Factors	Units	Levels	
		Low	High
Pressure (P)	N/m^2	1.0	2.50
Toasting time (T)	Min	30.00	60.00

The R^2 reveals the efficiency of the experiment, and thus, is expected to be very high (≈ 1), while the Adjusted R^2 (Adj R^2) and predicted R^2 (Pred R^2) were generated for the adjusted and predicted values by the software, respectively. Consequently, a suitable model for the optimization was characterized by the highest R^2 but not 'Aliased', lowest standard deviations, and the smallest differences between the generated Adj R^2 and Pred R^2. The Predicted Residual Error Sum of Squares (PRESS) measures the degree to which the model developed is likely to predict the responses in new experiments and, thus, it is desirable to have small values of PRESS (Montgomery, 2001).

2.3. Characterisation of Physicochemical Properties of Soy oil Samples

2.3.1 Acid value

The acid value and acidity of Soy oil were determined according to ISO standard 660. Alcohol (a mixture of 1/1(v/v) of 95% ethanol and diethyl ether) (25 mL) neutralized just before use with 0.1 M KOH solution in the presence of 3 drops of phenolphthalein was added to 0.5 mL of the Soy oil sample in a 250 mL conical flask. The flask was swirled for 2 mins, followed by the addition of 3 drops of indicator, and then the mixture was titrated against 0.1 mg/L solutions of the ethanolic potassium hydroxide until a permanent pink colour was attained (Amos-Tautua and Onigbinde (2013).

2.3.2. Peroxide value

The peroxide value of the Soy oil was determined using the ISO standard 3960. The soy oil (0.5 g) was dissolved in a solvent mixture of acetic acid and titrated against 0.05 M sodium thiosulphate using starch as an indicator (Amos-Tautua and Onigbinde (2013).

2.3.3. Iodine value

The iodine value (IV) was determined according to ISO standard 3961. The soy oil sample (0.2 g) was dispensed in a round neck bottle and mixed with chloroform (5 mL) and Wijj's reagent (8 mL), iodine trichloride (9 mL), and 10 g of iodine in chloroform (300 mL) /acetic (700 mL) solution. The bottle was shaken gently and placed in the dark for 1 h after which 7 mL of KI (100 g/1) and 75 mL of distilled water were added and titrated against 0.05 M sodium thiosulphate solution using starch as the indicator. A blank test was carried out simultaneously without the oil under the same conditions.

$$I.V = \frac{[(\text{Blank Titre} - \text{Sample Titre (mL)}] \times 0.01269}{mass\ of\ Sample\ ()g} \times 100 \quad (2)$$

2.3.4. Free Fatty Acid

The free fatty acid (FFA) content of the Soy oil extracted was determined using Eqn. 3 (Chai *et al.,* 2014) and this involves the use of the relationship that relates FFA to the acid value (AV) of an oil sample.

$$The\ free\ fatty\ acid\ (FFA) content = AV/\ 2 \quad (3)$$

2.3.5. Saponification value

The Saponification Value (SV) of the Soy oil sample was determined according to ISO standard 3961. The soy oil sample (1 mL) was poured into a conical flask and 25 mL of 0.1 M of ethanolic KOH was added to it. The mixture was boiled for 30 mins under reflux. Phenolphthalein (3 drops) was added to the warm mixture and titrated against 0.5 M HCl acid until the pink colour disappeared (endpoint). The same procedure was

administered to the blank sample and the SV was calculated from Eqn. 4 (Amos-Tautua and Onigbinde (2013).

$$\text{Saponification value } (mg/g) = \frac{(\text{BLI} - \text{EPI})}{\text{S}} \times \text{TF} \times \text{Cl} \times \text{K1} \quad (4)$$

Where EPI is Titration volume (mL), BLI is Blank level (25.029 mL), TF is Reagent (HCl) factor (1.006), Cl is concentration conversion coefficient (28.05 mg/mL), Kl is Unit conversion coefficient (1) and S is the Sample size (g).

