

Original Research Article

ULTRA VIOLET SPECTROPHOTOMETRIC QUANTIFICATION OF SUDAN-IV DYE IN ADULTERATED PALM OIL FROM MAJOR MARKETS WITHIN BENIN METROPOLIS

ABSTRACT

The deficit in the supply of palm oil in Nigeria over the years has been met in part through smuggling of the product from different sources into the Nigerian market by marketers of which some of the palm oil has been found to be adulterated with azo dyes. The aim of this study was to detect and quantify sudan dyes in adulterated palm oil from the open market using simple and inexpensive procedures such as analytical thin layer chromatography (TLC) and Ultra violet spectrophotometric methods respectively. Seven palm oil samples from the open markets within Benin metropolis labelled as (AUC, AUC2, IK1, IK5, OLK1, ADIT, and OB) were screened for the presence of Sudan-III and Sudan-IV dyes. Four of the samples (IK1, IK5, OLK1, ADIT) were confirmed to contain Sudan-IV dye with quantities of 22.5mg/L, 21.7mg/L, 29.5mg/L, 23.8mg/L respectively. This experiment shows that the use of easy and cheap methods such as TLC and UV-spectrophotometry can be used to detect and quantify sudan dyes in adulterated palm oil.

Keywords: *Ultra violet; spectrophotometry; quantification; palm oil; sudan-IV; adulteration; major markets; Benin; metropolis*

INTRODUCTION

Palm oil is extracted from the ripened mesocarp of the fruits of oil palm tree (*Elaeisguineensis*). (Gourichon, 2013). Crude palm Oil (CPO) is also called red palm oil due to its high carotene content which is responsible for the bright orange-red colour. About 90% of palm oil produced are consumed as food, while the remaining 10% are used in the manufacturing of soap and other useful derivatives (Maria et al., 2023). Palm oil can be extracted using different methods. These methods are grouped into four categories based on their scale and degree of mechanization. They are the traditional methods, small-scale mechanical units, medium-scale mills and large industrial mills (Gold et al., 2012). The fundamental operations involved in palm oil production include fruit sterilization, fruit loosening, digestion, oil extraction and clarification (Mba et al., 2015; Nwalieji and Ojike, 2018). The most common methods of palm oil extraction are either the wet or the dry processes (Mba et al., 2015). Nigeria is yet to achieve self-sufficiency in palm oil production despite being one of the largest producers of the product. Nigeria relies on imports from neighbouring African countries to make-up for the deficit in supply. According to USDA, (2023/2024) reports, It is forecasted that Nigeria's palm oil production in 2023/2024 will

reach 1.5 million metric tons (MMT) while consumption will reach 1.9 MMT leaving a deficit of 400,000 MMT. There has been widespread speculation that some palm oil marketers smuggle adulterated palm oil into the Nigerian market in a bid to bridge the demand-supply gap (Okogbenin et al., 2023). It is also speculated that local producers and marketers in Nigeria are in the business of adulterating low quality, degraded palm oil in order to make it look attractive (Okogbenin et al., 2023; Gold et al., 2023). These adulterators exploit the fact that colour is the first sensory quality by which foods are judged, giving unsuspecting consumers the impression that the palm oil is of high quality (Dias et al., 2012; Shiksha, 2012). Crude palm oil naturally has an orange-red colour due to the presence of carotene pigments. Degradation of CPO overtime, leads to a reduction in its carotene content and thus its attractiveness (Dias et al., 2012). Greedy palm oil dealers may decide to add certain artificial and most times harmful colorants to degraded CPO in order to improve its appearance and acceptability. Sudan dyes, especially Sudan-III and Sudan-IV dyes are the commonest dyes used in the adulteration of palm oil (Gold et al., 2023). These dyes are used due to their colour resemblance with CPO, inexpensiveness, wide availability and high solubility in CPO (Okogbenin et al., 2023). Sudan-III and Sudan-IV dyes are lipophilic azo dyes that have been found to have carcinogenic and genotoxic effects and as such are not permitted to be used in food (Li et al., 2018; Okogbenin et al., 2023). These dyes pose serious health risks and diseases including cancer, kidney and liver problems (Li et al., 2018; Okogbenin et al., 2023) as they are likely metabolised in the human body through certain biochemical mechanisms (Li et al., 2018). As the trend of adulteration of palm oil becomes rampant, there is need for regulatory bodies to be able to accurately detect and quantify the presence of Sudan dyes in palm oil. The detection and quantification are usually carried out using sophisticated tools such as HPLC plus UV diode array detection (HPLC-DAD), two-dimensional high-pressure liquid chromatography in conjunction with solid phase extraction (2D-HPLC-SPE) and surface-enhanced Raman spectroscopy (SERS) (DiAnibal e al., 2012; Haughey eg al., 2015). These instruments and techniques offer higher level of precision as they can detect very minute levels of dye in food samples but are quite expensive and are not readily available here in Nigeria.

