UNIVERSITY OF GHANA, LEGON
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IDENTIFICATION OF SUDAN DYES (I TO IV) IN PALM OIL USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS) FROM LOCAL MARKETS IN GREATER ACCRA REGION AFTER THE BAN BY THE FOOD AND DRUGS AUTHORITY(FDA)

BY

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DECLARATION

I hereby declare that this thesis is the product of my own original work and that no portion of it has been submitted for any other degree or recognition. Every section of the text and any findings derived from external sources or works is properly cited.

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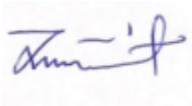

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DEDICATION

This work is dedicated to the Most High God, my dear parents, my husband, my friends, and my lecturers, whose guidance and encouragement have been invaluable throughout my training.

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I would like to show my heartfelt appreciation to all individuals whose contributions were instrumental in the successful completion of this project. I couldn't have achieved this without the crucial support and mentorship provided by my supervisor, Prof V. K Nartey. I would like to express my sincere gratitude to all personnel involved. I am very grateful to staff of Ghana Standards Authority – Pesticide residue lab for their direction and ongoing oversight, as well as for giving the project the information it needs. In addition, I want to sincerely thank my family for their wonderful support and encouragement, both of which were invaluable in getting this project finished.

ABSTRACT

This study investigates the persistent contamination of palm oil with carcinogenic Sudan dyes (I-IV) in Ghana's Greater Accra Region, assessing the effectiveness of the Food and Drugs Authority's (FDA) prohibition of these substances. Despite regulatory prohibitions, economic incentives for colour adulteration remain, posing significant public health risks. To address this, an efficient and robust analytical method using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) was developed and validated. The method demonstrated excellent linearity ($R^2 > 0.97$) across a range of 0.05 to 5 mg/kg, with high mass accuracy (< 5 ppm) ensuring definitive identification.

The method was applied to 104 palm oil samples collected from ten major markets. The findings reveal a critical public health failure: Sudan IV was detected in 61.5% of samples, with concentrations reaching an alarming 15,638.95 mg/kg, far exceeding international safety limits. Statistical analysis of variance (ANOVA) confirmed that adulteration was not random but geographically concentrated, with significant variation across markets ($F = 6.40$, $p < 0.001$), identifying specific hotspots. Sudan I and III were present in 5.8% and 12.5% of samples, respectively, while Sudan II was not detected.

The study concludes that the FDA's ban has been largely ineffective in the domestic market, revealing a stark enforcement gap between export-oriented controls and local consumer protection. The widespread and geographically clustered adulteration underscores an urgent need for a paradigm shift in food safety strategy. This research provides critical empirical evidence and a validated methodological framework to guide targeted regulatory action, enhanced surveillance, and public health interventions to mitigate this preventable risk to Ghanaian consumers.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Adulteration of palm oil is a globally prevalent issue that poses significant risks to public health and safety (Rahman et al., 2015). It is an inappropriate technique that is prohibited by international food safety rules (Charnley Berris, 2008). The variety of adulterated food products differs between countries and may encompass meat products, oils, flour, confectionery, fruit juices, and dairy items (Spink & Moyer, 2011). The global extent of food adulteration remains unclear. Adulteration is the process of deliberate addition, mixing, or substitution of substances to a product, particularly food or drink, in a way that reduces its quality, safety, or authenticity (Banti, 2020; Choudhary et al., 2020). When the material being added is disliked by the consumer, the term adulteration is applicable. Otherwise, the phrase "food additive" would be used (Essuman et al., 2022). Food is mostly tampered with to improve its appearance and mask the effects of using unhealthy ingredients, increase organoleptic features, and protect nutritional values; consequently, food adulteration generates economic gain for those who are involved in the practice (Choudhary et al., 2020). Palm oil is a key food product that is contaminated in Ghana and Sudan dyes have been identified in samples of palm oil (Teye et al., 2019).

