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PHYSICOCHEMICAL AND FORMULATION STUDIES ON PLAIN AND FORTIFIED CASHEW (Anacardium Occidentale Linn) NUT OIL

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Article Info

Abstract

The need for an alternative to replace already existing excipient in pharmaceuticals is becoming very important. This is because traditional excipient is being over exploited in pharmaceutical formulations. The aim of this research was to extract, characterize and evaluate the self-emulsifying property of the oil from cashew nut seed. The oil was extracted using Soxhlet and cold press methods. Physicochemical analysis of the extracted cashew nut oil (CNO) which include determination of pH, moisture content, viscosity, saponification value, iodine value and peroxide value were determined using standard methods as outlined by the Association of Official Analytical Chemists (AOAC, 2016),(IUPAC,1979) and British Standard Institute 1999. Quantitative analysis of the oleic acid content of CNO was determined using the High Performance Liquid Chromatography (HPLC) and GCMS. Emulsions containing CNO were prepared in the presence and absence of an emulsifying self-emulsifying Oleic acid at varying concentrations. The organoleptic properties, pH, viscosity, creaming index, globule size and emulsion type of the prepared emulsions were determined. Stability studies of the prepared emulsions stored at 25 oC and 7 oC were also evaluated on day 1, day 30 and 3 months to assess the stability of the emulsions. The results showed that the Soxhlet method gave the highest oil content (33 %) than the cold press (27 %) methods of extraction. Quantitative analysis of the oil using HPLC showed that CNO from Soxhlet method contained 18.7 % of oleic acid and cold press method 46.2%. Stability studies showed that the emulsions containing emulsifying agent were homogenous and stable at 25 o C and 7 o C after 24 h, day 30 and 90 and their physicochemical properties were not significantly altered. On the other hand, emulsions prepared without emulsifying agent were found to be stable after 24 h but pH, viscosities, creaming indexes and globule sizes were observed to increase significantly by day 30, 60 and 90 days with Phase separation and inversion as an indication of instability. In conclusion, this study has been able to show that cashew nut oil possesses self-emulsifying property 24hr after formulation but cannot be used to prepare stable emulsions on its own without the inclusion of self-emulsifying agent.

Keywords:
Emulsion, Globules sizes, Linoleic acid, Oleic acid, Palmitic acid, pH, Surfactant, Stearic acid, SEDDS, Viscosity.

1. Introduction

Nigeria is blessed with varieties of nuts and its oils such as arachis, cashew and groundnut oils. In recent times, there has been a need to optimize these locally available raw materials as excipients for the needs of Nigeria's growing Pharmaceutical manufacturing companies.

To reduce the need for more ingredients in pharmaceutical formulations, it will be important to have locally available oil that possesses surfactant and excipient properties. Oleic acid is a surfactant with HLB value 1.6 and Oil Containing reasonable quantities of Oleic acid may possess Self Emulsifying properties (Gillian, 2021). In this research work Cashew kernel oil is extracted and its physicochemical properties studied. Its Oleic acid component was quantified to qualify its self-emulsifying properties in the formulation of Self Emulsifying Drug Delivery System (SEDDS). SEDDS are oil and surfactant-based preparations with the help of slow agitation that can be emulsified rapidly in water (Park et al., 2023).

Cashew nut is readily available in the southern and middle parts of Nigeria. Cashew nut oil is rich in oleic acid (Dendena and Torsi, 2021), and can be exploited as a biomaterial for pharmaceutical applications. By lowering the interfacial tension between the oil and water interface with the aid of surfactants and co surfactants, the self-emulsification feature provided will boosts solubility by minimizing precipitation (Gillian, 2021). Provision of local and readily available materials with sufficient quantity of oleic acid will be an added value to the varieties of excipients available to the pharmaceutical industries to aid formulations. It is therefore paramount to identify, extract and characterize local oils and study its possible Self-emulsifying properties (Barkat et al., 2020). The aim of this work was to carry out physicochemical and emulsion formulation studies on plain and oleic acid fortified cashew kernel oil.

2.0 MATERIALS AND METHODS

2.1.1 Collection, Authentication and Processing of Cashew Nuts

Cashew nut (Anacardium occidentale linn) from Odape Farm Land Kabba Local Government area of Kogi State. Cashew nuts were collected from Odape farm land kabba Local Government area of Kogi state, Nigeria. It was authenticated in the in the Harbarium unit of the Department of Pharmacognosy and Ethnopharmacy Usmanu Danfodiyo University Sokoto, Nigeria and assigned a Voucher NO: PCG/UDUS/ANAC/0002 for future reference. The nuts were sun dried for 7 days and slit open with the help of a simple knife cutter, the kernels were removed from the shell and then roasted in an oven at 70 ° C for 30 min in order to dry the kernel and remove the test. The nuts were then blended using an automated blender to obtain cashew nut powder (CNP). This powdered material was placed in a desiccator until further use.

2.2 Extraction of cashew nut oil

Soxhlet Extraction

The process described by Yahaya et al., (2021) was adopted. A sample of the powdered nut (100 g) was placed into the extracting chamber of the Soxhlet extractor (see Plate 1), then the extracting solvent (200 mL of n-hexane) was added into the chamber and the process allowed to run for 7 h at 25oC. After the process was completed, the oil was recovered by means of air drying/laboratory ovum evaporator and the yield determined using equation I.

$0- 1/ 0 \times 100\%$---------- (I)

Where: W0 is weight of the powder sample

W1 is weight of the residue after extraction

Plate I: Soxhlet Extraction Chamber used for Extraction of CNO Cold press extraction

A slurry of known weight (100 g) of the blended cashew nuts was introduced into a 500 ml beaker and heated to 920 C in a water bath for 1 hour. The slurry was then transferred into a white cotton cloth and cold press at 250c in to a fabricated cold press bucket (Plate II). The oil was obtained through the pressure exerted on the powdered nuts by means of the press.

The yield of the oil was determined using equation I.

Plate II: Fabricated Cold Press Machine used for extraction of CNO

2.3 Physico-Chemical Characterization of Cashew nut oil

1. Determination of Kinematic viscosity

The method used was that of the British Standards Institute (1999). The Viscometer was placed in the 1000ml measuring cylinder filled to mark with water and regulated to the appropriate temperature. The tube was then filled up to graduation mark over the left storage bulb with the sample (oil). The sample (oil) was then sucked up to the higher storage bulb in the right left of the tube and then released. The time

taken for the sample (oil) to flow from the upper mark to the lower was observed and calculated. The kinematic viscosity of the sample is calculated using the formula below:

Kinematic Viscosity (Y) = Absolute Viscosity (ɳ)…… (II)

Density (δ)

Absolute Viscosity (ɳ) = $\frac{t - t0}{t0}$

Where; t = time of flow of the sample t0 = time of flow of the reference (water in this case)

Determination of Saponification Value (S.V)

The method used was that of the British Standards Institute (1999). The oil (2 g) was placed in a 250 ml conical flask and 25 ml of ethanolic potassium hydroxide solution was added. A reflux condenser was attached and the flask content refluxed for 30 minutes on a water bath while swirling until it simmered. The mixture was then titrated against 0.5M HCl using phenolphthalein indicator while still hot. A blank determination was also carried out under the same conditions and the saponification value was calculated as thus;

Saponification Value (S.V) = (B-S) x 28.05 ……(III)

W

Where;

B = titer value of blank

S = titer value of sample

W = weight of oil

Determination of Peroxide Value

The peroxide value was determining using IUPAC (1979) method. Exactly 2-4g Sample was weighed in ground neck flask and 10ml chloroform was added to dissolve the sample followed by addition of 15ml acetic acid and 1ml 15% KI solution. The mixture was shaken and kept in the dark for 5minutes. After which 25ml water will be added and titrate with

0.002M sodium thiosulphate.

