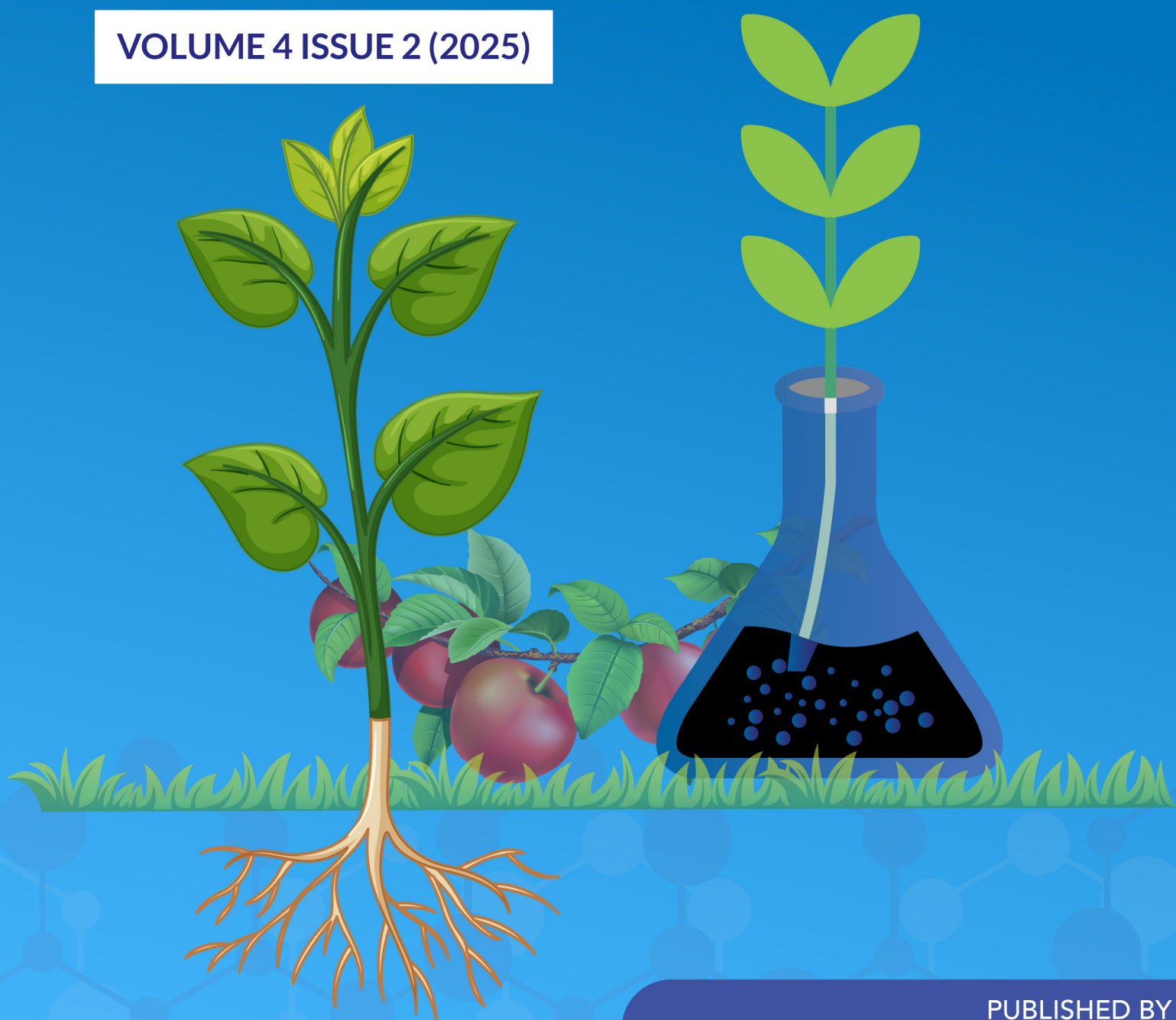




American Journal of Food Science and Technology (AJFST)

ISSN: 2834-0086 (ONLINE)

VOLUME 4 ISSUE 2 (2025)



PUBLISHED BY
E-PALLI PUBLISHERS, DELAWARE, USA

Quality and Safety of Soybean Oils in Makurdi: Physicochemical Properties, Contaminants, and Residues