Edible palm oil is a significant non-traditional export that brings in a substantial amount in foreign exchange for Ghana (Essuman et al., 2022). Recently, adulteration of palm oil has become more popular due to its expanding use, particularly in ready-to-eat meals (Banti, 2020). Palm oil is a reddish/orange oil extracted from the mesocarp of oil palm fruits (Hamid, 2008). It is extremely nutritious and has several applications all around the world. It is utilized in certain societies to cure ailments, usually vitamin A deficiency (Rao, 2000). Almost all ready-to-eat items use palm

oil, which is the most widely used oil in the food business (Goggin & Murphy, 2018). This market is supported by palm oil's low cost and ease of use, such as its ability to quickly decolorize and semi-solidify naturally at temperatures below 25 °C. Because of its high amount of β-carotene, palm oil from West African nations such as Nigeria and Ghana are regarded to be among the finest accessible (Absalome et al., 2020). They are distinguished by big, medium, and small-scale producers, processors, and marketers (Ofosu-Budu & Sarpong, 2013). This has made monitoring and detecting fraud inside the industry challenging. Conversely, global demand for vegetable oil is steadily increasing, with palm oil accounting for the lion's share, prompting countries that produce it to increase production while diverting more resources into formulating techniques to ensure quality and safety (Teye et al., 2019).

A tropical tree with fronds, the oil palm tree (*Elaeis guinensis*) is a shrub, or plant having a crown of pinnate or palmate leaves above a stem devoid of branches (Hamid, 2008). Native to the tropics and subtropics, oil palm has long been recognized for its benefits to humans through its forages and products (Pan et al., 2012), and it is considered one of the major oil-producing plants in West Africa. The fruit of the oil palm produces two types of oil: red crude palm oil, which is produced from the monocarp, and brownish crude oil, which is derived from the seed (kernel). While its brownish crude oil largely comprises Lauric acid, the red crude palm oil is mostly composed of Palmitic and Oleic acids. Of all natural sources, palm oil has the greatest content of tocotrienols and carotenoids (Gee, 2007; Pan et al., 2012). The current increased demand for palm oil in both industrial and domestic usage has resulted in an upsurge of palm oil adulteration (Okogeri & Otika, 2011; Pan et al., 2012). A common belief in Nigeria insinuates that palm oil is tainted by producers and bulk buyers to raise the oil's quality, without considering the possible consequences for the customers' health and the oil's quality (Rahman et al., 2015).

Food fraud is presently a global concern due to technological improvements, and every food item is susceptible, including palm oil (Choudhary et al., 2020). It is now illegal in almost every country to include Sudan I, II, III, and IV in food due to violations of US and EU rules. However, these dyes have been used illegally to intentionally adulterate a variety of products, including tomato paste, spices, and palm oil, to raise the perceived quality of these goods (Genualdi et al., 2016). Its regular usage as an adulterant in food products has also been documented (Sciuto et al., 2017). According to the European Union Rapid Alert System for Food and Feed (Rebane et al., 2010) a wide range of foods may be contaminated by Sudan dyes. These dyes are identified as category 3 carcinogens in general, and subsequent addition to palm oil samples has been reported worldwide. These dyes also pose a genotoxicity and carcinogenic risk, and this implies that any food product that contains these dyes is harmful and puts the health and safety of its consumers at risk (Gizaw, 2019). In response, the European Union implemented several laws starting in early 2003 that mandated analytical reports attesting to the importation of palm oil and chili spices free of Sudan I–IV (Genualdi et al., 2016). West Africa, which was formerly recognized for producing genuine palm oil and topping the global production list, is reportedly facing a significant problem with palm oil adulteration using Sudan dyes (Okogeri, 2013). For example, the Eastern regional Food and Drugs Authority Secretariat in Ghana disclosed that greater than 42% of palm oil samples gathered from multiple local stores revealed the presence of the cancer-causing Sudan dye (Business Day Ghana, 2015) (Essuman et al., 2022).