Calculation

Peroxide Value (P.V) = V x N x 100/W …… VI

Where; N = Concentration (Normality) of thiosulphate used

V = Volume of thiosulphate solution used

W = Weight in gram of test sample (g)

Determination of Iodine Value

Five milliliter (5 ml) of Chloroform Solution was taken and 5 ml of Dan's reagent (acetic acid + $CHCl_3$) was added, the solution was kept in fume cupboard for 10 min. Five milliliter (5 ml) of 10% potassium iodide was then added with 20 ml of distilled (H_2O), stirred several times to mix solution and titrate to a colorless end point with 0.025N $N_2S_2O_3$ (Eromosele, et al. 1994).

pH Determination

Procedure: The Mettler Toledo pH meter was standardized using a pH 7.0 phosphate buffer solution. The meter was dipped in 30 ml of the Sample Oil at room temperature and the pH was recorded. Triplicate measurements were recorded for each of the oil sample.

Moisture Content

Determination of moisture content

A quantity of the extracted oil; five (5) grams, was weighed into a previously dried and tarred porcelain dish. The lid of the dish was loosened to permit escape of moisture and heated in an oven at 105±1 o C for 1h. The dish was removed from the oven and the lid closed. The oil was cooled in a desiccator containing phosphorus pentoxide and then weighed. It was then heated in the oven for 1h, cooled and weighed again. This process was repeated until the change in weight between two successive observations was not more than one (1) milligram (AOAC, 2016). Triplicate determination was carried out and moisture content (%) was calculated using equation 4:

$$\text{Moisture \%} = 1 - 2\ 1 \times 100 \text{---------- (VII)}$$

Where,

W1-W2 = Loss in weight of the material on drying

W1 = Initial weight of oil and crucible

W2 = Final weight of oil and crucible

2.4 Extraction of Fatty acids from Cashew nut oil

The cashew nut oil (5 g) was added to 50 mL of 0.5 M NaOH at 100oC for 1hr to achieve hydrolysis of the oil in a reflux chamber. The mixture was cooled and 50 mL of petroleum ether added twice to remove any unsaponifiable fraction. The remaining fractions were acidified with 1 M HCl until a pH of 2.9 was achieved. After this, the free fatty acid was extracted twice with 50 mL petroleum ether. The petroleum ether was then removed at 40oC in a water bath. The acids were finally recovered with 4 mL of methanol and filtered with 0.22 μm Millipore filter. This was stored at room temperature for further HPLC analysis (Guarrasi et al., 2022)

Preparation of Standard Solution

A stock solution of oleic acid standard solution was prepared by dissolving 500mg of the standard in 1000cm3 of methanol.

Working solution 40, 80 and 120mg/L was then prepared from the stock solution (Guarrasi et al., 2022) using dilution formular:

$C_1V_1=C_2V_2$……..(VIII)

High Performance Liquid Chromatography (HPLC) of fatty acids in cashew nuts oil

Agilent HPLC (USA) 1260 infinity consisting of quaternary pump and a UV detector equipped with sampler TCC under computer control was used. The sample (10 µL) was analyzed in a column (250 × 4.6 mm i.e., particle size 5 µm). The mobile phase consisted of acetonitrile/water (85:15 v/ v) and was degassed to remove air bubbles. Total run time for the

HPLC analysis was 2 min at a flow rate of 1 mL/min while UV detection was carried out at 364 nm. The solution was acidified with 0.2 % acetic acid to stabilize the fatty acids. The composition of the fatty acids was determined after calculation of each content of the fatty acid from the cashew nut oil based on the calibration graph prepared using standard fatty acids (Table 1 and 2) (Guarrasi et al., 2022).

Table 1: Formular for Preparation of Cashew Nuts Oil Emulsions fortified with and without Oleic Acid as Surfactant

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
CNO (g)	10	20	30	40	10	20	30	40
Water (ml)	5	10	15	20	5	10	15	20
Propylene glycol (ml)	2.5	5	7.5	10	2.5	5	7.5	10
Oleic acid (g)	-	5.0	5.0	-	-	5.0	5.0	-
Water (ml) to	100	100	100	100	100	100	100	100

Table 2: Experimental Factorial Design

S/N	Nature of Oil (A)	Fortified or Not (B)	Speed (C)
F1	-	-	-
F2	-	+	-
F3	-	+	+
F4	-	-	+
F5	+	-	-
F6	+	+	-
F7	+	+	+

F8	+	-	+

KEYS

A- = COLD PRESS

A+ = SOXHLET

B+ = ADD OLEIC ACID

B- = ZERO OLEIC ACID

C- = 600 rpm (LOW SPEED)

C+ = 1200 rpm (HIGH SPEED)

2.5 Preparation of Cashew Nuts Oil Emulsions with and Without Oleic acid as Surfactant

Four (4) batches of emulsions (F1, F2, F3, F4,) containing 10, 20, 30 and 40 % v/v respectively of CNO (Cashew Nut Oil) extracted by Cold Press Method were prepared. Formulation F2 for example was prepared by measuring 20 mL of the CNO into a 500 mL beaker, Oleic acid 5mL was then added into the beaker and the contents were mixed gently by shaking. Distilled water (5mL) was transferred into the beaker and the contents shaken vigorously in a unidirectional manner with a glass rod until a uniform mixture was obtained. The resulting mixture was further diluted with distilled water (10mL) and propylene glycol (5ml) was added as co-surfactant and the volume was made up to 100ml with distilled water. The mixture was mixed using magnetic stirrer at 600 rotations per minute (rpm) for 30 min at room temperature. The resulting emulsion was packaged into glass bottles and stored at 25 o C for further analysis (Anukam et al., 2020).

The remaining Four (4) batches of emulsions (F5, F6, F7, F8) containing 10, 20, 30 and 40 % v/v respectively of CNO (Cashew Nut Oil) extracted by Soxhlet Method were prepared according to the composition in Table 3 & Experimental Design in Table 4.Batch F8 was prepared as follows; CNO (40 mL) extracted by Soxhlet was measured into a 500 mL beaker and propylene glycol 10ml were added into the beaker and the volume was made up to 100 mL with distilled water, the mixture was mixed using magnetic stearer 1200 rpm for 30 min at room temperature. The resulting emulsion was packaged in a glass container and stored at 25 o C for further analysis.

2.6 Evaluation of the Emulsions

2.6.1 Organoleptic characterization

All the formulations were observed for colour, texture and phase separation immediately after preparation. The feel of the preparations after application on the skin were also determined.

2.6.2 Determination of pH

The Mettler Toledo pH meter was standardized using a pH 7.0 phosphate buffer solution. The meter was dipped in 30 mL of the emulsion at room temperature and the pH was recorded.

