Asian Journal of Research in Biochemistry

Volume 12, Issue 2, Page 33-39, 2023; Article no.AJRB.97933
ISSN: 2582-0516

The Use of Chemical Bleaching and Thin-layer Chromatographic Methods for the Detection and Identification of Sudan-III Dye in Adulterated Palm Oil

E. A. Okogbenin [a], N. U. Asiriuwa [a*], B. O. Imoisi [a], D. C. Onyia [a*], T. Okunwaye [a], V. Ezoguan [a], I. L. Gold [a], J. U. Obibuzor [a], H. Anemene [a], O. I. Akpose [a] and E. E. Osawaru [a]

[a] Biochemistry Division, Nigerian Institute for Oil Palm Research (NIFOR), P.M.B. 1030, Benin City, Edo State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRB/2023/v12i2232

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97933

Original Research Article

Received: 07/02/2023
Accepted: 09/04/2023
Published: 13/04/2023

ABSTRACT

The deleterious effect of consuming azo dye adulterants in palm oil is well documented including cancer. The presence of Sudan-III dye in palm oil cannot be detected by mere visual inspection. This study was aimed at developing a simple, cheap and convenient protocol for detection and identification of Sudan-III and other azo dyes in adulterated palm oil. The results revealed that the refractive index could be used to screen for azo dye adulteration in palm oil samples as the parameter increases with increasing concentration of Sudan-III dye in palm oil and were statistically

**Corresponding author: E-mail: onyiachimad@gmail.com, nathasiriuwa@gmail.com;*

different ((p<0.05) from crude unadulterated palm oil samples. Analytical thin layer chromatography and chemical bleaching using 20% v/v each of concentrated sulphuric acid and 30% hydrogen peroxide in palm oil was effective in detecting Sudan III dye adulteration in concentrations of 10mg/L and above.

Keywords: Palm oil; Sudan-III; detection; thin layer chromatography; colour.

1. INTRODUCTION

The African oil palm (*Elaeis guineensis Jacq*) is the oil palm of commerce worldwide and has been useful to humanity for centuries [1,2]. It is an oleaginous tree crop that produces two different kinds of oils in commercial quantities, namely; the crude palm oil from the fleshy mesocarp of the palm fruit, and palm kernel oil, from the kernel or seed. These two primary products from the oil palm are very different in their fatty acid composition and characteristics [2-5]. Palm oil is high in palmitic acid (C16 fatty acid) while palm kernel oil is high in lauric and myristic acids (C12 and C14) fatty acid respectively. Palm oil has a good mix of saturated and unsaturated fatty acids together with important phytonutrients such as carotenoids, tocopherols and tocotrienols and as such is good for human health. Palm kernel oil main applications are in non- edible products such as detergents and cosmetics [5]. Palm oil is the most consumed edible oil worldwide and the largest vegetable oil by volume. Palm oil and palm kernel oil contributes the largest percentage to the world vegetable oil output [6].

In recent years, the high demand for palm oil for both domestic and industrial use has been backed up by an increase in adulteration [1,4]. Nigeria's total national palm oil output is currently 1.40 million metric tons annually while the national palm oil consumption is estimated at 1.84 million metric tons annually [6]. Therefore, there is a deficit of 440,000 metric tons which is imported into the country via different routes, and Nigeria spends 500 million USD for palm oil importation annually [7]. The distinctive red-orange colouration of freshly milled crude palm oil is due to the presence of carotenoids. Palm oil contains the highest amount of plant-derived carotenoids, ranging from 100-1000 ppm depending on the oil palm fruit type with the dura virescens oil being the most pigmented with the highest carotene contents [8]. In recent years, the high demand for palm oil for both domestic and industrial utilization has been backed up by an increase in adulteration [2,4].

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. Some marketers of the product add azo dyes to poor quality palm oil with characteristic off colour in a bid to improve the colour of the oil, making it attractive to unsuspecting buyers and consumers of the product, thereby maximizing profit without considering the health of the consumers [2,9,10]. Such adulterated palm oil is unsafe for human consumption and poses serious health risks and diseases including cancer, kidney and liver problems [11].