Azo-dyes are frequently utilized as adulterants (Alim-Un-Nisa & Akhlaq, 2015; Cristina, 2014). Azo-dyes are organic molecules containing the diazinyl (R-N=N-R') functional group, with R and R' often being aryl. Azo-dyes are the most common source of synthetic color in textiles. These dyes are likely to break down when they are in the hydrazine state by skin surface microflora

which possesses the enzyme azo-reductase. The interaction between aryl diazonium salt (ArN_2X^-) and secondary aromatic molecule yields the synthetic colors of azo-dyes (Alim-Un-Nisa & Akhlaq, 2015). Azo dyes produce a wide spectrum of vibrant colors and a very popular because they are widely accessible and inexpensive (Benkhaya et al., 2020). The process may be easily scaled up and down due to the reaction's simplicity, which is always an important factor in chemical cost (Mohamed et al., 2016; Cristina, 2014). Azo dyes are used to color diverse materials in industries (textiles, leather, cosmetics, and plastics) (Selvaraj et al, 2021). Furthermore, azo dyes are employed in the food industry as food additives. The classifications are Sudan I, II, III, and IV (Li et al., 2018). These dyes are employed for many purposes and are available in a range of hues. Sudan II is orange, Sudan III and IV are both red, while Sudan I is yellowish in hue. The most popular Sudan Azo-dye used in the adulteration of palm oil is Sudan III and Sudan IV dye with the latter being the most detected in palm oil samples (Nyorkeh et al., 2024). In addition, the dye may be utilized as a stain for lipoproteins, lipids, and triglycerides, and lipoproteins as well as to color textiles, polymers, wax, and floor polish (Alim and others, 2016). The International Agency for Research on Cancer (IARC) declared this dye as having carcinogenic properties, therefore are not permitted for use in food. Nevertheless, due to its vivid color and inexpensiveness, it has been intentionally exploited as artificial color for palm oil (Okogeri, 2013). Regrettably, the application of Sudan IV as an additive for its colour enhancing property of palm oil is increasing. According to Andoh et al. (2020), formerly, it was thought that the second most prevalent dye in food was Sudan IV. Sudan IV dye is an azo-dye that dissolves in fat and is utilized as a dye in industries in polishes, tiles etc. These colours are not permitted to be added to food in any form according to the International Agency for Research on Cancer (IARC) and quantities because of shown carcinogenic and mutagenic properties, as there is no known daily dosage that

can be tolerated (Loprieno, 1975). Some unscrupulous individuals use Sudan IV dye to improve palm oil colour because of its inexpensive cost, vibrant hues, wide availability, and ease of access (MacArthur et al., 2021). The detection of Sudan IV in palm oil presents significant analytical challenges, similar to those encountered in other complex liquid matrices. Palm oil adulteration, on the other hand, has become a consumer confidence, public health, and worldwide concern, resulting in increased interest in formulating procedures for verifying the authenticity of palm oil and identifying possible adulterations of Sudan IV (Gizaw, 2019). The World Health Organization has established maximum permissible residue levels for various colours in food products and the Food and Agriculture Organization (FAO) (F. A. O., & WHO, 2016). Nonetheless, the covert addition of Sudan dyes to palm oil remains a concern, straining regulatory agencies and jeopardizing public health.

Although edible palm oil is susceptible to adulteration, its authenticity can be verified through various analytical techniques. These procedures include Liquid chromatography-mass spectrometry (LC-MS)/mass spectrometry (MS), high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FTIR), and mid-infrared spectroscopy (MIR). These techniques produce results that are incredibly accurate, repeatable, and effective (Lohumi et al., 2015).

It is vital to create quick and effective techniques for verifying and detecting oil adulteration. This study dives into the essential issue of recognizing Sudan Dyes in adulterated palm oil utilizing novel high-resolution Liquid Chromatography Tandem Mass Spectrometry (LC-MS) techniques, focusing on ten local markets in Accra. The study intends to shed light on the prevalence of such adulteration and the possible health hazards connected with it, as well as to investigate the efficacy of modern analytical technologies in guaranteeing food safety in a local, real-world environment.