2.3.6. Refractive Index

The refractive index of the extracted Soy oil samples with the azeotropic solvent was determined based on the relationship between refractive index and iodine value (IV) as proposed by Pekins (1995) (Eqn. 5).

$$\text{Refractive Index} = 1.45765 + 0.0001164 \times (\text{IV}) \quad (5)$$

2.4. Determination of Physicochemical Properties of Defatted Cake Samples

2.4.1. Moisture content

The cake sample (5 g) was weighed into pre-weighed aluminium drying dishes and dried to constant weight in an oven (MEMERT) at 50 ^{o}C for 24 h. (Lui *et al.,* 2013). The moisture content was determined and calculated as follows

$$Moisture\ content = \frac{M_1 - M_2}{M_1 - M_0} \times 100 \quad (6)$$

Where M_0 is the weight of the aluminium dish, M_1 is the weight of the fresh sample + dish, and M_2 is the weight of the dry sample + dish.

2.4.2. Ash content

The cake sample (5 g) was weighed into a porcelain crucible previously ignited and weighed. Each seed sample was charred by igniting the materials on a hot plate in a fume cupboard. The crucible was placed in a muffle furnace and maintained at 600 ^{o}C for 6 hr. The resulting materials were cooled in a desiccator and later re-weighed to determine their percentage ash content according to Eqn. 7 (Lui *et al.,* 2013).

$$\%\ Ash = \frac{(crucible\ weight + Ash) - (Empty\ crucible\ weight)}{Sample\ weight} \times 100\% \quad (7)$$

2.4.3. Crude fat

The cake sample (5 g) was placed in thimbles and plugged with cotton wool into a Soxtec System (HT2). The extraction cup was dried and weighed, and then 25 mL petroleum ether was added into each cup, which was inserted into the Soxtec system for 45 minutes in a rising position (Lui *et al.,* 2013). The percentage of fat in the sample was calculated according to Eqn. (8).

$$\%\ Fat = \frac{W_3 - W_2}{W_1} \times 100\% \quad (8)$$

Where $W_1$the weight of the sample, W_2 is the weight of the empty cup and W_3 is the weight of the sample and the cup.

2.4.4. Carbohydrate

The total percentage of carbohydrate content in the cake sample was determined by the different methods as recommended by (Lui *et al.,* 2013). This method involved the subtraction of the sum of crude protein, lipid, crude fibre, moisture, and ash constituent values from 100 (Eqn. 9). The value obtained is the percentage carbohydrate constituent of the sample.

$$\%\ Carbohydrate = 100 - (Moisture + Ash + Crude\ fiber + Crude\ protein + Fat) \quad (9)$$

2.4.5. Crude protein

The Kjeldahl nitrogen method was used to quantify the protein content of the cake sample. The sample (5 g) was introduced into the digestion flask and five Selenium tablets of Kjedahl catalyst were added to the sample in which 20 mL of concentrated acid was added and then digested for 8 h until a clear solution was obtained. The cooled digest was transferred into a 100 mL volumetric flask and made up to mark with distilled water.

The distillation apparatus set was rinsed for 10 min by boiling and 20 mL of 4% Boric acid was pipetted into a conical flask, then 5 drops of methyl red were added to each flask as an indicator. The sample was diluted with 75 mL of distilled water and 10 mL of the digest was made

alkaline with 20 mL of NaOH (20%) before distilling. The Boric acid solution was changed to green and the mixture was further distilled for 15 min, then the filtrate was titrated against 0.1N HCl (Lui *et al.*, 2013). The percentage of total nitrogen was calculated as:

$$\%\,Total\ Nitrogen = \frac{Tire \times Normality \times 0.014}{Sample\ weight} \times 100\% \quad (10)$$

$$Crude\ protein = \%Total\ Nitrogen \times Conversion\ fat \quad (11)$$

Normality = 0.14, Conversion Factor = 6.25

2.4.6. Crude Fiber

The crude fiber of the cake sample was determined using the method of (Lui *et al.*, 2013). The cake sample (5 g) was placed in a filter crucible, which was inserted into the hot extraction unit (Hot Extractor, Model-1017). A sufficient amount of pre-heated 0.128 M H_2SO_4 was added to the reagent in the heating system and few drops of ethanol were added through the valves. The mixture was digested for 30 min, then washed with boiling water and filtered to remove the acid content. The residue in the flask was boiled with the required amount of 0.223 M KOH for 30 mins and then filtered with subsequent washing in boiling water and acetone. The residual content was oven-dried at 105 °C and then ignited in the muffle furnace at 550 °C for 3 h. It was transferred to a desiccator and weighed as W_1, then burnt in a muffle furnace at 500 °C for 6 h., allowed to cool and reweighed as W_2. Then percent crude fibre was calculated from Eqn. 12.