Azo dyes are industrial colourants which comprises; lipophilic, acidic and basic azo dyes [12]. The above-mentioned azo dyes are commonly used as colourants in the chemical industries including oils, waxes, petrol, textile, leather, printing floor polishing, spirit varnishing, etc. [13,14]. The lipophilic azo dyes include mainly the following; Sudan 1 – IV, Sudan red B, Sudan red 7B, Sudan red G, Sudan orange G, methyl yellow, and para red. Sudan dyes have intense red-orange coluor, wide availability, low cost, and chemical stability [12,13,15]. Their use in foodstuffs and beverages, at any level, is forbidden by the European Community and many other countries and organizations [16]. Sudan I, II, III, IV, 7B, basic orange 2, and orange G have been found to have carcinogenic effects, belonging to group 3, namely animal carcinogens [17]. Azo dyes have the functional group R-N=N-R', and the R and R' are usually aryl. They exist in the hydrazine form and are more likely to be broken down. They can be reduced by azo-reductase in the intestinal bacteria cells and skin surface microflora [18]. Sudan III is a diazo and lysochrome dye which has been reported to have carcinogenic and genotoxic effects, and as such is not permitted to be used in food [19-21].

Proper regulation and monitoring by regulatory agencies such as National Agency for Food and Drugs Administration and Control (NAFDAC), is therefore imperative in order to forestall the

unwholesome practice of adulteration of palm oil and safeguard the health of the populace.

Adulterated palm oil is difficult to identify by mere visual inspection, hence, the need to develop a simple analytical method for detection cannot be overemphasized. Therefore, this study seeks to develop simple tests and procedure that is cheap, fast and convenient for the detection of adulterated palm oil which can be adopted by all stakeholders in the local palm oil industry.

2. MATERIALS AND METHODS

2.1 Collection of Sample

The crude palm oil sample used for this study was processed in the Biochemistry Division, NIFOR, and kept at room temperature for two months before use. Sudan-III dye was purchased from Pyrex Scientific Chemical Company, Nigeria.

2.2 Sample Analysis

The unadulterated crude palm oil and adulterated samples were analyzed using the following physicochemical parameters; moisture content, free fatty acids, specific gravity and refractive index, using AOCS official methods [22]. All physicochemical analyses were carried out within one week of adulteration with Sudan-III dye.

2.3 Sudan III Dye Detection Methods

2.3.1 Chemical bleaching detection of Sudan-III dye

Five (5) ml each of palm oil samples (unadulterated and adulterated) previously melted at 70°C was taken into 10ml test tubes labelled A-J. Tube A contained unadulterated crude palm oil, while tube B contained bleached palm oil without Sudan-III dye as control. Tubes C-J, contained 1mg/L, 10mg/L, 20mg/L, 30mg/L, 40mg/L, 50mg/L, 100mg/L and 200mg/L of Sudan III dye in palm oil respectively. 1ml of concentrated sulphuric acid was added to tubes B-J and mixed thoroughly using a glass rod, and allowed to stand for 5 minutes followed by the addition of 1ml of 30% hydrogen peroxide to the components in the tubes B-J, mixed together and allowed to stand for 5 minutes.