In today's globalized and interconnected world, the quality and safety of consumable products, particularly in the food industry, is a paramount issue for both consumers and regulatory authorities. One of the persistent challenges in this regard is the detection of contaminants in food items, which can cause significant health risks to consumers (Haughey et al., 2015)

The economic significance and high increased demand for palm oil have led to the emergence of unethical practices in the industry (Goh et al., 2017). One such practice is the adulteration of palm oil with unauthorized substances, such as synthetic dyes like the Sudan series (I to IV), which pose a serious threat to consumer safety given their potential carcinogenicity. These dyes are metabolized into aromatic amines, which are hepatotoxic and genotoxic, potentially leading to liver disease and cellular mutations (Di Anibal et al., 2015).

Sudan dyes are synthetic organic compounds originally developed for use in the textile and petrochemical industries. Due to their vivid coloration, they have found illicit use as food colorants, especially in chili products and palm oil. However, their use in food is strictly regulated due to health concerns. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set maximum residue limits for these dyes in food products (Ávila et al., 2011).

The formulation of fast and effective procedures for the verification and detection of oil adulteration is critical (Basri et al., 2017; Khamchum et al., 2013). This study delves into the critical issue of identifying Sudan Dyes in adulterated palm oil employing innovative high-resolution Liquid Chromatography Tandem Mass Spectrometry (LC-MS) techniques, focusing particularly on the local markets in Greater Accra Region, Ghana. The research aims to shed light on the prevalence of such adulteration and the potential health risks associated with it, while also

exploring the effectiveness of advanced analytical methods in ensuring food safety and quality in a local, real-world context.

1.2 STATEMENT OF THE PROBLEM

In Ghana, palm oil is extensively used in culinary applications, with a substantial proportion of the production being exported to European markets. Palm oil from Ghana was discovered to be tainted with Sudan IV dye. The European Commission accumulated 83 notifications about Sudan IV dye contamination in Ghanaian palm oil via the Rapid Alert System for Food and Feed (RASFF) between July 2004 and May 2005. In view of the concern, Ghana's Food and Drugs Authority (FDA) has maintained a policy since 2005 to guarantee that palm oil exported to the European Union is uncontaminated by Sudan IV dye. This requirement remains in force, as evidenced by a 2022 FDA notice reiterating that all palm oil exports to the EU must be accompanied by an official certificate attesting to the absence of Sudan dyes, without which they will be rejected upon arrival (Food and Drugs Authority, 2022). The agreement stipulated that each shipment of palm oil intended for export to the EU must be tested and accompanied by a Certificate of Analysis before export.". The Ghana FDA has explicitly stated that “Sudan IV dye is not a food additive and must not be found in food products, including palm oil,” and that its adulteration constitutes a criminal act under national food safety regulations (Citi Newsroom, 2024; Modern Ghana, 2021). However, palm oil intended for the domestic market is not subjected to the same rigorous testing for Sudan dyes, creating a significant gap between national regulatory stance and enforcement for local consumers. This suggests a compromised safety of palm oil sold and consumed within Ghana. Guided by a food fraud mitigation framework, this study posits that the lack of effective control measures for the domestic market is the key driver of this public health risk. This framework conceptualizes adulteration as a function of motivation (economic gain) and opportunity (weak

enforcement). While the motivation to adulterate palm oil with cheap, vibrant Sudan dyes remains clear, the critical opportunity is created by a regulatory asymmetry: a stringent, enforced testing regime for exports versus a largely unmonitored domestic market. This research therefore operates on the premise that this enforcement gap has facilitated widespread adulteration, exposing local consumers to preventable carcinogenic risks. Consequently, this study seeks to empirically investigate this gap and assess the true effectiveness of the current regulatory prohibitions for the Ghanaian public. As a result, the goal of this research is to investigate the prevalence of Sudan dye adulteration in palm oil from certain marketplaces in the Greater Accra Region.