$$Percentage\ Crude\ Fibre = \frac{W_1 - W_2}{W_0} \times 100 \quad (12)$$

3. Results and Discussion

3.1 Design Matrix Evaluation for Mixture Quadratic Model

The Std. Dev. obtained for the available models (Linear, Quadratic, Special Cubic, and Cubic), embedded in the software are 1.73, 0.31, 0.35, and 0.000, respectively, while the corresponding R^2 was 0.5644, 0.9921, 0.9924, 1.0000 (Table 2). Similarly, their Adj R^2 was 0.4400, 0.9821, 0.9771, and 1.0000, but only Linear, Quadratic, and Cubic had Pred R^2 values of 0.1679, and 0.8352, and 0.5954, respectively, while their PRESS was 39.97, 7.92 and 19.43, respectively. The cubic model demonstrated the highest R^2, lowest Std dev., and minimum deference (0.000) between its Adj R^2 (1.0000) and Pred R^2 (1.0000). However, the ed that it was 'aliased' and as a result may need to be discarded as a suitable model for the study (Aremu *et al*, 2019). The option of the quadratic model for the study was buttressed based on its relatively high R^2 (0.9921), a small difference (0.1469) between the Adj R^2 (0.9821) and Pred R^2 (0.8352), smallest PRESS value of 7.92, and low standard deviation (0.31) of the data obtained to the mean values (Montgomery, 2005).

Table 2. Model Summary Statistics for the Responses from Ternary Mixture developed

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	Adj R^2 and Pred R^2 Differences	PRESS
Linear	1.73	0.5644	0.4400	0.1679	0..2721	39.97
Quadratic*	0.31	0.9921	0.9821	0.8352	0.1469	7.92*
Special Cubic	0.35	0.9924	0.9771	0.5954	0.3817	19.43
Cubic^	0.000	1.0000	1.0000	ND	ND	ND

* Suggested, ^ Aliased, ND- Not Define

3.2. Responses from experimental data

Run 6 with 9.17%, 6.67%, and 84.17% (Water, Ethanol and Ethyl acetate) mixture, gave the highest Soy oil yield of 15.56 %, while Run 5 (7.50% Water, 10.00% Ethanol and 82.50% Ethyl acetate) gave the least Soy oil yield (9.15%), respectively (Table 3). The yield from this study is higher than 14.51% and 15.2% reported for the use of petroleum ether and ethanol by Amos-Tautua and Onigbinde (2013)

and Dagostin *et al.*, (2018), respectively, for the extraction of soy oil from soybean, but less than 18.8% and 21.6% obtained from the use of 5:95 and 10:90 ethanol: ethyl acetate mixtures by Dagostin *et al.*, (2018). However, the use of pure ethanol or a high proportion of ethanol in solvent mixtures for oil extraction is affected by issues of solubilization of the extracted oil (Baumler *et al.*, 2016). The diagnostic case studies of the soy oil yield response (Table 3) indicate that Runs 1, 2, 3, 7, 8, and 10 gave positive residual values while Runs 4, 5, 6, 8, and 9 gave negative residual values.

Table 3. Result of Response (Soybean oil yield) from Experimental data

Run	Components			Response
	A: Water (%)	B: Ethanol (%)	C: Ethyl acetate (%)	Soybean oil yield (%)
1	5.00	10.00	85.00	14.14
2	10.00	5.00	85.00	12.82
3	10.00	10.00	80.00	10.26
4	9.17	9.17	81.67	10.05
5	7.50	10.00	82.50	9.15
6	9.17	6.67	84.17	15.56
7	7.92	9.17	82.92	12.25
8	6.67	9.17	84.17	14.88
9	10.00	7.50	82.50	10.05
10	10.00	10.00	80.00	10.26