Therefore this study seeks to explore the use of simple, cheap, available and reliable method such as UV- spectrophotometry for the quantification of Sudan dyes present in adulterated palm oil samples.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

The samples analysed in this study were collected from randomly selected major markets within Benin metropolis, Edo State, Nigeria. Seven palm oil samples collected from the open markets were used for this study. The samples were coded as AUC, AUC2, IK1, IK5, OLK, ADIT, and OB. Crude unadulterated palm oil sample from NIFOR oil mill labelled as CUS was used for the preparation of standards.

DETECTION OF ADULTERATION IN PALM OIL SAMPLES USING ANALYTIC THIN LAYER CHROMATOGRAPHY

Adulteration of Palm Oil Samples

The palm oil sample collected from NIFOR oil mill was self-adulterated in order to prepare standards for TLC analysis. Analytical grade Sudan-III and Sudan-IV dyes obtained from PYREX -IG Scientific Company, Nigeria, were used. Self-adulteration was done by separately preparing 0.1% w/v solution of Sudan-III dye and Sudan-IV dye in palm oil. The standard samples were labelled as (S3, S4, and CUS), representing Sudan-III adulterated palm oil, and Sudan-IV adulterated palm oil and crude unadulterated palm oil sample from NIFOR, void of any adulteration (Gold et al., 2023).

Screening/Detection of Adulteration of Palm Oil Samples from the Open Market

Reagents and Materials

Screening/detection of sudan dyes was carried out according to the method as described by (Okogbenin et al, 2023; Gold et al., 2023).

Analytical grade Acetonitrile, hexane, chloroform, and acetic acid were used. The solvent system was made up of hexane, chloroform, and acetic acid in the ratio of (60:40:2). Aluminium plate of 20×20 cm dimension, pre-coated with Silica gel 60 TLC grade was used.

Sample Preparation

The samples were prepared by solvent extraction method. In solvent extraction, the extraction efficiency of the analytes from food strongly depend on the solvents. Acetonitrile has been mostly chosen because of its good extraction efficiency and lower fat solubility (Li et al., 2018)

5ml of sample was dissolved in 5 ml of acetonitrile. The mixture was agitated vigorously for 1 minutes and allowed to stand for 15 minutes. The top layer of acetonitrile containing the extracted dye was decanted and ready for spotting (Gold et al., 2023)

Thin-layer chromatographic (TLC) detection of adulteration

Analytical Thin Layer Chromatographic detection of Sudan dyes in palm oil samples was carried by spotting 1 μ L of the decanted supernatant layer within 2cm on one edge of the TLC plate with a micro glass capillary spotter unto a pre-coated 20 × 20cm aluminium plate with silica gel 60 of 0.25mm thickness.

Ten spots were made on the TLC plate which corresponds to; NIFOR (unadulterated crude palm oil), S3 (0.1% Sudan-III dye in palm oil), S4 (0.1% Sudan-IV dye in palm oil), and seven open market samples labelled as AUC, AUC1, IK1, IK5, OLK, ADIT, OK, and OB. All the analytes were allowed to run in a previously saturated chromatographic tank containing a shallow pool of mobile phase made up of the solvent systems (Hexane, Chloroform and

glacial acetic acid) in a ratio of 60:40:2 respectively. After appropriate development, the plate was air-dried and the coloured spots which are visible were circled using a lead pencil and the retention values (R_f) were calculated (Okogbenin et al., 2023).

ULTRA VIOLET(UV) SCANNING OF SUDAN-IV DYE IN ACETONITRILE SOLVENT

In order to quantify the dye present in the palm oil samples from the open market, the wavelength of maximum absorption of Sudan-III and Sudan-IV dyes in acetonitrile was determined. This was done by dissolving 0.01g of Sudan-III and Sudan-IV dyes separately in 25mL Acetonitrile solvent. The solutions were further diluted using a dilution factor of 10. UV-VIS scanning was done within the wave length range of 190 – 1000nm using a VWR UV-630PC Double Beam Spectrophotometer.