1.3 JUSTIFICATION

Palm oil is a widely used food ingredient in Ghana. Nearly every Ghanaian household uses palm oil in one way or another. Popular dishes include palava sauce (Kontomire stew), and mashed yam (Etoo) beans, gari, and fried plantain with palm oil (Red-Red).

Unfortunately, the regulatory measures implemented following the detection of Sudan dyes in Ghanaian palm oil exported to the EU failed to address the domestic vending and consumption by residents. This suggests that the quality of palm oil available in the Ghanaian market cannot be assured.; hence, assessing the safety of palm oil as it pertains to Sudan dye contamination on Ghanaian markets is crucial. Such an initiative will spur on the development of directives and regulations to protect public health and safety, thereby reducing risks to both local and international trade. To mitigate this issue, it is essential to evaluate how widespread the practice is, identify the source of the dye, and determine where adulteration occurs along the value chain of palm oil.

1.4 AIM

This study's aim is to identify and quantify the presence of Sudan dyes (I to IV) in palm oil samples from local markets in the Greater Accra region.

1.5 OBJECTIVES

I. To determine the prevalence of Sudan Dyes in palm oil sold in various markets in the Greater Accra region of Ghana.

ii. To quantify the levels of Sudan Dyes in contaminated palm oil samples applying Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS).

iii. To assess the effectiveness of the FDA's prohibitions on the use of Sudan Dyes in palm oil within the Greater Accra region.

iv. To provide recommendations for improving regulatory enforcement and consumer safety regarding palm oil adulteration.

1.6 SIGNIFICANCE OF THE STUDY

This research provides critical, empirical data on the prevalence of Sudan dye adulteration in palm oil within Ghana's local markets. By applying a robust LC-MS/MS method, the study directly assesses the effectiveness of current regulatory measures for protecting domestic consumers, an area previously overlooked in favor of export controls. The findings will offer Ghana's Food and Drugs Authority (FDA) evidence-based insights to guide more targeted enforcement strategies and public awareness campaigns. Furthermore, the validated methodology can serve as a reliable reference for future monitoring efforts. Ultimately, this work contributes to safeguarding public health in Ghana by highlighting a specific food safety risk and informing actions to mitigate it.

CHAPTER 2

LITERATURE REVIEW

2.1 BOTANY OF PALM OIL

Palm oil, a consumable vegetable oil, is obtained from the reddish pulp of the oil palm fruit's mesocarp (Hamid, 2008). The oil is utilized in the making of food, cosmetics, and biofuel. The common African oil palm (*Elaeis guineensis*) belongs to the Palmaceae family (Gee, 2007). Its broad, erect stem may reach a height of 30 meters when completely mature (Hamid, 2008). It's a monoecious plant, which means it produces both male and female flowers. When the palm has four spear-shaped leaves, it is ready to tap. When the leaf's base is removed, the product is a drupe that generates two kinds of oil: kernel oil and oil extracted from the mesocarp. The exudates are known as palm wine or palm sap.

Red palm oil is Africa's principal domestic or edible oil source (Bakoumé, 2016). Vegetable oil is an important and valuable raw material in both the food and non-food sectors. The bulk of the oil is employed in the production of margarines, spreads, ice cream, confectionary fats, emulsifiers, and vanaspati (Ofosu-Budu & Sarpong, 2013). Because palm oil is a vegetable oil, it has no cholesterol. It has a unique triacylglycerol and fatty acid makeup. Because it is naturally semi-solid at room temperature, it does not need to be hydrogenated for use as a food ingredient (Rao, 2000). Palm oil is high in carotenoids and tocopherols, which give natural stability against oxidative degradation (Gee, 2007). Mechanical or traditional milling procedures yield crude palm oil that is orange-red in hue (Hartley, 1977). Bleached palm oil is more refined than red palm oil. Red palm oil is excellent for food applications since it loses less nutrients throughout the refining process. Palm Olein and Super Olein would be produced by fractionating and refining raw palm

oil. Partitioning leads to a decrease in palmitic acid, the principal saturated fatty acid, and consequent rise in monounsaturated oleic acid (Edem, 2002).