3.3 Analysis of Variance (ANOVA) and Regression statistics

The Soy oil yield has a Model F-value of 100, (Table 4), which implies a significant model, with only a 0.03 % chance of occurrence due to noise. The linear mixture components and model terms (AB, AC, and BC) are significant with p-values of 0.0002, 0.0007, and 0.0137, respectively, which is less than 0.05 ($p<0.05$). R^2 obtained for soy oil yield response was 0.9921 while the Adj R^2 value was 0.9821 (Table 4) and the closeness of these values implies that there is a good correlation between observed and predicted values in the model (Anbia and Amirmahmoodi, 2016; Khani *et al.*, 2016). The adequate precision is 25.882, which is greater than 4, thus indicating an adequate signal of the model, applications (Montgomery, 2005). The PRESS is 7.92, which shows the suitability of the model in predicting the responses in new experiments however, small values are desirable (Montgomery, 2005). The Coefficient of Variations (CV) obtained for this model is 2.59, which is less than 10 percent and suggests a high tendency of reproducibility of the model (Agarry and Ogunleye, 2012). Low CV and SD indicate the accuracy with which the experiment was conducted and high precision in predicting the Soy oil yield.

Table 4. Analysis of Variance (ANOVA) for Soybean oil yield response for the development of Ternary Mixture

Source	Sum of Squares	Df	Mean Square	F Value	p-value (Prob > F)
Model	47.65	5	9.53	100.00	0.0003*
Linear Mixture	27.11	2	13.56	142.24	0.0002*
AB	16.44	1	16.44	172.50	0.0002*
AC	8.29	1	8.29	87.04	0.0007*
BC	1.68	1	1.68	17.61	0.0137*

Residual	0.38	4	0.095		
Lack-of-Fit	0.38	3	0.13		
Pure Error	0.000	1	0.000		
Cor Total	48.03	9			

NA- Not Applicable, *Significant at 0.05< (prob>F) < 0.1

3.4. Model equations of responses for the development of Ternary Mixture

The coded and actual terms as generated by the software are represented in the positive and negative coefficients of a model quadratic equation (Eqn. 13) indicating the positive and negative effects of the independent variables on the selected responses (Alade *et al.,* 2012). The coefficients +22.94, +1.71, and +2.60 obtained for model terms A (water), B (Ethanol), and C (ethylacetate) indicate that soy oil yield was highly influenced by the three solvents used (Araromi *et al.,* 2017). Similarly, the coefficient obtained for the mixtures of water and ethanol has a positive coefficient of 1.29 and this suggests that the mixture of the two solvents has a stronger influence on soy oil extraction. The negative coefficients of AC (α_{13}= -0.48) and BC (α_{13}= -2.3) indicate that the combined components were not as effective as the mixtures of water and ethanol components (Aremu *et al.,* 2019). It has been suggested that mixed polarity solvent systems with alcohol usually gave better overall oil yields than purely hydrocarbon-based solvents for extractions (Long, and Abdelkader, 2011).

$$Soy\ oil\ yield = 22.94A + 1.71B + 2.60C + 1.29AB - 0.48AC - 0.23BC \quad (13)$$

Where A is the coded variable for water, B is the coded variable for Ethanol and C is the coded variable for Ethyl acetate. α_0 is the intercept term, α_1, and α_2, are the influences of A and B on the process while α_{12} is the combined effect of A and B on the process.