QUANTIFICATION OF SUDAN-IV DYE IN ADULTERATED PALM OIL SAMPLES USING SPECTROPHOTOMETRIC METHOD

Preparation of Standards and Plotting of Calibration Curve

Six different concentrations of Sudan-IV dye adulterated crude palm oil samples were prepared by dissolving accurately weighed masses of the dyes in appropriate volumes of unadulterated crude palm oil samples. The concentrations prepared are 1mg/L, 10mg/L, 20mg/L, 30mg/L, 40mg/L, and 50mg/L. 0.1g each of the different concentrations was dissolved in 25mL acetonitrile. UV-absorbance readings at wave length 322nm were taken and recorded for each of the standards. A standard curve of absorbance against concentration was plotted.

Quantification of Sudan-IV Dye in Palm oil Samples from the Open Market

The palm oil samples in which sudan dyes were detected were further selected for quantification using UV-spectrophotometric method. 0.1g each of the selected palm oil samples was dissolved in 25mL acetonitrile. UV-absorbance readings at wave length 322nm were taken and recorded for each of the samples.

RESULTS AND DISCUSSION

Analytical Thin Layer Chromatographic

A set of samples comprising of, in-laboratory adulterated palm oil samples and untreated open market palm oil samples collected in Benin, Edo State, Nigeria were analysed by Analytical Thin Layer Chromatographic (TLC). TLC techniques are quick and relatively cheap methods enabling the detection of adulteration in palm oil. In Image. 1 below, the chromatogram of the standard self-adulterated and open market palm oil samples is presented. Each spots separated out along the TLC plate. Samples OLK1, IK1, ADIT, 1K5 were observed to contain dyes. The R_f values of these samples correspond with that of sudan-IV (S4) confirming that they were adulterated with Sudan-IV dye. In a recent study conducted by Gold et al, (2023), the limit of detection of Sudan-III and Sudan-IV dyes by analytical TLC is up to 10mg/L and 18mg/L respectively. This is supported by a similar result by

Okogbeninet al, 2023 who used chemical bleaching and TLC techniques for the detection of the presence of Sudan-III dyes in palm oil samples.



Image 1. Chromatogram of Standards and palm oil samples from open markets

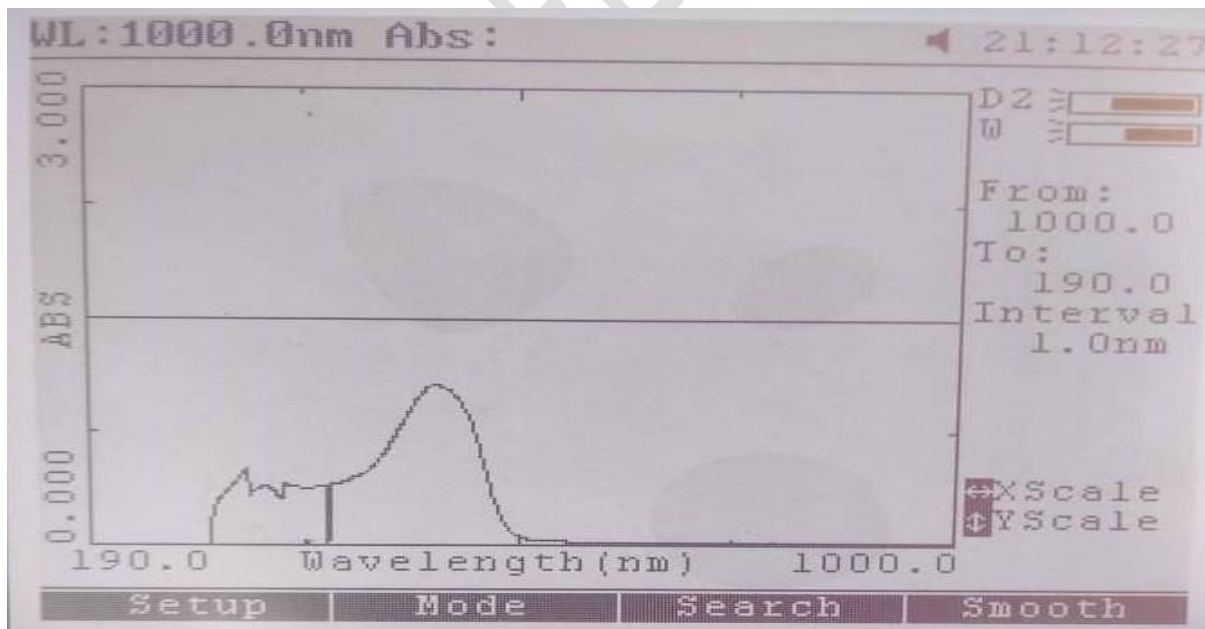


Image 2. UV-VIS Scanning of Sudan-IV Dye in Acetonitrile Showing the Absorption Spectra within a range of 190 – 1000nm