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Article Information

Received: February 20, 2025

Accepted: March 26, 2025

Published: August 08, 2025

Keywords

*Aflatoxins, Heavy Metal,
Physicochemical Properties,
Refining, Soybean Oil*

ABSTRACT

The study aimed to investigate the physicochemical properties, heavy metals, total aflatoxins, and solvent residues in soybean oils produced in some parts of the Makurdi metropolis. The oil samples were collected from “Seraph” and “Hule and Sons” vegetable oil factories in Makurdi. Samples include: Seraph crude soybean oil (A1), Seraph Refined soybean oil (A2), Hule and Sons crude soybean oil (B1), and Hule and Sons refined soybean oil (B2). Analysis showed that the physicochemical properties varied between the refined and unrefined samples. Specific gravity increased from 0.807 to 0.923, 0.822 to 0.931 for A1 and A2 and B1 and B2 respectively. The peroxide value reduced from 9.71 to 9.6 (mEq.O₂.kg⁻¹) after refining. Iodine value and moisture content also reduced with refining. Cadmium, arsenic, and lead were detected only in the crude soybean oil (A1 and B1) copper content reduced from 5.3 to 2.6 mg/kg, and nickel decreased from 0.55 to 0.01 mg/kg upon refining. Crude soybean oil content (A1 and B1) total aflatoxins of 6.05 ppm and 7.10 ppm respectively. No aflatoxin was detected in the refined oil samples. Major chemical compounds and solvent residues in refined soybean oil (A2) composed of 21.10% 2,4-Decadienal, 18.16% n-Hexadecanoic acid, 4.66% Bicyclo [3.1.1] heptane, 4.45% 1,6,10,14-Hexadecatetraen-3-ol, 4.17% p-xylene, 4.22% cyclohexane, 5.05% cyclopentane, 1.69% 9-Eicosyne and 3.88% toluene. While B2 composed mainly of 2,4-decadienal (27.92%), 2-pentene (17.42%), Bicyclo [3.1.1] heptane (2.10%), 9-octadecyne (1.45%), 2-methyl-E-7-octadecane (1.31%), 2-Heptenal (2.17%), Cyclohexane (2.33%) and Hexanal (2.90%). Refining has a significant role in the physicochemical properties of soybean oil. Refining greatly reduces heavy metal content and aflatoxin in crude vegetable oils.

INTRODUCTION

Soybean oil belongs to the popular vegetable oils used in food, cosmetic, and pharmaceutical industries (Mohdaly *et al.*, 2017). It is considered a good source of the essential fatty acids and lipid-soluble vitamins. Predominantly, soybean oil is made up of unsaturated fatty acids like oleic, linoleic and linolenic acids (Makni *et al.*, 2015). Soybean seeds are one of the dominant oilseeds in the world. Its high-quality protein yield and edible oil give soybeans peculiar characteristics. This vegetable oil is rich in nicotinic acid, riboflavin, thiamine and minerals. Oleo-chemical application includes; cosmetic, soap manufacture, biodiesel production and methyl esters.

Refining vegetable oils is essential to ensure the removal of gums, phosphatides, pigments and getting rid of odorants (Mohdaly *et al.*, 2017). Crude soybean oil contains a large amount of non-glyceride materials consisting chiefly of phosphatide and other impurities. Crude oil has a lighter amber colour which upon refining is reduced to the light yellow colour of most vegetable oils. Refined oil is usually clear, odourless, and resistant to rancidity. Reports state that refining crude vegetable oil affects the physicochemical properties of the oils. Changes have been observed in oxidation indices, phenolic compounds and fatty acid composition during refining of vegetable oils. Nevertheless, refining increases the value of soybean oils, but refining could modify the chemical constituents of

these oils to the point which could be injurious to health (Mohdaly *et al.*, 2017).

Heavy metal contamination of vegetable oils has been a great concern related to human health problems (Gu *et al.*, 2019). Studies have shown that these metals contaminate soils and trace levels above required limits have been estimated in oil-extracting seeds (Asemave *et al.*, 2012; Onakpa *et al.*, 2018). Trace elements found in edible oils can be a result of environmental contamination, extraction and refining process of the oil (Kalappurayil & Joseph, 2017).

Aflatoxin contamination in foods is a global food safety issue (Aghemwenhio *et al.*, 2017; Karunarathna *et al.*, 2019). Environmental conditions, conventional agricultural practices, and illiteracy are the main factors which favour the production of AFs in food (Waqas *et al.*, 2019). There are a number of related fungal metabolites found mostly in cereals, maize grains, peanuts and animal fodders mainly by the fungi *Aspergillus flavus* and *Aspergillus parasitica* (Idris *et al.*, 2010). Aflatoxin B1 is a lethal naturally occurring, it is a potent liver carcinogen (Negash, 2018). Food and Agriculture Organization of the United Nations (FAO) states that about 25% of the world's crops are contaminated with mycotoxins (Aflatoxin) during the growth or storage period (Pankaj *et al.*, 2017).