2.3.2 Thin-layer chromatographic (TLC) detection of Sudan-III dye

Analytical Thin Layer Chromatographic detection of Sudan-III dye in palm oil samples was carried out by dissolving appropriately and spotting 1 µL of the analytes within 2cm on one edge of the TLC plate with a microcapillary spotter unto a precoated 20 × 20cm aluminium plate with silica gel 60 of 0.25mm thickness. Nine spots were made on the TLC plate which corresponds to; 1 (Sudan-III dye), 2 (unadulterated crude palm oil), 3 (1 mg/L Sudan-III dye in palm oil), 4 (10 mg/L Sudan-III dye in palm oil), 5 (20 mg/L Sudan-III dye in palm oil), 6 (30 mg/L Sudan-III dye in palm oil, 7 (40 mg/L of Sudan-III dye in palm oil), 8 (50 mg/L of Sudan-III in palm oil, 9 (100 mg/L of Sudan-III dye in palm oil), and 10 (200 mg/L of Sudan-III dye in palm oil). 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 (Iso-octane, diethyl ether and glacial acetic acid) in a ratio of 70:30:1 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 (Rf) were calculated. The spots representing distinct chemical groups were compared from the Rf values obtained and a conclusion was drawn from the Rf value [23,24].

> Retention value (Rf) = Distance travelled by compounds (cm) / Distance travelled by solvent front (cm)

2.3.3 Colour code

The colour code of the unadulterated and adulterated palm oil samples was developed based on the colour of the oil.

3. RESULTS AND DISCUSSION

Table 1 below shows the results of palm oil samples analyzed for some physicochemical properties.

Free fatty acid (FFA) levels of all the samples were higher than acceptable levels of ≤5%, indicating that the oil was already getting oxidized prior to analysis. The result shows that there was no significant difference ($P>0.05$) between in the FFA values of the unadulterated and adulterated palm oil samples; CPO, APO1, APO2, APO3, and APO7, which were significantly different from APO4, APO5, APO6 and APO8 ($P<0.05$ but greater than APO1. The presence of Sudan-III dye did not lead to substantial cleavage of fatty acids from their parent triglycerides at the time of sample

analysis. Hence, no significant increase the FFA levels of the palm oil samples. Free fatty acids are primary oxidation products and are a key index used in the determination of oil quality and pricing in the international market.

Analysis of the moisture content shows that the moisture content of the unadulterated CPO and all the adulterated samples were not statistically significant (*P>0.05*). The difference in density between CPO and the other samples was not statistically significant (*P>0.05*).

The refractive index of all the samples showed a trend of increase with increasing concentration of Sudan-III dye in the oil, and were all higher and significantly different from CPO and APO1.

This may suggest refractive index is a key physical property in palm oil adulteration studies.

It can be seen from Image 1 below that addition of concentrated sulphuric acid and 30% hydrogen peroxide to the unadulterated palm oil sample in tube b led to the degradation of the carotenoids in the oil, thus, bleaching the oil with a change in colour from red-orange to pale yellow. The same effect was observed in sample c containing 1mg/l of sudan-iii dye in palm oil. This shows that the chemical bleaching method could not detect the Sudan-iii dye at a concentration of 1 mg/l. Tubes d-j contained sudan-iii dye in palm oil in concentrations of 10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, 100 mg/l and 2 00mg/l respectively, and retained the red colour of the dye, indicating that the bleaching had effect only on the carotenoids in the oil and not the synthetic azo dye. Thus, chemical bleaching can detect the presence of sudan iii dye in oil in concentrations of 10 mg/l and above within 10 minutes of the reaction.