Table 1: Showing Absorbance of different concentrations of Sudan-IV Dye

Conc (mg/L)	1	1 0	2 0	3 0	4 0	5 0
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Abs @ 322nm	0.029	0.06	0.08	0.1	0.135	0.141
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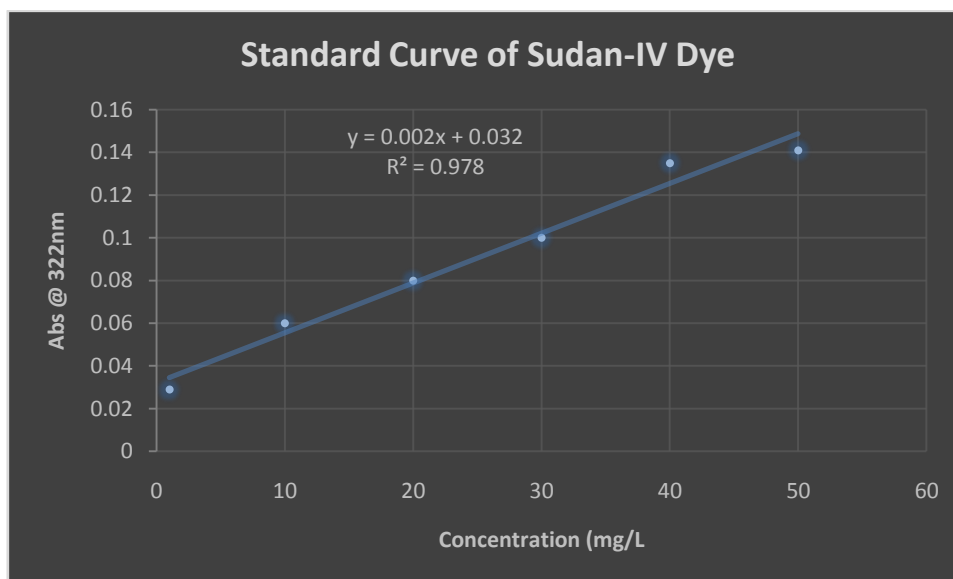


Image3. Standard Curve of Showing the Absorbance Value of Different Concentrations of Sudan-IV Dye in Self-Adulterated Palm Oil.

Table 2: Showing concentrations of the Palm Oil Samples from Open Markets

S a m p l e s	C U S	OLK1	I K 1	ADIT	I K 5
A b s @ 3 2 2 n m	0 . 0 0 6	0 . 1	0.084	0.087	0.082
Concentration (mg/L)	Undefined	29.5	22.5	23.8	21.7

Quantification of Sudan-IV Dye

We decided to quantify the amount of dye present in the palm oil samples confirmed to contain Sudan-IV dye. In Image 2. UV-VIS Scanning of Sudan-IV Dye in acetonitrile was done within the frequency range of 190 – 1000nm. The UV-VIS spectrum shows that the maximum absorption of Sudan-IV dye is within a frequency range of 262nm – 367nm with maximum peak at 322nm.

Table 1, shows the Absorbance of different concentrations of Sudan-IV Dye In self-adulterated palm oil samples. This was used to plot a standard curve for the quantification of sudan dyes in palm oil samples from the open markets. The standard curve in Image 3 has an R-squared value of 0.978 which shows that there is correlation between the concentrations and absorbance and that this relationship can be trusted.

The concentrations of the palm oil samples bought from open markets were determined by using the line equation, $y = 0.0023x + 0.0322$. Each absorbance value is substituted for Y in equation in order to determine the concentration, X of each palm oil sample. Table 2 shows the calculated concentrations of the market palm oil samples. CUS sample from NIFOR gave

a negative value which was recorded as undefined meaning it is void of Sudan-IV dye adulteration. OLK1 gave the highest amount with 29.5mg/L, IK1, ADIT and IK5 gave 22.5mg/L, 23.8mg/L and 21.7mg/L respectively.

CONCLUSION

It has been demonstrated from this study that the presence Sudan-III and Sudan-IV dyes can be detected and quantified using TLC techniques and UV-spectrophotometry respectively.

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