Triplicate measurements were recorded for each emulsion formulation.

2.6.3 Determination of viscosity

The viscosity of the emulsion was determined using Brookfield viscometer with spindle number S-06. The emulsion (30 mL) was transferred into a 50 mL beaker, the spindle was placed in the beaker and viscosity of the emulsion at room temperature was obtained at 100 rpm. Triplicate determinations were obtained for each formulation of emulsions.

2.6.4 Creaming index

The formulated emulsions (10 mL) was transferred into universal bottles and tightly sealed with a cap and then stored at room temperature for 24 h. The height of total emulsion (HE) and the height of the droplet-depleted lower layer (HD) was measured using a meter rule.

Creaming index was then calculated using equation IX (Gillian, 2021).

Creaming Index = 100(H)⁄ …… (IX)

2.6.5 Globule Size Determination

The droplet size/globular size was determined using the capillary rise method. The capillary tube was slowly lowered into a container of emulsion sample and wait till it rose up the tube.

The globule size was then calculated using the formular:

D= V x t/A ……..(X)

A=Cross sectional area of the capillary

V=Volume of flow in capillary

T=Duration of one droplet in sec

Identification of emulsion type

Test using methylene blue

The formulated emulsion was viewed under a microscope to determine their types (o/w or w/o). A drop of the emulsion was placed on a slide and mixed with a drop of methylene blue (water soluble dye) with the aid of a spatula, the slide was covered with a cover slip and the colour of the droplets observed against the background under the microscope.

2.6.6 Stability study

The formulated emulsions (100mL) were stored in a refrigerator (7oC) and at 25oC (relative humidity of 75%) for 30 days, 60 days and 90 days. After this period, the emulsions were evaluated for organoleptic properties, pH, viscosity, globule size and creaming index (Gillian, 2021). Factorial regression analysis using Minitab Software was then used as statistical studies for the formulations.

3.0 RESULTS AND DISCUSSION

3.1 Percentage Yield

The yields of the oils extracted by the Soxhlet and cold press method were 33% and 27 % respectively.

3.2 Physical and Chemical Properties

The colour of the oils obtained was golden yellow for the Soxhlet extraction and dark yellow for the cold press method as shown in Table 3. The moisture content, viscosity, saponification value, iodine value, peroxide value and pH of the oils are shown in Table 3

Table 3: Physiochemical Properties of Cashew Nut Oil Parameter CNOS CNOC

CNOA official limits

Parameter	**CNOS**	**CNOC**	**Official limits**
Extraction Yield (%)	33	27	-
Color	Golden Yellow	Dark Yellow	
Moisture Content (%)	2.45	2.30	0.5-2.5
Saponification Value (mgKOH/g)	130.43	140.25	<180mgKOH
Iodine Value (gI2/100g)	98.52	90.32	80-100
Peroxide Value (MeqKOH)	0.35	0.67	<1
Viscosity (mPas)	2.0	3.2	-
PH	6.52	6.31	4-6.8

KEYS:

CNOC-Cashew nut oil from cold press extraction

CNOS-Cashew nut oil from Soxhlet extraction

3.3 Fatty Acid Composition of Cashew Nuts Oil

High performance liquid Chromatography (HPLC) was used as a means of studying the fatty acid composition of cashew nut oil. Peaks were obtained as shown in the chromatogram (Figure 1). The fatty acid peaks were identified and quantified from the calibration curve of the respective standard. The percentages Oleic acid, linoleic acid, palmitic acid and stearic acid were quantified to be 18.71, 40.20,

39.02 and 5.70 respectively for CNO from Soxhlet method and 46. 10, 51.3, 48.7 and 20.02 for CNO from Cold press method (Table 4 and Table 5 respectively).

Table 4: Soxhlet Extracted Fatty Acid Composition of Cashew Nut Oil

S/N	Fatty acids	Composition (g/100g)
1	Oleic acid	18.70
2	Palmitic acid	39.20
3	Stearic acid	5.70
4	Linoleic acid	40.20

Table 5: Cold Press Extracted Fatty Acid Composition of Cashew Nut Oil

S/N	Fatty acids	Composition (g/100g)
1	Oleic acid	46.2
2	Palmitic acid	48.7
3	Stearic acid	20.02
4	Linoleic acid	51.3

Results of Formulated Emulsion

The emulsion formulated at different concentrations were found to be white, smooth to touch and non-gritty on application to the skin (Plate V).

3.4 pH

The pH of the different emulsions from plain CNO at 24 hr. were (F1=5.8, F4=4.9, F5=5.7, F8=5.2 slightly acidic while that of fortified CNO formulation (F2=2.9, F3=2.6, F6=2.4, F7=2.3) were acidic. However, the pH was found to decrease after storage for 30 days and 3 months at both 25 o C and in the 7oC as shown in Figure 1 & 6.

3.5 Viscosities of Formulated Emulsion

The viscosities of the formulated emulsions were taken at different storage conditions. The result revealed a decrease in viscosity at 25 o C, however, viscosity was found to increase for emulsion stored at 7 o C in the refrigerator as shown in Figure 3.

3.6 Creaming Index of Formulated Emulsions

The creaming index of the emulsions at 24hr after formulation are shown in Table 7 and Table 8. Creaming index was found to decrease with increase in concentration of CNO in formulation. However, phase

separation was observed for the batches of emulsions that do not contain Oleic acid when stored at 25 o C and in the refrigerator at 7 o C for 30 days, 60 days and 90 days.

3.7 Globule Size

The average globule sizes of the formulated emulsions with oleic acid were seen to be in the range of 0.8µm-1.2µm at 24hr as in Fig 4.8. Also the average globule size of the emulsion formulated without oleic acid were seen to be higher than emulsions formulated with oleic acid. A similar trend was observed after storage for 30 days, although the formulations stored at 7oC were seen to have smaller globule sizes. No further increase in globule size was observed when the emulsions were stored for 90 days. However, those emulsions containing oleic acid were observed to have smaller globule sizes than those prepared without oleic acid.

Emulsion Type

All the formulated emulsions were observed to be oil-in-water (o/w) type after preparation and on storage for 30 days, 60 days and 90 days at 7oC and 25oC as revealed by the up-take of the methylene blue dye .Formulations containing oleic acid were found to maintain the o/w consistency, on storage for 30 days, 60 days and 90 days at 25oC and 7oC, while those without oleic acid were observed to have inverted to the water-in-oil (w/o) type.