The extraction of vegetable oils usually employs the use of n-hexane. Reports state that solvent residues in consumable food are undesirable. Generally in edible

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oils, hexane residues could be considered as undesirable substances when the maximum residual limits are exceeded (Yousef & Hosseini, 2017). Even though the recovery process of these compounds is ensured, a good percentage of solvent is lost in the process which may either be retained in the oil or undergo other reactions to produce other compounds. These derivatives are known to be toxic at certain dosages (Cerutti *et al.*, 2017).

There is a paucity of information on the heavy metal content and the level of aflatoxins in crude and refined soybean oil produced and sold in Makurdi. Therefore, there is a great necessity to investigate the physicochemical properties, some heavy metals, aflatoxins contents and solvent residues in soybean oil from “Seraph” and “Hule and Sons” oil factories in Makurdi.

MATERIALS AND METHODS

Reagents and chemicals include; Hydrochloric acid (HCl), Sodium hydroxide (NaOH), Potassium hydroxide (KOH), Iodobromine (IBr), Sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$), Potassium iodide (KI), Acetic acid (CH_3COOH) Nitric acid (HNO_3) and, Hydrogen Peroxide (H_2O_2). All reagents were of analytical grade. Instruments used includes; Watman No 1 filter paper, ELISA READER, atomic absorption spectrophotometer (AAS), GC-MS (Agilent USA5975C), refractometer, electric Oven, water bath.

Seraph and Hule and Sons Vegetable oils (soybean oils) were purchased at their factories in Makurdi and Wannune respectively. The oil samples were stored at room temperature away from direct lights. Samples were coded A1, A2, B1 and B2: Seraph crude vegetable oil, Seraph Refined vegetable oil, Hule and Sons crude vegetable oil and Hule and Sons refined vegetable oil.

Determination of Physicochemical properties

The Specific gravity, refractive index, the peroxide value, saponification values, the moisture content, of the samples was determined using the method of (AOAC, 2015). The acid value was measured by titration (Bello, 2015). The smoke point of the oils was determined as described in literature. The temperature at which 10 mL of the sample gave off a thin bluish smoke continuously was recorded as the smoke point (Kenechi *et al.*, 2017).

Determination of Heavy Metals Contents

Heavy metals assay of the samples was done using

atomic absorption spectrophotometer (AAS) following the Methods described by (AOAC, 2015). The lamps were allowed to warm up for 15 minutes and the monochromator was positioned to a wave length corresponding to the metals needed (217.0 nm for lead, 232.0 nm for nickel, 324.7 nm for copper, 213.9 nm for iron, 228.8 nm for arsenic and 328.4 nm for iron

Determination of Total Aflatoxins

Determination of total Aflatoxins was done by ELISA method (Murshed *et al.*, 2019). Extraction of aflatoxins was done using 10 mL of oil sample in 200 mL of 70% ethanol in an orbital shaker for 3 hours.

GC-MS Analysis of Solvent Residues in Refined Soybean Oil

A gas chromatography- mass spectrophotometer (Agilent USA 5975C) with triple axis detector equipped with an auto injector (10 μL syringe) was used to analysed the solvent residues. Helium was the carrier gas. Capillary column specifications includes: length; 30m, internal diameter 0.2 μm , thickness; 250 μm , treated with phenyl methyl silox. Other GC-MS conditions are ion source temperature (EI), 250 $^{\circ}\text{C}$, interface temperature; 300 $^{\circ}\text{C}$, pressure; 16.2 psia, out time, 1.8 mm, 1 μL injector in Split mode with split ratio 1:50 with injection temperature of 300 $^{\circ}\text{C}$ the column temperature started at 35 $^{\circ}\text{C}$ for 5 min and changed to 150 $^{\circ}\text{C}$ at the rate of 4 $^{\circ}\text{C}/\text{min}$. The temperature was raised to 250 $^{\circ}\text{C}$ at the rate of 20 $^{\circ}\text{C}/\text{min}$ and held for 5mins. The total elution was 47.5minutes. Mass spectrophotometer solution software provided by supplier was used to control the system and to acquire the data. Identification of the compounds was carried out by comparing the mass spectra obtained with those of the standard mass spectra from NIST library (NISTII).