2.1.1 Carotenoids and its influence on crude palm oil

Unquestionably a prevalent and essential pigment in living things, carotenoids got their name since they are the primary pigment of carrot root, Daucus carota. Many vegetable oils, including olive, barley, sunflower seed, cottonseed, rapeseed, ground nut, soybean, and yellow maize (corn) oil, may contain these carotenoids (Ong & Tee, 1992). These vegetable oils frequently have low amounts of carotenoids—below100 ppm. Among all commonly used vegetable oils, Palm oil has the largest known quantity of carotenoids. produced through agriculture (Tan, 1989). The world's largest natural plant source of carotenes is crude palm oil, measured in units of retinol (provitamin A) equivalent. It contains 15 to 300 times the amount of retinol equivalents compared to carrots, leafy green vegetables, and tomatoes, all of which are also high in provitamin A activity (Tan 1989). These carotenoids are what give crude palm oil its orange-red hue. Carotenoids' nutritional qualities are gaining attention. The expectation of carotenoids to increase in value has necessitated its extraction from palm oil and its byproducts.

Palm fruit oil's red color is owing to its high carotenoid concentration, notably beta-carotene and lycopene (Hamid, 2008). The intensity of the hue is frequently employed to assess the quality of the oil. Palm oil with a brilliant red color is preferred by consumers and industry because it is linked with freshness and high carotenoid content. It typically accentuates the reliability and authenticity of crude palm oil. They operate as natural antioxidants, preventing oil oxidation, which can lead to rancidity and a loss in quality (Amira et al., 2014). Carotenoids and other antioxidants protect the oil from oxidative degradation, increasing its shelf life. The carotenoid content in crude palm oil can impact its market value. Higher carotenoid levels in oils are

frequently sought in the market because they are regarded healthier and more visually attractive (Basiron & Weng, 2004). The hue of palm oil may also be used as a quality factor by processors and producers. Farmers may improve the carotenoid content of palm oil by ensuring optimum soil fertility and pH levels, as these factors impact nutrient absorption by the palm plants (Edem, 2002). A sufficient supply of key nutrients, such as nitrogen, phosphorus, and potassium, is required for optimum development and carotenoid synthesis. Ample and continuous irrigation is also essential to minimize water stress, which can reduce carotenoid accumulation. Nonetheless, collecting palm fruits at the proper maturation stage is critical for producing fruits with the highest carotenoid concentration. Lower carotenoid levels may arise from premature harvesting (Adhikari, 2021).

2.1.2 Red Palm Fruit Oil versus Palm Kernel Oil

Eating the oil extracted from ancient or virgin red palm fruit is considered a holy and therapeutic meal in many cultures (Bakoumé, 2016). It is one of the most nutrient-dense edible oils available. Using it with palm kernel oil is not advised. Because of its deep, rich color when left untreated in nature, "red palm oil" is another name for the oil sourced from oil palm tree's fruit (*Elaeis guineensis*) (Hamid, 2008). The seed or kernel of a palm tree is used to obtain palm kernel oil (Amira et al., 2014). Palm fruit oil is roughly 50% saturated and mostly formed of lauric acid, as compared to palm kernel oil, which is more than 89 percent saturated and primarily composed of palmitic and oleic acids (Gee, 2007). A significant exclusion in today's diet may be due to the common misconception that palm fruit oil and palm kernel oil may be used interchangeably. Until now, the stigma linked to the kernel has kept the fruit hidden. In any case, unrefined organic sustainable red palm fruit oil is a complete meal. Red palm fruit oil provides higher health benefits than palm kernel oil (Hartley, 1977). High carotenoid content of the palm fruit confers its oil a bright red color, specifically beta-carotene and lycopene (Hamid, 2008). These strong antioxidant