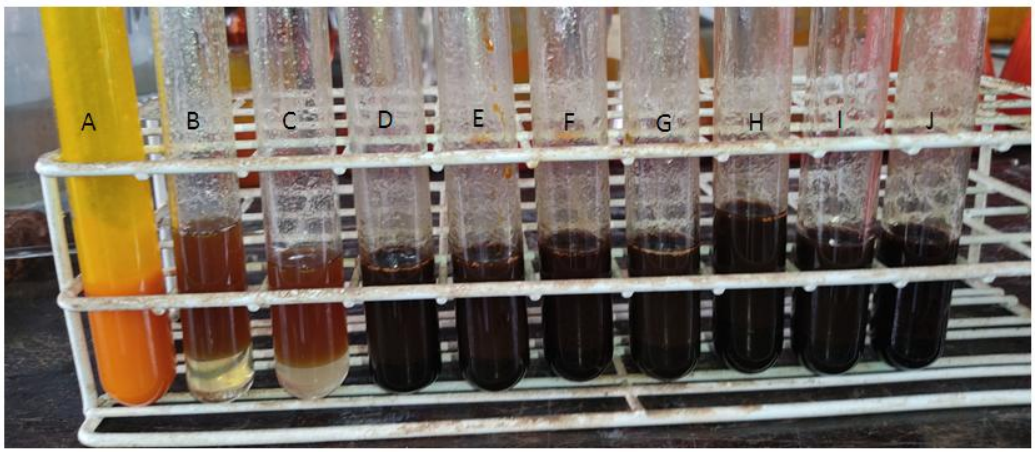


Image 1. Effect of chemical bleaching on the colour of unadulterated and adulterated palm oil samples

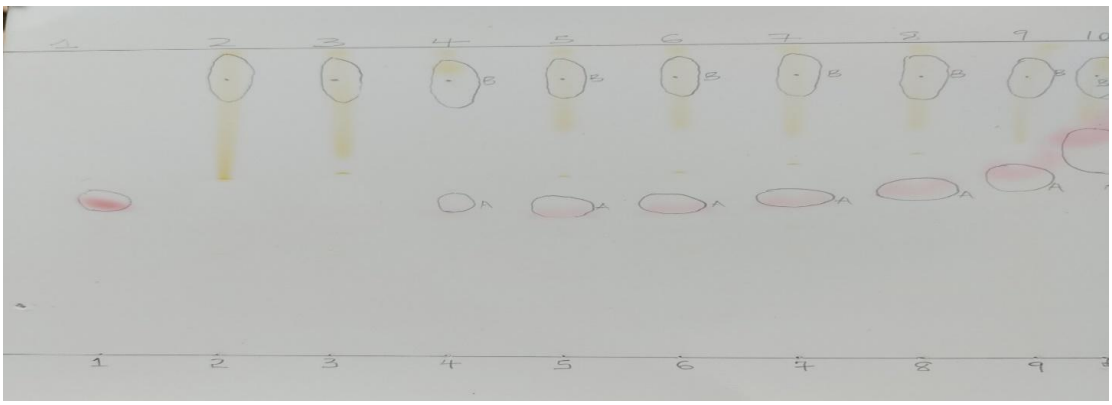
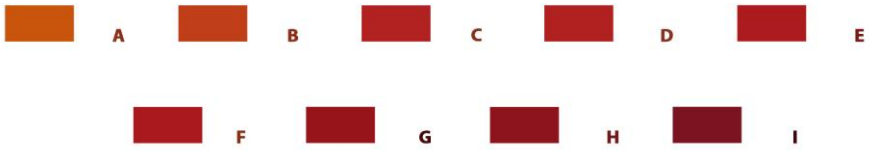


Image 2. Chromatogram of Sudan III dye in adulterated palm oil samples

Table 1. Results of physicochemical analysis

Parameters	CPO	APO1	APO2	APO3	APO4	APO5	APO6	APO7	APO8
FFA (%)	6.405± 0.005^{ab}	6.450± 0.050^{ab}	6.410± 0.010^{ab}	6.600± 0.100^{ab}	6.250± 0.050^{a}	6.050± 0.150^{a}	6.100± 0.200^{a}	6.500± 0.100^{ab}	6.250± 0.050^{a}
Moisture (%)	0.200± 0.02^{a}	0.230± 0.050^{a}	0.200± 0.010^{a}	0.210± 0.010^{a}	0.200± 0.020^{a}	0.225± 0.010^{a}	0.225± 0.010^{a}	0.210± 0.0.01^{a}	0.200± 0.010^{a}
Refractive Index np 50^{0}c	1.465± 0.003^{a}	1.468± 0.001^{a}	1.475± 0.002^{ab}	1.476± 0.002^{ab}	1.481± 0.001^{ab}	1.491± 0.004^{b}	1.516± 0.001^{b}	1.619± 0.002^{c}	1.671± 0.008^{c}
Specific Gravity g/ml at 50^{0}c	0.931± 0.014^{a}	0.938± 0.031^{a}	0.934± 0.011^{a}	0.938± 0.013^{a}	0.940± 0.014^{a}	0.942± 0.012^{a}	0.937± 0.011^{a}	0.940± 0.010^{a}	0.939± 0.012^{a}