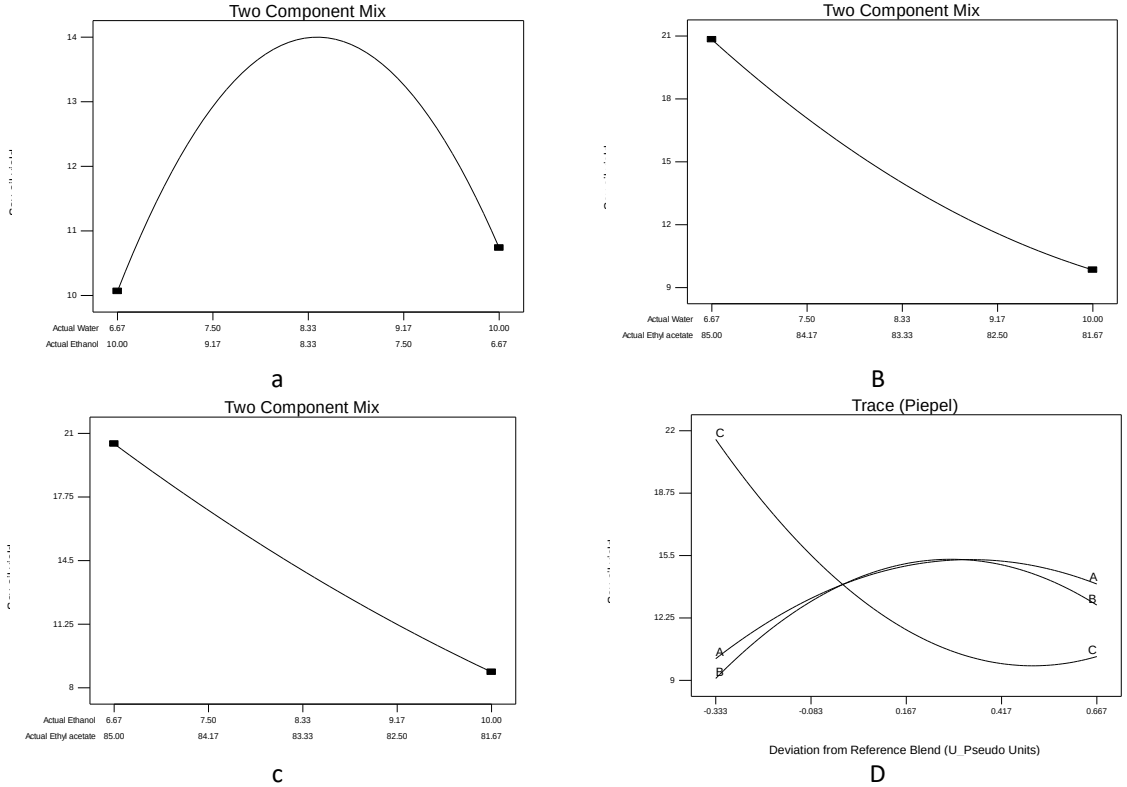

Figure 1. Plot of Two-Component Mix and Trace (Piepel) for the solvent mixtures

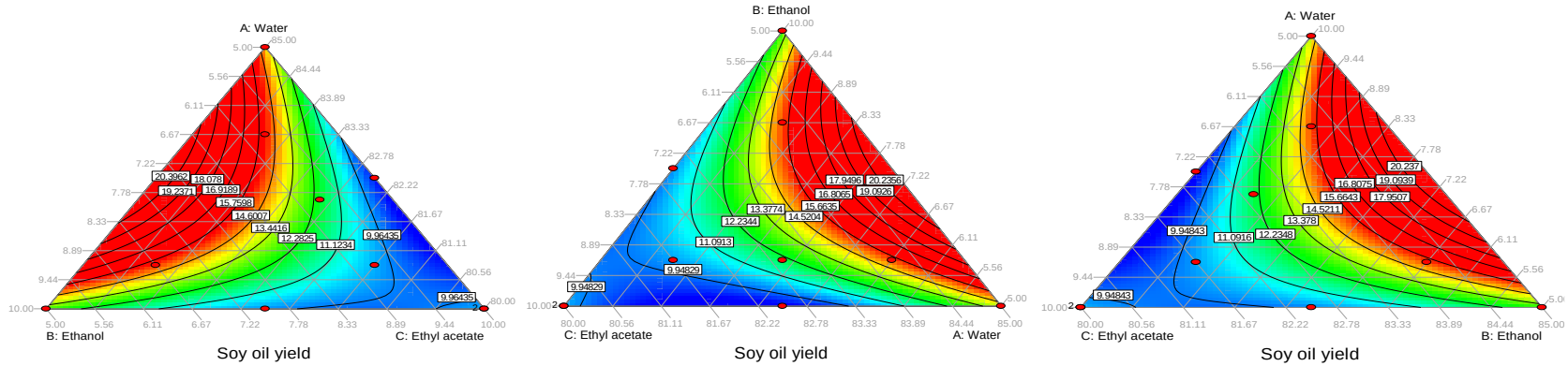

Figure 2. Contour plot of Soybean oil yield

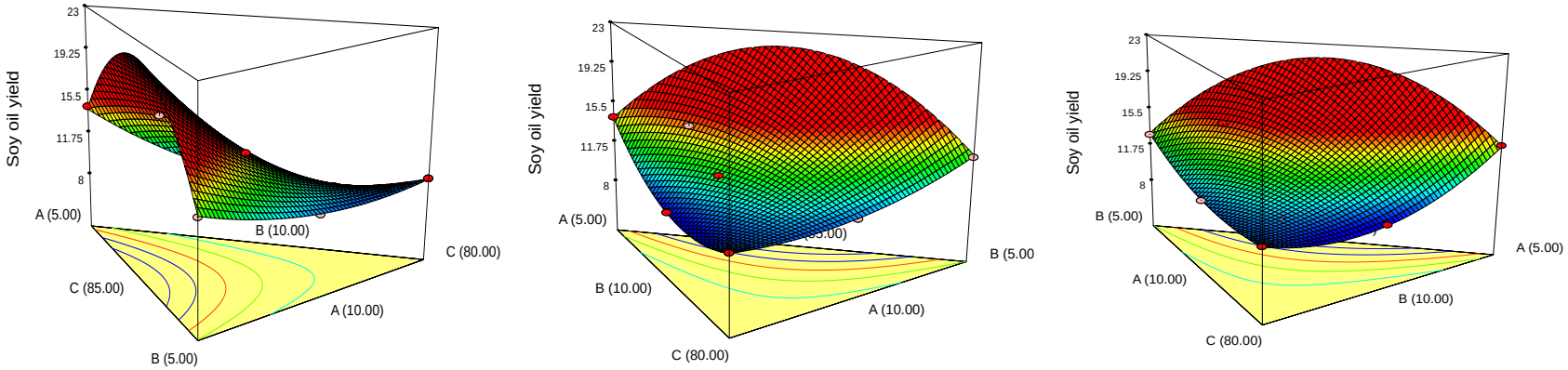

Figure 3. 3D plot of Soybean oil yield

3.5. Model graphs for the response to the development of Ternary Mixture

The interaction between water and ethanol components (Figure 1a) showed that soy oil extraction increased from 10 to 14% yield with increased volume of water and decrease the volume of ethanol until 8.33 % and 8.33% volume of the two components was reached at the maximum, at constant ethyl acetate volume (83.33 %) and then decreased as the volume of water increased to 10% while the volume of ethanol decreases to 6.67 %. This development suggests that an equal amount of water and ethanol may be effective in a ternary mixture involving ethyl acetate for the effective extraction of soy oil from its seed. The interaction between water and ethyl acetate components (Figure 1b) showed that soy oil extraction decreased from 21% to 9% with increased % volume (6.67 to 10) of water and decreased volume (85 to 81.67 %) of ethyl acetate, at constant ethanol volume (8.33 %). It could be observed that a ternary mixture involving a high volume of ethyl acetate and a low volume of water, in an azeotropic mixture with ethanol, is effective for the extraction of soy oil.