Plate V: Visual Appearance of plain CNO Emulsions at 24hr after Formulation

Plate VI: Visual Appearance of plain CNO Emulsions Stored at 25oC for 30 Days

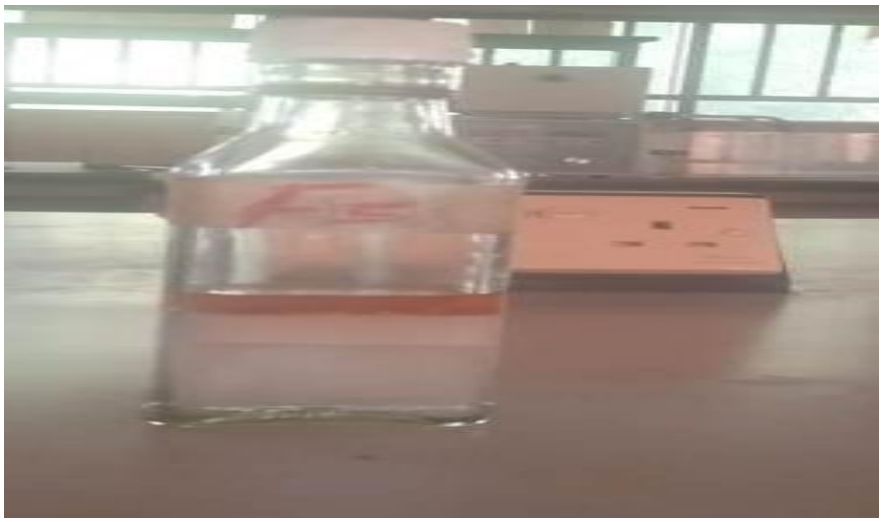


Plate VII: Visual Appearance of plain CNO Emulsions Stored at for 250C for 60 Days

Plate VIII: Visual Appearance of plain CNO Emulsions Stored at for 250C for 90 Days

Plate IX: Visual Appearance of oleic acid fortified CNO Emulsions Stored at for 250C for 24hours

Plate X: Visual Appearance of oleic acid CNO Emulsions Stored at 250C for 30 Days

Plate XI: Visual Appearance of oleic acid fortified CNO Emulsions Stored at 250C for 60 Days

Plate XII: Visual Appearance of oleic acid fortified CNO Emulsions Stored at 250C for 90 Days

Table 6: Organoleptic Properties of CNO Formulation at 24 h, 30 Days, 60 days and 3 Months

FORMULATION	24hrs	30 days	60 days	90 days
F1	C,W,P	C,W,Mp	C, W, P	C, W, P
F2	C,W,P	C,W,Mp	C, W, P	C, W, P
F3	C,W,P	C,W,Mp	C, W, P	C, W, P
F4	C,W,P	M, Wb, Mp	M, Wy, Mp	M, Wy, Mp
F5	C,W,P	M.Wb, Mp	M, Wy, Mp	M, Wy, Mp
F6	C,W,P	M.Wb, Mp	M, Wy, Mp	M, Wy, Mp
F7	C,W,P	M.Wb, Mp	M, Wy, Mp	M, Wy, Mp
F8	C,W,P	M.Wb, Mp	M, Wy, Mp	M, Wy, Mp

KEY:

C= Cloudy

W= Whitish

P= Pleasant

M= Milky

Mp= Mildy pleasant

Wb= Whitish brown

Wy= Whitish yellow

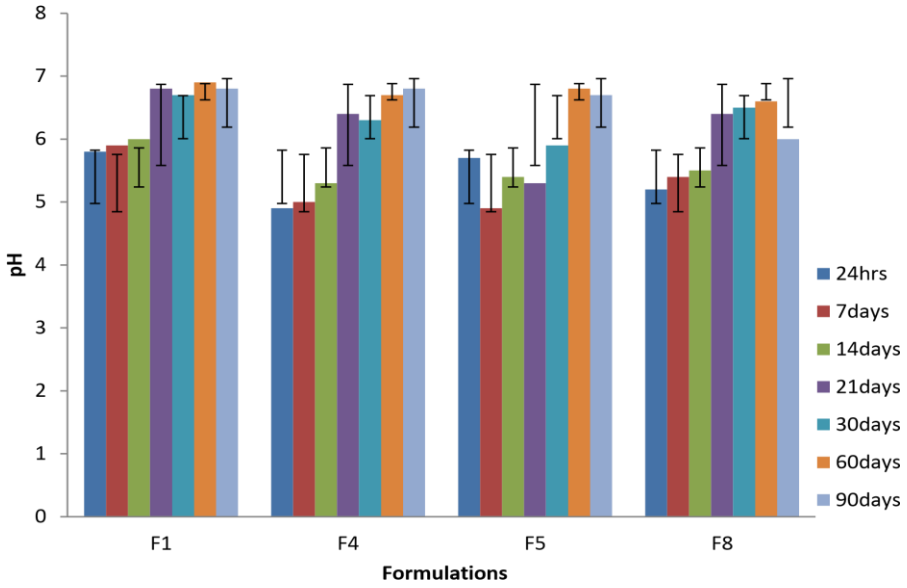


Figure 1: Result of pH of Plain CNO Formulation

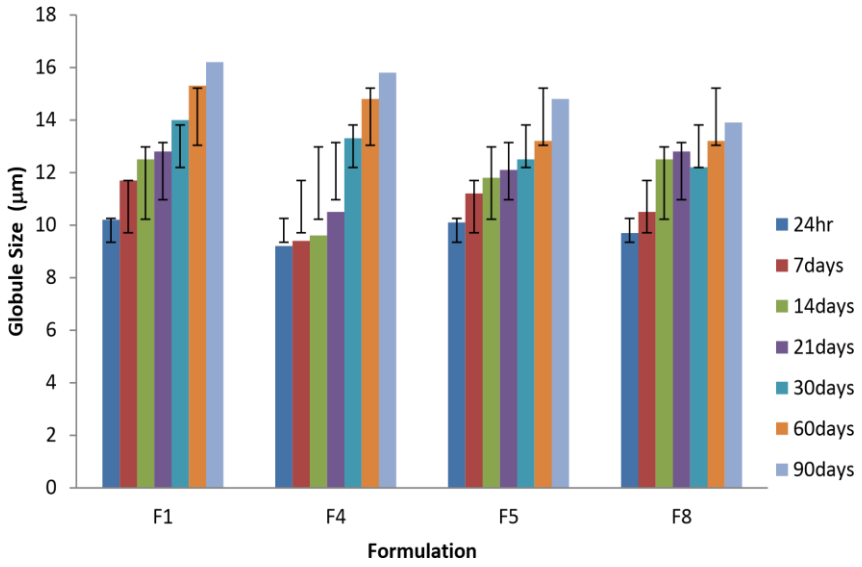
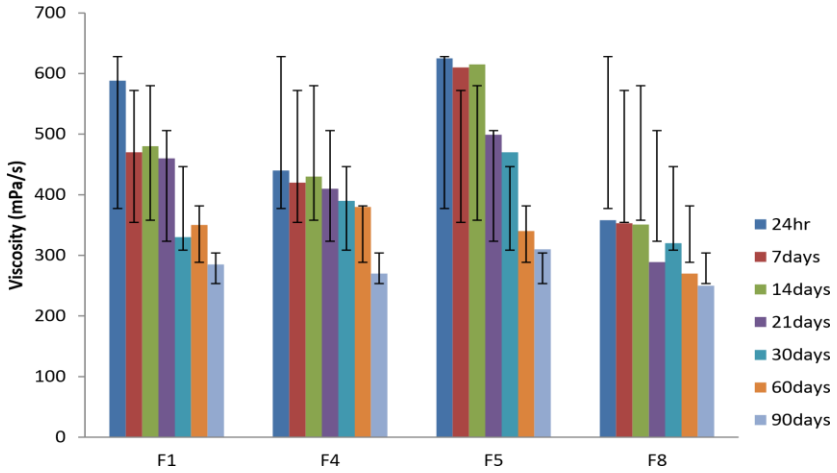