Statistical Analysis

Values were reported in mean $\pm$ standard deviation of triplicate determinations. Mean values were compared using One Way analysis of Variance (ANOVA) at $p < 0.05$ using statistical package for social sciences (SPSS) software version 22. Duncan multiple range test (DMRT) was used for mean separation

RESULTS AND DISCUSSION

Table 1: Physicochemical properties of crude and refined soybean oil

Physicochemical properties	Samples			
	A ₁	A ₂	B ₁	B ₂
Specific gravity	0.807 ^b $\pm$ 0.02	0.923 ^a $\pm$ 0.01	0.822 ^b $\pm$ 0.01	0.931 ^a $\pm$ 0.01
Refractive index at 40 $^{\circ}\text{C}$ ($^{\circ}\text{Bx}$)	1.482 ^a $\pm$ 0.01	1.468 ^b $\pm$ 0.03	1.489 ^a $\pm$ 0.01	1.470 ^b $\pm$ 0.02
Acid value (mgKOHg^{-1})	2.04 ^a $\pm$ 0.01	0.58 ^b $\pm$ 0.01	2.11 ^a $\pm$ 0.01	0.60 ^b $\pm$ 0.01
Saponification value (mgKOHg^{-1})	191.00 ^b $\pm$ 0.02	193.06 ^a $\pm$ 0.03	192.50 ^b $\pm$ 0.04	194.64 ^a $\pm$ 0.02
Peroxide value ($\text{mEq.O}_2\text{kg}^{-1}$)	9.71 ^a $\pm$ 0.01	9.67 ^b $\pm$ 0.02	9.70 ^a $\pm$ 0.02	9.61 ^b $\pm$ 0.01
Iodine value (Wijj's)	146.07 ^a $\pm$ 0.01	136.94 ^b $\pm$ 0.01	145.87 ^a $\pm$ 0.02	137.54 ^b $\pm$ 0.01

Moisture (%)	1.02 ^a ±0.01	0.18 ^b ±0.02	1.26 ^a ±0.01	0.20 ^b ±0.03
Smoke point (°C)	216.0 ^b ±0.01	234.0 ^a ±0.01	215.0 ^a ±0.02 ^a	234.0 ^a ±0.01
Ester value(mgKOH/g)	188.96 ^a ±0.01	192.48 ^b ±0.01	190.39 ^a ±0.01	194.04 ^b ±0.01

KEY: A_1 = Seraph crude vegetable oil, A_2 = Seraph Refined vegetable oil, B_1 = Hule and Sons crude vegetable oil, B_2 = Hule and Sons refined vegetable oils. Means with different superscript on same column are significantly different at $p < 0.05$.

Table 2: Heavy metals content of crude and refined soybean oil

Metals	Samples (mg/kg)			
	A_1	A_2	B_1	B_2
Cadmium	0.82±0.01	ND	0.86±0.01	ND
Arsenic	0.46±0.01	ND	0.50±0.01	ND
Copper	5.36 ^a ±0.01	2.64 ^b ±0.01	5.35 ^a ±0.01	2.65 ^b ±0.01
Nickel	0.55 ^a ±0.01	0.01 ^b ±0.01	0.57 ^a ±0.01	0.01 ^b ±0.01
Lead	0.08±0.01	ND	0.09±0.01	ND
Iron	6.23 ^a ±0.01	4.95 ^b ±0.05	6.31 ^a ±0.01	4.97 ^b ±0.01

A_1 = Seraph crude vegetable oil, A_2 = Seraph Refined vegetable oil, B_1 = Hule and Sons crude vegetable oil, B_2 = Hule and Sons refined vegetable oil, MPL = maximum permissible limits, ND=Not Detected. Means with different superscript in the same column are significantly different at $p < 0.05$.

Table 3: Aflatoxin content of soybean oils

Oils	Sample	Total aflatoxin concentration
Soybean oil		
	A_1	6.05±0.01
	A_2	ND
	B_1	7.10±0.01
	B_2	ND

A_1 = Seraph crude vegetable oil, A_2 = Seraph Refined vegetable oil, B_1 = Hule and Sons crude vegetable oil, B_2 = Hule and Sons refined vegetable oil, ND=Not Detected.