The interaction between ethanol and ethyl acetate components (Figure 1c) showed a similar trend observes in Figure 1b. Soy oil extraction decreased from 20% to 8% with increased volume (6.67 to 10 %) of ethanol and decreased volume (85 to 81.67 %) of ethyl acetate at constant water volume (8.33 %). The combined effect of this development is well illustrated in the trace (Piepel) diagram (Figure 1d) where curves A, B, and C represent the water, ethanol, and ethyl acetate components. The graphical illustration selected 8.33%, for each component.

The 3-D plots (Figure 2-3) indicate a quadratic model due to the curvy nature of the graph as also indicated in their contour plots (Alade *et al.,* 2012). Precisely, 3-D plots of the ternary azeotropic solvent mixture are expected to be characterized by a saddle curve as obtained in this study (Montgomery, 2009).

3.6. Numerical optimization studies

The SOY response selected was set to "maximize", while the solvent components [water (A), ethanol (B), and ethyl acetate (C) were all set within their percentage ranges (5-90%), (5-90%), and (80-85%). The numerical optimization of the data obtained was conducted using the software and the highest desirability is 1.000. The highest limit for SOY was 15.56 while the lowest limit was 9.15. Therefore, the optimum value suggested for water (A), ethanol (B) and ethyl acetate (C) components are 9.09 %, 5.91 %, and 85.00 %. The experimental value for soy oil yield was 15.56 while the numerical value was 17.928 from which the error difference gave 13.22 % (Table 5).