Figure 2: Result of Globule Size for Plain CNO Formulation



Formulations

Figure 3: Result of Viscosity of Plain CNO Formulation

Table 7: Results of Cream index of plain CNO Emulsion

Formulations	24hr	7 Days	14 Days	21 Days	30 Days	60 Days	90 Days
F1	81	84	85	90	PS	PS	PS
F4	75	78	80	88	PS	PS	PS
F5	64	63	66	89	PS	PS	PS
F8	69	71	73	90	PS	PS	PS

KEYS:

PS=Phase Separation

Table 8: Result of Cream Index of Oleic acid fortified Formulation

Formulations	24hr	7 Days	14 Days	21 Days	30 Days	60 Days	90 Days
F2	7.33	8.33	29.20	38.30	43.20	6.10	6.33
F3	0.67	0.80	8.25	8.70	10.0	6.20	50.30
F6	6.33	0.33	8.20	8.50	9.30	9.90	11.33
F7	5.0	6.50	7.90	8.30	8.90	23.40	26.67

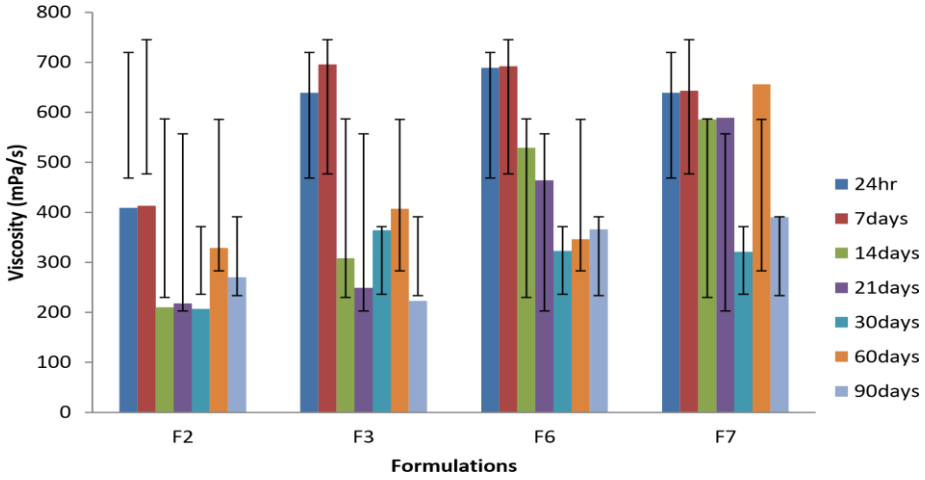


Figure 4: Result of Viscosity of Oleic Acid Fortified CNO Formulation

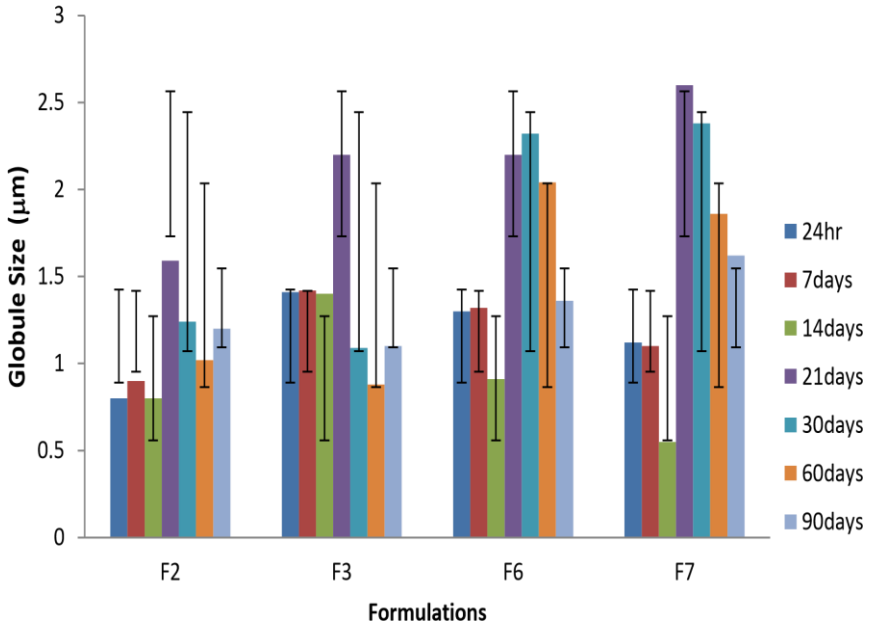


Figure 5: Globule Size of Oleic Acid Fortified CNO Formulation

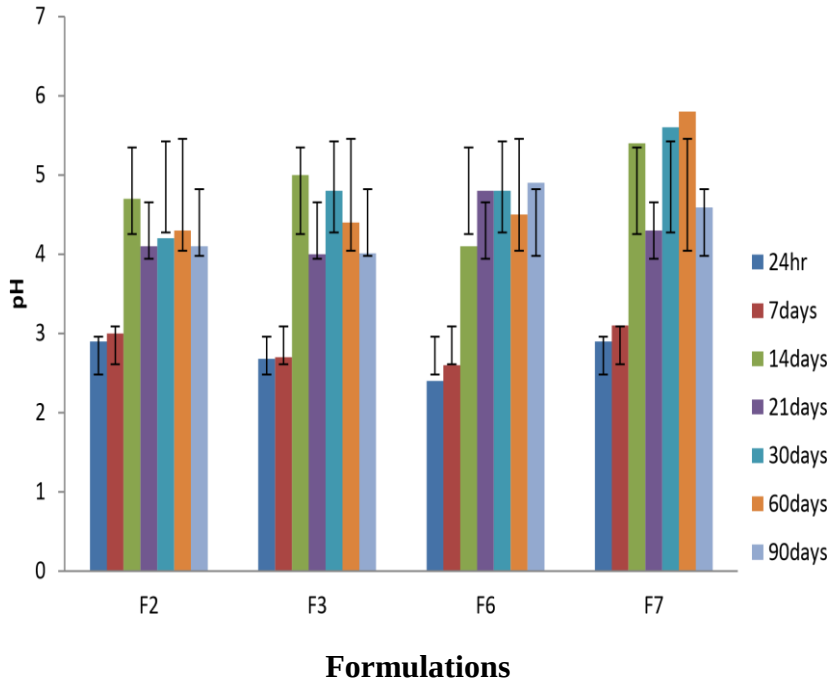


Figure 6: Result of pH of Oleic acid Fortified CNO Formulation

4. Discussion

The result obtained indicates that, extraction using the Soxhlet process gave higher yield (33%) than the cold press (27%). The extraction yield of the Soxhlet process can be ascribed to enhanced solvent action on the oil by Soxhlet process which involves heating the solvent to reflux. This result implies that employing the Soxhlet method of extraction can be considered for commercial production of cashew nuts oil (CNO) in Nigeria but for small scale production of Cashew not oil Cold Press Method is more economical.