Table 4: Major Chemical constituents of refined soybean oil (GC-MS) (GCMS)

	Compound	Retention time	% composition
Sample A_2	(E-E) 2,4-Decadienal	10.341	21.10
	n-Hexadecanoic acid	14.426	18.16
	1,6,10,14-Hexadecatetraen-3-ol	14.173	4.45
	Bicyclo [3.1.1] heptane	13.722	4.66
	p-xylene	4.651	4.17
	Cyclohexane	13.947	4.22
	Cyclopentane	2.285	5.05
	9-Eicosyne	11.581	1.69
	Toluene	3.496	3.88
Sample B_2	(E-E) 2,4-Decadienal	10.342	27.92
	2-pentene	2.285	17.42
	Bicyclo [3.1.1] heptane	13.722	2.10
	9-octadecyne	11.581	1.45
	2-Methyl-E-7-octadecane	13.102	1.31
	2-Heptenal	5.750	2.17
	Cyclohexane	2.510	2.33
	Hexanal	3.834	2.90

A_2 = Seraph Refined vegetable oil, B_2 = Hule and Sons refined vegetable oil

Physico-Chemical Properties of Crude and Refined Soybean Oil in Benue State

Result of the physicochemical properties of crude and refined soybean oil is presented in Table 1. The specific gravity of the oil increased with refining from 0.807 for A₁ to 0.923 A₂. It also increased from 0.822 in crude Hule and Sons vegetable oil (B1) to 0.931 in refined Hule and Sons vegetable oil. This trend was also reported in refined oils by Gando *et al.* (2014). Rather, others reports that specific gravity decreased after refining (Mohdaly *et al.*, 2017). Refractive index, Acid value, and peroxide decreased progressively with refining. The refractive index decreased from 1.482 °Bx to 1.468 °Bx and from 1.489 °Bx to 1.470 °Bx for Seraph and Hule and Sons vegetable oils respectively.

It is often stated generally that the refractive indices of oils increase with increase in the number of double bonds (Mohdaly *et al.*, 2017). The acid value of seraph oils decreased from 2.04 mgKOH⁻¹ to 0.58 mgKOH⁻¹ and 2.11 mgKOH⁻¹ (sample B₁) to 0.60 mgKOH⁻¹ (sample B2) in Hule and Sons vegetable oils. Peroxide values ranges from from 19.71 mEq.O₂kg⁻¹ to 9.67 mEq.O₂kg⁻¹ and 21.01 mEq.O₂kg⁻¹ to 12.21 mEq.O₂kg⁻¹ from crude to refined oils. Refining increases the oxidative stability of the oil (Mohdaly *et al.*, 2017). The moisture content reduced by 17% when the oil was refined. Most of the physicochemical properties of soybean oil reduce upon refining.

Heavy Metals Content of Crude and Refined Soybean Oil in Benue State

Heavy metals content of crude and refined soybean oil is presented in Table 4. Crude soybeans oil from Seraph and Hule and Sons oil contained 0.82 mg/kg and 0.86 mg/kg of cadmium, respectively, which were all higher than the maximum permissible limit (0.1 mg/kg) of the metal as stipulated by CODEX (Codex Alimentarius Commission, 2009). The high cadmium content may be attributed to soil contamination on which the soybean was cultivated. Reports highlight that the frequent use of phosphorous fertilizers (Lagerwerff & Aspecht, 2017), and the leaching of paints into the farm could be the main coursed of cadmium contamination to the environment. However, cadmium was not detected in the refined soybean oil samples. It has earlier been reported that the refining process removes heavy metals in vegetable oil (Lagerwerff & Aspecht, 2017; Ikebueze *et al.*, 2009). Cadmium is a toxic element. Its presence in consumable food products is highly undesirable as it creates serious health concerns on the consumer's health.

Measures of 0.46 mg/kg and 0.50 mg/kg of arsenic were recorded in Seraph and Hule and Sons crude soybean oils respectively. These values were higher than the 0.1 mg/kg maximum permissible limits stipulated by NAFDAC (Ohimain *et al.*, 2013). Arsenic occurs naturally in the soil but the major source of Arsenic in farm fields is through the use of herbicides (Gando *et al.*, 2014). It was observed that the refining process reduced the arsenic content

to a point of not detected. This result highlights the importance of refining vegetable oils for consumption and expressed the effectiveness of the refining process in removing heavy metals like arsenic in oils refined for human consumption.