Key: CPO= Crude Palm Oil; APO1=1mg/L Sudan-III in palm oil; APO2=10mg/L Sudan-III in palm oil; APO3= 20mg/L of Sudan-III in palm oil; APO4= 30mg/L of Sudan-III in palm oil; APO5=40mg/L Sudan-III dye in palm oil; APO6=50mg/L of Sudan-III dye in palm oil; APO7=100mg/L of Sudan-III dye in palm oil; APO8= 200mg/L of Sudan-III dye in palm oil



KEYS:

A = Unadulterated Crude Palm oil

B = Bleached adulterated palm oil containing 1mg/L of sudan III dye in oil

C = Bleached adulterated palm oil containing 10mg/L of sudan III dye in oil

D = Bleached adulterated palm oil containing 20mg/L of sudan III dye in oil

E = Bleached adulterated palm oil containing 30mg/L of sudan III dye in oil

F = Bleached adulterated palm oil containing 40mg/L of sudan III dye in oil

G = Bleached adulterated palm oil containing 50mg/L of sudan III dye in oil

H= Bleached adulterated palm oil containing 100mg/L of sudan Iii dye in oil

I = Bleached adulterated palm oil containing 200mg/L of sudan Iii dye in oil

Image 3. Colour code showing different concentrations of adulterated crude palm oil

The Rf value of Sudan-III dye in spot 1 was 0.5 while that of the triglycerides in the unadulterated crude palm oil sample in spot 2 was 0.9. Spot 3 which contains 1mg/L of Sudan III dye in palm oil had just one component separating with Rf value of 0.9. This shows that thin-layer chromatography could not detect the presence of the dye in oil at the concentration of 1mg/L. Each of spots 5-10 had two separated components A and B with retention values of 0.5 and 0.9 respectively, indicating that the analytes contained Sudan-III dye. Thus, thin-layer chromatography was able to detect Sudan-III dye in adulterated palm oil at concentrations of 10 mg/L and above.

4. CONCLUSION

It has been demonstrated from this study that the presence of Sudan-III dye of concentrations 10 mg/L and above in adulterated palm oil can be detected by chemical bleaching and the use of thin-layer chromatography (TLC). Both methods can detect Sudan-III dye within 10 minutes of the procedure and as such, can be used for the detection of adulterated palm oil from the open market in the laboratory. The TLC detection of adulterated palm oil is a simple technique, and does not require visualizing with spray reagents or the use of UV lamp as the compounds are coloured and visible to the eyes, plus the added advantage of the possibility of using TLC to quantify the amount of azo dye in adulterated palm oil. The TLC is a simple, cheap, fast and convenient method, and can be used to analyze multiple samples.

The colour code that can be used to suspect adulterated palm oil in the open market has also been developed based on the different colours of adulterated palm oil from this study. We found from this study that a refractive index higher than 1.468 in palm oil samples could be used to predict Sudan-III dye adulteration in palm oil.