The color of CNO obtained from the two (2) extraction techniques ranged from golden yellow to dark yellow, Table 3. The dark yellow color of the cold press method could be ascribed to the action of heat involved in the process of removing the test a while the yellow coloration of the Soxhlet method could be due to the retention of β-carotene in addition to the fact that the oils are not refined. These characteristic colors are usually not found in refined oils because carotenoids are absent in them. However, this unrefined oil has the advantage of retaining all its qualities compared to refined oil (Halim et al., 2023). Similar findings were also reported by Yahaya et al., (2021).

The moisture content of oil is the quantity of water in a given sample of that oil (Yahaya et al., 2012). Presence of moisture in oils can predispose it to microbiological contamination as well as lipid oxidation which can eventually lead to rancidity. Moisture contents of the oil extracted by Soxhlet, and cold press were found to be 2.5 and 2.3 % respectively, (Table 3) which is within the acceptable range of 0.5-2.5 % reported by AOAC (2016). These variations could be a result of regional differences in cashew production and differences in the extracting conditions. However, the moisture contents observed for CNO via Soxhlet and cold press methods imply that the oil can be preserved for a long period without going rancid.

Viscosity of oil is the resistance offered by the oil to flow (Maphosa and Victoria, 2018). These results revealed that CNO obtained by Cold press method produced more viscous oil (3.2 mPas) than those obtained by the Soxhlet (2.0 mPas) method, Table 3. The high viscosity of the cold press extraction method could be attributed to the solid contents such as powdered cashew nuts present in the oil and will mean that emulsions formulated with this oil will not flow readily which could lead to irregular dosing of active pharmaceutical agent (Maphosa and Victoria, 2020).

Saponification value gives information about the molecular weight of a given fatty acid, (Mahato and Victoria, 2020). The saponification value of the oil extracted by the Soxhlet method was found to be lower (130.4 mgKOH/g), while those extracted via the cold press 140.25mgKOH/g respectively, Table 3. Official specifications show that oils that have saponification values equal or above 180 mgKOH/g comprise mainly of fatty acids with low molecular weight while those with values less than 180 mgKOH/g contain fatty acids with high molecular weight. Table 4.2 shows that cashew nut oil contains high molecular weight fatty acids with large number of carboxylic acid which will provide suitable functional groups for reaction with alkali in the formulation of emulsions.

Iodine value gives information about unsaturation of the oil and portrays that the oil can absorb oxygen on exposure to the atmosphere (Halim et al., 2023). It also indicates the extent of adulteration in the oil and the drying properties as well. The iodine values obtained for the Soxhlet and cold press method 98.52 and 90.32 g I2/100g respectively (Table 3). These values are within the acceptable limits of 80-100 g I2/100 g specified by AOAC (2016). Idah et al., (2021) reported a value of 86 g I2/100 g for Soxhlet extraction. As an indicator of unsaturation, the value obtained indicates that CNO is a non-drying oil, as such, will not harden when exposed to air and when formulated into a pharmaceutical emulsion.

The peroxide value indicates the ability of an oil to withstand oxidation. The peroxide value for the Soxhlet, and cold press methods are 0.35, and 0.67 MeqKOH/g respectively (Table

3). These fall within the acceptable range of < MeqKOH/g required by the AOAC (2016). This shows that CNO extracted by the above two methods will be stable to oxidative degradation. As such, the stability of formulations will be preserved when this oil is used for preparation of emulsion.

The pH value of a topical preparation in relation to that of the skin should results to a secretion that is slightly acidic which helps in protecting the skin, as against alkalis preparation which makes the skin dry

and harsh (Barkat et al., 2020). The pH values for CNO extracted from the Soxhlet, and cold press methods are 6.52 and 6.31 (Table 3). Saeed and Shola (2019) reported a value of 5.96 for Soxhlet extracted CNO. This shows that when CNO is applied to the skin, it will not disrupt the normal pH level of the skin. This is important in pharmaceutical and cosmetic formulations like emulsions because highly acidic or alkaline formulations will disrupt the skin's protection against pathogenic bacteria (Barkat et al., 2020). The fatty acid composition of CNO extracted by Soxhlet method revealed the presence of oleic acid (18.70 %), linoleic acid (40.20 %), palmitic acid (39.20%) and stearic acid (5.7 %) (Table 4). The fatty acid composition of CNO extracted by cold press method revealed the presence of oleic acid (46.20 %), linoleic acid (51.30 %), palmitic acid (48.70%) and stearic acid (20.02 %) (Table 4). These differences in fatty acid concentrations can be ascribed to the process involved in the extraction such as choice of solvent and heating by reflux.

As observed from this study both CNO from Soxhlet and cold press method contained significant quantities of oleic acid and could possess self-emulsifying properties (Gillian, 2021) but the oleic acid concentration may not be sufficient to formulate a stable emulsion.

All the emulsions formulated from oils extracted by Soxhlet method (Soxhlet method produced the highest yield of the extracted oil, 33 %) were white in color, smooth non-gritty in appearance with mildly pleasant odor 24hr after preparation (Table 5). However, on storage at 25 o C for 30 days and 3 months the color of the emulsions formulated without Oleic acid surfactant (F1, F4, F5, F8) showed a slight change to whitish-brown with a mildly pleasant Oduor. Similarly, the emulsions containing the oleic acid (F2, F3, F6, F7) changed color from white to whitish-yellow with a mildly pleasant odor. When the emulsions containing the Oleic acid (F2, F3, F6, F7) were stored for 30 days and 3 months at 8 o C they were observed to retain their organoleptic properties while those without surfactant (F1, F4, F5, F8) were found to be milky, whitish-yellow and mildly pleasant. In addition, the intensity of the coloration was found to increase with increase in the concentration of the oil from 20 to 40 %. This shows that the stability of an emulsion is influenced by its storage condition, concentration and the presence or absence of a surfactant. Pharmaceutically, the emulsions prepared without surfactant will lose acceptability by users which will result in noncompliance of the formulation.

The pH as a physicochemical parameter plays an important role in molecular, metabolic and cell regulating processes (Gillian, 2021). The pH requirements for topical preparations is recommended to be between 4 and 6.8 (Gillian, 2021); changes in the pH of formulations affect the barrier functions of the epidermis and cause skin damages or more untoward effects on the skin. In this present study, the pH of all the formulated emulsions with oleic acid is between 2.9-5.9 while those of plain CNO 4.9-6.9 (Figure 1) and Figure 4 across the storage conditions and time. There was no significant difference in the pH values between the different batches of emulsions 24hrs after formulation. There was also no significant difference ($p>0.05$) in pH between emulsion with Oleic acid (F7) and those without oleic acid (F5) (Appendix 1). The statistical difference was significant when compared between emulsions at 24hr and those stored at 7 o C for 3 months. However, the pH of emulsion containing oleic acid (F2, F3, F6, F7) were found to be slightly lower pH (4.9) than those prepared without oleic acid (F1, F4, F5, F8) pH (6.9). In addition, increase in the concentration of the oil from 20 to 30 %, did not result to appreciable increase or decrease in the pH of the formulations. This implies that storing the emulsion at high temperature increases the rate of instability as the formulations were found to be more stable at lower temperature of 7oC.