Copper content in the crude oils was 5.36 mg/kg and 5.35 mg/kg for Seraph and Hule and Sons respectively. Refining these crude oils significantly ($p < 0.05$) reduced the copper content to 2.64 mg/kg in Seraph refined oil and 2.65 mg/kg in Hule and Sons refined oil. Values of copper in refined oil recorded in this study also agreed with some earlier reports (Andersson & Lingnert, 2008). Lead measures from 0.08 mg/g and 0.09 mg/g in crude Seraph and Hule and Sons oils respectively but the metal was not detected in any of the refined oil samples. Iron content reduced from 6.23 mg/g in the crude Seraph oil to 4.95 mg/g in the refined oil. Crude Hule and Sons oil had an iron content of 6.31 mg/g which reduced to 4.97 mg/g after refining of the oil.

The 0.55 mg/kg and 0.57 mg/kg of nickel recorded in crude soybean oil from Seraph and Hule and Sons oils were slightly higher than the stipulated limit. Refining the crude vegetable oils reduced the nickel content to 0.01 mg/kg in both Seraph and Hule and Sons oils which were all lower the FAO/WHO maximum permissible limit of 0.5 mg/kg. The nickel content of the refined oils reported in this study was safe and competes favourably with other oils like Power oil and Kings Oil which were all reported to contain 0.02 mg/kg nickel (Mehmood *et al.*, 2012).

Total Aflatoxin Content of the Oils

Crude soybean oil from Seraph and Hule and Sons recorded total aflatoxin contents of 0.05 and 0.10 respectively which implies the oils were not safe from consumption. No aflatoxin was recorded in the refined oils which implied that the oxidizing agent (sodium bisulfite) added to the oils during the refining process could significantly reduce the aflatoxin. Low level of aflatoxins recorded in the extracted soybean oil is an indication that the soybean was properly handled at the harvesting and post-harvest stages of the production line as soybeans are hardly contaminated by aflatoxins during cultivation in the fields. Aflatoxins, toxic metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* fungi, are naturally occurring contaminants of food (PACA, 2015). Consumption of oils contaminated with aflatoxins is highly dangerous as it causes aflatoxicosis. Severe conditions can lead to death (Sowley, 2016).

Major Chemical Constituents and Solvent Residues of Refined Soybean Oil

Major chemical components of refined vegetable oil from Seraph and Hule and Sons are presented in Table7. (E-E) 2,4-Decadienal was the major chemical component of both oils having constituted 21.10% in Seraph oil and 27.92% in Hule and Sons oil. This compound is the major component of refined vegetable oils. It occurs naturally in oil seeds and is a natural flavouring component contained

in soybeans (Zhang *et al.*, 2020; WHO, 2019). Other compound detected includes Bicyclo [3.1.1] heptane and cyclohexane. Bicyclo [3.1.1] heptane has been reported to be a product of the isomerization of n-hexane that causes eye damage at doses above 10%. The result showed Seraph oil and Hule and Sons oil contained 4.66% and 2.10% Bicyclo [3.1.1] heptane respectively which were all lower than the 10% reported (Okechalu *et al.*, 2011). Cyclohexane was common to both oils Seraph oil contained 4.22% cyclohexane whereas Hule and Sons oil contained 2.33%. Cyclohexane is known to be toxic at concentrations above 10 ppm. Even though most of the n-hexane used as a solvent in oil extraction is recovered, a reasonable amount of the solvent undergoes aromatization to produce undesirable products like cyclohexane, bicycle [3.1.1] heptane and many others as oil constituents (WHO, 2019). Bicycle [3.1.1] heptane, cyclohexane, p-xylene and Toluene have been reported in refined vegetable oils (WHO, 2019). n-hexane lost during processing of the oil has also been reported Srbinska *et al.*, 2020). 2-pentene (17.42%) was recorded in Hule and Sons refined oil. This compound does not occur naturally in soybean and its presence in the refined soybean oil may also be traced to the reactions of lost n-hexane that occur in the refining line. The presence of 2-pentene however could not be of much concern as no health risks have been linked to the compound (WHO, 2019). Other studies reported 21% of 2-pentene in cottonseed oil extracted using n-hexane as solvent (Attah & Ibemesi, 1990).

CONCLUSION

The physicochemical properties of crude and refined soybean oils from Seraph and Hule and Sons oil factories varied significantly at $p > 0.05$. Findings revealed that refining had a significant role in enhancing the physicochemical properties of the oils. The heavy metals and aflatoxins content were greatly reduced upon refining. The refined oil contained some residual chemicals like (E-E) 2,4-Decadienal, Bicyclo [3.1.1] heptane and cyclohexane, 2-pentene, p-xylene and Toluene. However, these residues were found at levels that could not pose an immediate harm to health.

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