3.7. Physicochemical Properties of the Solvent Extraction Products

3.7.1. Physicochemical properties of the soy oil sample

The acidity of the soy oil extracted is 16.81 mgKOH/g which is slightly lower than the 19.21 mgKOH/g reported in soybean oil extracted with petroleum ether (Amos-Tautua and Onigbinde, 2013) (Table 6). The acid value obtained is higher than 0.37, 1.66, and 4.69 mgKOH/g of oil extracted from Cottonseed (CS), groundnut (GN), and melon seed (MS), but less than 65.50 mgKOH/g of maize seed oil (MZO) (Saxena *et al.,* 2011; Amos-Tautua and Onigbinde, 2013, Kadurumba *et al.,* 2018). The acid value is well related to the quality of fatty acid in the oil sample and the extent of storage on the oil quality. A high acid value suggests that the oil sample is susceptible to instability and rancidity over a long period (Aremu *et al.*, 2015; Kadurumba, *et al.*, 2018), and such oils are suitable for high cooking (Akintayo, 2004, Kadurumba *et al.,* 2018). The degree or extent of suitability of oil samples for consumption depends on their percentage of Free Fatty Acid (%FFA), and a lower level of % FFA indicates good quality. The % FFA value of the soy oil is 8.39%, which is relatively low, thus, suggesting the soy oil is suitable for human consumption, unlike MZO which has a relatively high % FFA (32.96%).

Table 6. Physicochemical Characteristics of Soy oil Extracted with Azeotropic Solvent mixture

Properties	**GNO**	**MZO**	**MSO**	**CSO**	**Soy Oil**
AV	4.69	65.50	1.66	0.37	16.81
FFA	2.33	32.96	ND	ND	8.39
SV	227.49	211.37	193.61	194.3	56.12
IV	38.65	47.25	112.53	106.8	15.17
PV	ND	ND	6.82	ND	27.00
RI	1.4622	1.4632	ND	1.4641	1.459

AV is Acid Value, FFA is Free Fatty Acid, SV is Saponification Value, IV is Iodine Value, PV is Peroxide Value, RI is Refractive Index. CSO is Cottonseed oil, GNO is Groundnut oil, MSO is Melon seed oil, MZO is Maize seed oil, and ND is Not Determined

Saponification value (SV) is an important physicochemical property of the oil and it indicates the extent of deterioration of oil during storage due to the oxidation effect (Aremu *et al.*, 2004, Kadurumba *et al.*, 2018). The SV (56.12 mmHg) of the soy oil is lower than 193.61, 194.5, 211.37, and 227.49 mgKOH/g were obtained for CSO, MSO, MZO, and GNO (Amos-Tautua and Onigbinde, 2013; Kadurumba *et al.*, 2018). The disparity between the SV reported in this study and the works of Amos-Tautua and Onigbinde, (2013) could only be linked to the solvent used, where the latter involved petroleum ether. The relatively low value of SV reported for soy oil indicates that the oil is less volatile and may not be suitable for the production of oil-based products such as ice cream, shampoo, and soap (Kadurumba *et al.*, 2018) nor applicable as drying oil (Araromi *et al.*, 2017).

The iodine value of soy oil is 15.17, which is lower than 38.65, 47.25, 106.8, and 112.53 reported for CSO, MZO, CSO, and MSO (Saxena *et al.*, 2011, Amos-Tautua and Onigbinde, 2013) and became more unsaturated. The low IV (15.17) of soy oil indicates that the oil is relatively saturated compared to others. The periodic value (PV) for the soy oil is 27.00 M/mol.kg which is higher than the 6.82 M/mol.kg obtained for MSO. Generally, refined oils are characterized by low PV while high PV indicates a high degree of oxidative rancidity of the oil sample. Furthermore, oil with high PV is highly depleted of antioxidant and thus require some level of antioxidant fortification to meet commercial grade (Kadurumba *et al.*, 2018). The refractive index (RI) of GNO, MZO, CSO, and soy oil are 1.4622, 1.4632, 1.4641, and 1.4069 respectively, which are within the ranges reported for various edible oils (Saxena *et al.*, 2011, Amos-Tautua and Onigbinde, 2013). The RI is an important oil characteristic that specifies the degree of conjugation of unsaturation as well as the length of the fatty acid chain and molecular weight of the oil sample (Amos-Tautua and Onigbinde, 2013).