Viscosity is a measure of a fluids resistance to flow (Barkat et al., 2020). Generally, viscosities of the formulations with oleic acid present a regular pattern. Viscosities of the formulated emulsions 24 h after preparation were between 409 mPas and 639mPas at a shear rate of 100 rpm and was found to increase

with increasing concentration of the oil phase (Figure 3). Viscosity of the emulsions were observed to decrease with increasing concentration of the oil (for formulations without oleic acid). However, viscosities of emulsions prepared with oleic acid were observed to be higher than those prepared without the oleic acid. This shows that oleic acid increase viscosity of emulsion.

The result shows that there is no significant difference between the batch of emulsions containing oleic acid (F7) and those without (F4) on storage for 30 days and 3months. These uneven viscosities in formulations prepared without oleic acid implies that the emulsion will not flow as required during dispensing which will lead to irregular dosing. Creaming occurs when there is a density difference between the two phases of an emulsion. It is the precursor of coalescence which will eventually lead to phase separation (Maphosa and Victoria, 2020). This means that for an emulsion to be stable, the rate of creaming should be slow. Increasing the concentration of oil in all the emulsions was found to decrease the creaming rate (Table 6). In addition, emulsions prepared with the oleic acid (F2, F3, F6. F7) were found to have lower creaming rates (between 7.33 and 50.33 %) than those prepared without the oleic acid F1, F4, F5 and F8 which were between 63 and 90 %. This can be attributed to the reduction in interfacial tension brought about by the presence of the oleic acid. The creaming rate of the emulsions prepared with oleic acid was found to decrease upon storage at 25oC and at 7oC and can be attributed to the effect of the oleic acid. However, those prepared without the oleic acid were found to completely separate out (phase separation) upon storage at 25oC and at 7oC which is as a result of the absence of oleic acid. This shows that the emulsions are unstable without oleic acid. It also infers that these emulsions prepared without oleic acid will lose their aesthetic nature and acceptance by users.

The globule sizes are used to differentiate an emulsion from nano emulsion or micro emulsion (Maphosa and Victoria, 2020). An emulsion will be stable if the globule sizes are uniformly distributed throughout the continuous phase. Aggregation of globules, will lead to flocculation and finally phase separation. The average sizes of the emulsion globules ranges between 0.8µm and 2.52µm (Figure 5) and 9.2-15 µm .There is no significant difference in the globule sizes of the emulsion 24hr after formulation ($p>0.05$) and on storage for 30 days and 3 months at 25oC and 7oC for oleic acid fortified formulations. Also, globule sizes of the formulation were found to increase with increase in the concentration of the oil phase from 20 to 30 %. As a result, emulsions containing oleic acid are stable and are able to retain uniformity of active pharmaceutical ingredients as against those formulated without oleic acid. The dye test reveal whether an emulsion is water-in-oil or oil-in-water and can also reveal the change that occurs in emulsion phases i.e. when an emulsion changes from oil-in water (o/w) to water-in-oil (w/o) as a result of change in surfactant affinity or temperature difference. Figure 6 shows that the emulsions were formulated as oil-in-water (o/w). However, on storage for 30 days and 3 months at 25oC and 7oC, the formulations with oleic acid (F2, F3, F6, F7) were seen to remain as o/w while formulations without oleic acid (F1, F4, F5, F8) changed to w/o. This means phase inversion has taken place in the formulations without oleic acid as such will not be considered fit for pharmaceutical use. Results from statistical test 23 factorial regression analysis conducted using Minitab Software on the formulated emulsions shows that the main factor Fortified or Not (B) affect the response pH Value with ($p=0.000$) of all the emulsions (F2, F3, F6, F7) stored at 25oC and 7oC for 24hrs, 30 days,60 days and 3 months. Other factors and interactions are not statistically significant ($p>0.05$) as in appendix II. Also Two ways and 3 ways Anova shows $p=0.310$, $p=0.55$ and $p=0.498$ which are not statistically significant. Also Fortification significantly reduce the globule size with ($p=0.000$) of the emulsions stored at same storage conditions above while the Nature of oil and Speed of rotations are not statistically significant with $p>0.05$. However, a marginal interactions between the nature of oil and fortification was observed. Residual diagnostics identified outliers that may impact the results of oleic acid formulations (F2, F3, F6, F7). The model explains only 29.55 % of variability in viscosity of all the emulsions. However, there is significant interactions between Fortified or Not (B) and Speed (C) with $p=0.041$ while there is no significant interactions among the three factors on the viscosity of the

formulated emulsions with $p>0.05$. It can be inferred that emulsion formulated from oleic acid fortified CNO (F2, F3, F6, F7) will possess pH, Globule Size and Viscosity that will offer excellent stability over the duration of the stability studies.

5. CONCLUSION

The Cold Press Extraction method is the most economical method of extracting CNO at less Cost. Soxhlet extraction can be employed for large scale production of Cashew Nut oil producing the highest yield. CNO from Cold Press Method can be exploited in the formulation of stable emulsions at a concentration of 10, 20, 30 and 40% in the presence of self-emulsifying oleic acid.

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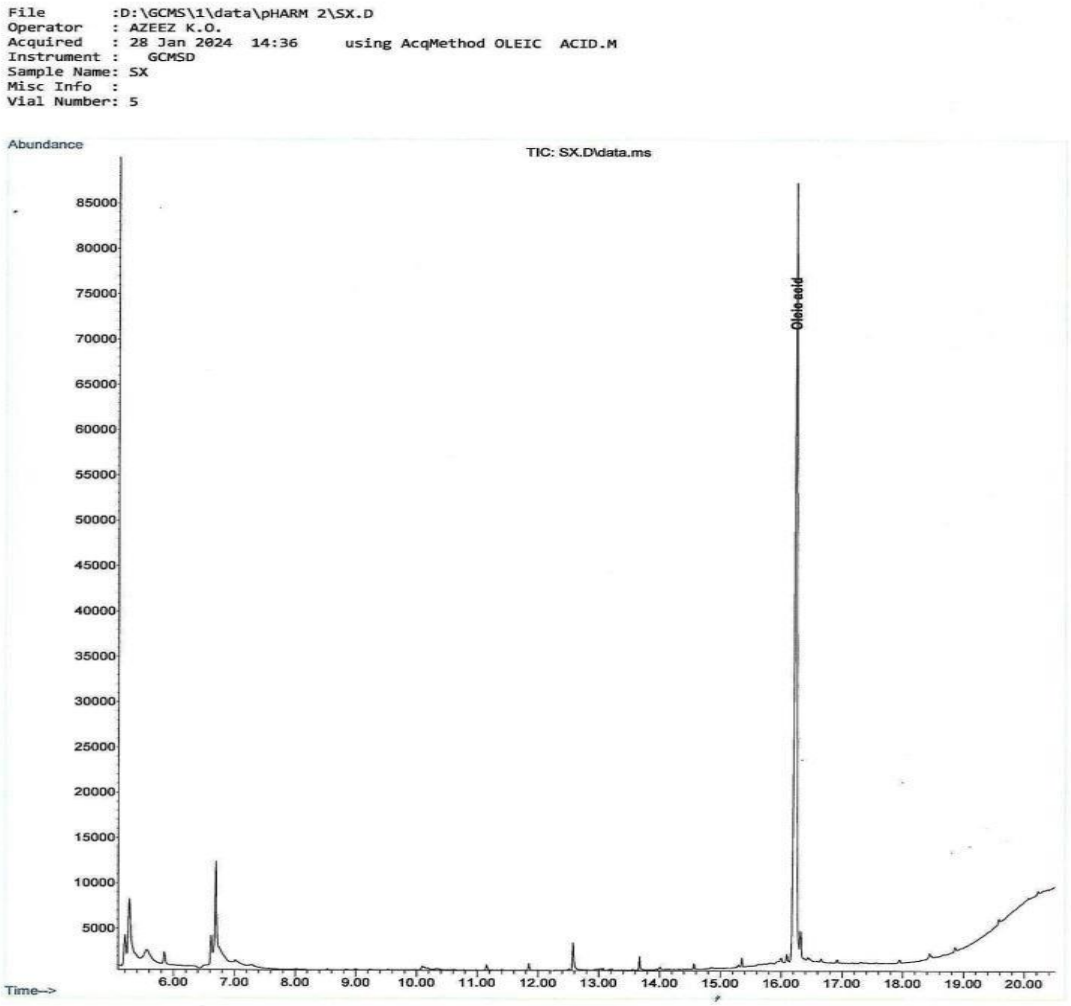
APPENDIX I