Further studies for an easy “on-the-spot” strip for adulterated palm oil detection is ongoing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Henson IE. A brief history of the oil palm. In: Lai OM, Tan C, Akor CC, editors. Palm oil production, processing, characterization and uses. 1st ed. Urbana, IL: AOCS Press. 2012;1-26.
2. Otu O. Adulteration of crude palm oil with red dye from the leaf sheath of *Sorghum bicolor*. Food Sci Quant Manag. 2013;17:1-6.
3. Gunstone FD. Vegetable oils. In: Shahidi F, editor. Bailey’s industrial oil and fat products. 6th ed. 2005;213-67.
4. Okogeri O, Otika FN. Quality parameters of crude palm oil obtained by traditional processing techniques. Eur J Lipid Sci Technol. 9th Euro Fed Lipid Congress, Rotterdam. 2011;2:117-21.
5. MPOC. Malaysia Palm Oil Company. Palm oil and palm kernel oil applications; 2011.
6. USDA. United States Department of Agriculture: oil seeds and Products Annual Report-. Nigeria; 2022.
7. Central Bank of Nigeria annual report; 2019.
8. Obibuzor JU, Asiriuwa NU, Okogbenin EA, Okunwaye T, Odewale JO, Anemene H. A Comparative Study of the Carotene Contents of the Nigerian Oil Palm Fruit Types and Forms and its Implication in Industry. Chem Tech Journal. 2017;12: 51-56.
9. Imai C, Watanabe H, Haga N, Ii T. Detection of Adulteration of Cottonseed oil by Gas chromatography. J Am Oil Chem Soc. 1974;51(7):326-30.
10. Axon A, May FEB, Gaughan LE, Williams FM, Blain PG, Wright MC. Tartrazine and sunset yellow are xenoestrogens in a new screening assay to identify modulators of human oestrogen receptor transcriptional activity. Toxicology. 2012; 298(1-3):40-51.
11. Kola-Ajibade IR, Atere G, Olusola AO. Effects of azo dye adulterated palm oil on the expression of inflammatory, functional and antioxidant markers and body weights in albino rats. J Toxiocol Risk. Asses. 2021;7(1):12-35.
12. Li Y, Yang Y, Yin S, Zhou C, Ren D, Sun C. Inedible azo dyes and their analytical methods in foodstuffs and beverages. J AOAC Int. 2018;101(5):1314-27.
13. Samar E. Sudan III azo dye; Oxidative stress with possible GeNO and Hepatotoxic effect in male rat. Int J Sci Res. 2013;16:3-5.
14. Susie G, Shaun M, Katherine R, Samantha F, Nicole S, Lowri D. Method development and survey of Sudan I- IV in palm oil and chilly spices in the Washington, DC, HH Public Health. 2016;33(4):583-91.
15. Cristina G. Toxicological effect of food additives- azo dye. J Vet Public Health. 2014;12:1-20.
16. Stuart BL, Inger RF, Voris HK. High level of cryptic species diversity revealed by sympatric lineages of Southeast Asian forest frogs. Biol Lett. 2006;2(3):470-4.
17. IARC World Health Organisation, International Agency for Research on Cancer. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to man: Some aromatic amines and related nitro compounds – hair dyes, coloring agents and miscellaneous industrial chemicals. 1978;16:97-109.
18. Bafana A, Devi SS, Chakrabarti T. Azo dyes: past, present and the future. Environ Rev. 2011;19:350-71.
19. IARC World Health Organisation, International Agency for Research on Cancer. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to man: Some aromatic azo compounds. 1975;8:79-90.
20. Lu D, Zhao X, Zhao Y, Zhang B, Zhang B, Geng M et al.. Binding of Sudan II and Sudan IV to bovine serum albumin: comparison studies. Food Chem Toxicol. 2011;49(12):3158-64.
21. Pielesz A, Baranowska I, Rybakt A, Włochowicz A. Detection and determination of aromatic amines as products of reductive splitting from selected azo dyes. Ecotoxicol Environ Saf. 2002;53(1):42-7.
22. AOCS. Official methods and recommended practices of the American Oil Chemists’ Society. 6th ed. American Oil Chemists Communications’ Society: Champaign. IL; 2009.

23. Satpute SK, Banat IM, Dhakephalkar PK, Banpurkar AG, Chopade BA. Biosulphatants, bioemulsifiers and exopolysaccharides from marine microorganisms. Biotechnol Adv. 2010; 28(4):436-50.

24. Das A, Chakraborty B, Sood AK. Raman Spectroscopy of graphene on different substrates and influence of defects. Bull Mater Sci. 2008;31(3): 579-84.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/97933