3.7.2 Physicochemical Properties of Defatted Soybean Cake Sample

Moisture content (1.46 %) of the raw soybean sample (*Glycine Max (L*) used in this study is lower than 8.22 and 11.67 % reported for Gamasugen 1 (G1) and Gamasugen 2 (G2) varieties of soybean as well as sunflower seed (10.29 %) (Lisanti and Arwin, 2019) (Table 7). The high moisture content of seeds makes them prone to microbial attack and rapid deterioration, thus the low moisture content of the varieties of the soybean used in this study is very desirable (Buba *et al.*, 2015). The Ash content of the soybean sample is 16.75 %, which is higher than the 4.61 and 4.86 % of the G1 and G2 varieties of soybean respectively (Lisanti and Arwin, 2019). Percentage Ash content (%) is related to the amount of minerals present in the biomaterials.

Table 7. Physicochemical Characteristics of Soybean cake Defatted with Azeotropic Solvent mixture

Samples		Physicochemical Properties (%)				
	Moisture Content	Ash Content	Fibre Content	Lipid Content	Crude Protein	Carbohydrate Content
*Raw Soybean	1.456	16.75	4.64	20.30	33.87	22.99
*Defatted Soybean Cake	16.75	4.85	5.00	2.60	31.54	39.86
G1 Soybean	8.22	4.61	14.56	14.50	37.65	20.46
G2 Soybean	11.67	4.86	9.96	15.51	37.34	20.66
RUSSM	10.29	5.46	26.35	12.40	27.02	19.52
SESSM	8.44	4.96	18.4	6.45	45.31	17.77

G1 is Gamasugen 1, G2 is Gamasugen 2, RUSSM is Raw Undehulled Sunflower Seed Meal, and SESSM is Solvent Extracted Sunflower Seed Meal, * This study

The fiber content (4.64 %) of the soybean used is lower than the 14.56 and 9.96 % of G1 and G2 varieties of soybean. The lipid content of the three varieties ranged between 14.50 and 20.3 %, thus corroborating the submission of Rodrigues *et al.,* (2010) that categorized soybeans as low oil-containing biomass. The crude protein content (37.65 and 37.34 %) of the G1 and G2 soybean varieties is very close to the protein content (33.78 %) of the *Glycine Max (L*) used in this study. Soybean is one of the protein-rich oilseeds that has attracted wide cultivation in the world (Amos-Tutu and Onigbinde, 2013). The carbohydrate contents of the *G. Max (L*), as well as G1 and G2 soybean varieties, are 22.99, 20.46, and 20.66 %, respectively. These values are very close, it can therefore be suggested that variations in the proximate composition of different varieties of soybean may be due to the influence of environmental, genetic, and processing conditions (Grieshop and Fahey, 2001; Karr-Lilienthal *et al.,* 2004; Kumar *et al.,* 2006).

The trend of changes in the proximate compositions of the soybean cake after the azeotropic solvent extraction was monitored and documented in Table 7. This was compared with the changes observed in Sunflower seeds, subjected to the solvent extraction process. Essentially, there are differences between the proximate composition of the raw seed samples and the resulting (defatted) cake samples after the solvent extraction process. Distinctly, the difference in the lipid content before (20.30 % and 12.40 %) and after (2.00 % and 6.45 %) solvent extraction for the soybean and sunflower seeds, is very wide, thus indicating the impact of the solvent extraction. The two seed samples shared similar trends in their ash content after extraction. The increase in the moisture content of the soybean cake after extraction may be attributed to the water content in the azeotropic solvent mixture composition.

4.Conclusions

The composition of the azeotropic solvent mixture developed with the D-optimal Design under the Mixture Methodology was 9.17%, 6.67%, and 84.17% of water, ethanol, and ethyl acetate, respectively and was capable of extracting soy oil from its bean sample. The extraction process is best described by a Quadratic model and the physicochemical properties obtained indicate that the soy oil extracted is an oil sample fit for cooking.

5.References

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