Chromatogram of Linoleic, Palmitic, Stearic and Oleic acid From CNO obtained by Soxhlet Method.



APPENDIX II Statistical Analysis of Formulated Emulsions Factorial Regression: Globule size (Output) versus Nature of Oil (A), Fortified or Not (B), Speed (C)

Coded Coefficients SE T- P-

Term Effect Coeff Coeff Value Value

Constant	6.958	0.333	20.92	0.000
Nature of Oil (A)	-0.652 -0.326	0.333	-0.98	0.337

Fortified or Not (B)	- 11.072	- 5.536	0.333	-16.64	0.000
Speed (C)	0.231	0.115	0.333	0.35	0.732
Nature of Oil (A)*Fortified or Not(B)	1.311	0.655	0.333	1.97	0.060
Nature of Oil (A)*Speed (C)	0.653	0.327	0.333	0.98	0.336
Fortified or Not (B)*Speed (C)	-0.207	- 0.103	0.333	-0.31	0.759
Nature of Oil (A)*Fortified or Not(B)*Speed (C)	-0.684	- 0.342	0.333	-1.03	0.314

Term	**VIF**
Constant	
Nature of Oil (A)	1.00
Fortified or Not(B)	1.00
Speed (C)	1.00
Nature of Oil(A)*Fortified or Not(B)	1.00
Nature of Oil(A)*Speed (C)	1.00
Fortified or Not(B)*Speed (C)	1.00
Nature of Oil(A)*Fortified or Not(B)*Speed (C)	1.00

Model Summary R- R-

S	R-sq	sq(adj)	sq(pred)
1.88160	92.21%	89.94%	86.15%

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	7	1005.76	143.680	40.58	0.000
Linear	3	984.52	328.172	92.69	0.000
Nature of Oil(A)	1	3.40	3.400	0.96	0.337
Fortified or Not(B)	1	980.69	980.691	277.00	0.000
Speed (C)	1	0.43	0.426	0.12	0.732
2-Way Interactions	3	17.50	5.832	1.65	0.205
Nature of Oil(A)*Fortified or Not(B)	1	13.74	13.742	3.88	0.060
Nature of Oil(A)*Speed (C)	1	3.41	3.413	0.96	0.336
Fortified or Not(B)*Speed (C)	1	0.34	0.342	0.10	0.759
3-Way Interactions	1	3.75	3.747	1.06	0.314
Nature of Oil(A)*Fortified or Not(B)*Speed (C)	1	3.75	3.747	1.06	0.314
Error	24	84.97	3.540		
Total	31	1090.73			

Values are Significant at P<0.05

Factorial Regression: pH output versus Nature of Oil (A), Fortified or Not (B) and Speed (C)

Term	Effect	Coeff	SE Coeff	T-Value	P-Value
Constant		5.266	0.159	33.05	0.000
Nature of Oil (A)	0.193	0.097	0.159	0.61	0.550
Fortified or Note (B)	-2.181	-1.090	0.159	-6.84	0.000
Speed (c)	0.107	0.053	0.159	0.34	0.740

Nature of Oil(A)*fortified or Not (B)	0.331	0.165	0.159	1.04	0.310
Nature of Oil (A) *Speed (C)	0.193	0.097	0.159	0.61	0.550
Fortified or Not (B)*Speed (C)	0.219	0.110	0.159	0.69	0.498
Nature of Oil (A)*Fortified or Not(B)*Speed (C)	0.056	0.028	0.159	0.17	0.863

Value is significant at P<0.05

Minitab Output for 23Factorial Regression- To examine the effect of nature of oil (A), Fortification (B) and Speed (C) on Viscosity of Emulsion using factorial regression analysis Coded Coefficients

Term	**Effect Coeff**	**SE Coeff**	**T-Value**	**P-Value**
Constant	39.52	2.36	16.74	0.000
Nature of Oil(A)	5.61 2.81	2.36	1.19	0.246
Fortified or Not(B)	3.71 1.86	2.36	0.79	0.439
Speed (C)	1.84 0.92	2.36	0.39	0.701
Nature of Oil(A)*Fortified or Not(B)	7.36 3.68	2.36	1.56	0.132
Nature of Oil(A)*Speed (C)	-3.91 -1.96	2.36	-0.83	0.415
Fortified or Not(B)*Speed (C)	10.21 5.11	2.36	2.16	0.041
Nature of Oil(A)*Fortified or Not(B)*Speed (C)	1.39 0.69	2.36	0.29	0.771
Term	**VIF**			
Constant				
Nature of Oil(A)	1.00			
Fortified or Not(B)	1.00			

Speed (C)	1.00			
Nature of Oil(A)*Fortified or Not(B)	1.00			
Nature of Oil(A)*Speed (C)	1.00			
Fortified or Not(B)*Speed (C)	1.00			
Nature of Oil(A)*Fortified or Not(B)*Speed (C)	1.00			

Coded Coefficients

Term	Effect Coeff	SE Coeff	T-Value	P-Value
Constant	39.52	2.36	16.74	0.000
Nature of Oil(A)	5.61 2.81	2.36	1.19	0.246
Fortified or Not(B)	3.71 1.86	2.36	0.79	0.439
Speed (C)	1.84 0.92	2.36	0.39	0.701
Nature of Oil(A)*Fortified or Not(B)	7.36 3.68	2.36	1.56	0.132
Nature of Oil(A)*Speed (C)	-3.91 -1.96	2.36	-0.83	0.415
Fortified or Not(B)*Speed (C)	10.21 5.11	2.36	2.16	0.041
Nature of Oil(A)*Fortified or Not(B)*Speed (C)	1.39 0.69	2.36	0.29	0.771

Term VIF

Constant

Nature of Oil(A) 1.00

Fortified or Not(B) 1.00

Speed (C) 1.00

Nature of Oil(A)*Fortified or Not(B) 1.00

Nature of Oil(A)*Speed (C) 1.00

Fortified or Not(B)*Speed (C)	1.00
Nature of Oil(A)*Fortified or	1.00 Not(B)*Speed (C)