components also contribute to the orange and rich red colors of tomatoes, carrots, and other fruits and vegetables. You could be surprised by the crimson palm fruit. Palm oil has a considerable amount of nutrients than tomatoes or carrots, which may surprise you. Tocotrienols, a form of vitamin E that is highly concentrated in red palm fruit oil (Akinola et al., 2010), are also prevalent. Even though processed palm oil does not contain cholesterol, its "thickness" at room temperature causes it to behave similarly to hydrogenated fats (the harmful trans isomer of cis fatty acids) in packaged foods (Pan et al., 2012; Gee, 2007; Rao, 2000). It is an excellent alternative for hydrogenated oils in many food manufacturers' snack items. When this occurs, processed foods may have labels such as "trans fat-free" and "cholesterol-free." However, refined and treated red palm oil loses its medicinal and nutritional properties, as well as its red hue (Absalome et al., 2022). When it is processed, it loses the powerful antioxidants and health benefits that are only found in raw palm oil.

2.1.3 Palm Oil, an Important commodity in the oil sector

Oil palm (*Elaeis guineensis*) is the crop that produces the most oil per unit area planted. Thus, utilizing palm oil to generate hard currency represents a viable economic strategy for Ghana, given the country's advantageous climatic conditions for the farming of oil palm trees. (Ofosu-Budu & Sarpong, 2013). Since the 1970s, palm oil and oil from its kernel have been the fastest growing sectors in the Oils and fats industry. Palm oil is extracted from palm tree fruit and is used in various culinary applications (Hamid, 2008). The total global output of palm oil is approximated to be more than 45 million tons, and the world's largest producers and exporters being Indonesia and Malaysia. The top three importers are India, China, and Europe (Ofosu-Budu & Sarpong, 2013; Edem, 2002).

Approximately 80% of global palm oil production is allocated to culinary uses such as baked goods, noodles, margarines, and cooking oil (Okogeri, 2013). Additionally, palm oil is utilized in manufacturing non-food products, including soaps, cosmetics, detergents, surfactants, biofuels, and pharmaceuticals (Oti, 2021). Ghana's current annual output is anticipated to be 109,000 metric tons, a decrease from 121,000 in 2006. The amount consumed domestically is projected to be 230,000 tons. Imports compensate for the shortfall (Ofosu-Budu and Sarpong, 2013).

2.2 PRODUCTION OF PALM OIL

According to Essuman et al. (2022), the palm tree (*Elaeis guineensis*) evolved in the West African tropical rainforest. Over a millennium, people in Africa have processed palm oil fruit into crude and refined oil. Carotenoids, a naturally occurring red pigment, give palm oil its rich red colour (Hamid, 2008). Palmitic acid, a saturated fatty acid, is a key component of its glycerides. It is fluid semi-solid in tropical ambient temperatures and solid fat in temperate regions (Gee, 2007). Palm oil production starts with palm oil seeds (Bakoumé, 2016). Oil palms have a productive life span of 25-30 years, therefore seed selection will have a long-term effect on output. Seeds are planted in a nursery and nurtured for eight months before transplanting to plantations. The trees in the plantations are irrigated and fertilized as needed during their growth. The trees mature after 30 months and due for harvest which happens every 7 to 10 days (Sharma, 2013).

Palm trees are grown in regions with abundant rainfall and tropical climates (1,200 mm per year) because of their economic worth as a source of edible oil. The palm fruit arrives in bunches of 10 to 40 kg (Hamid, 2008). Individual fruits are composed of a mesocarp and an exocarp, which is the outer layer, and range in size from 6 to 20 grams. It contains a nut in the centre that is composed of an endocarp shell and a kernel that contains oil that is distinct from palm oil.

An efficient palm oil mill processes 100 tons of fresh fruit bunches (FFBs) into an average of 22 tons of crude palm oil. The palm oil tree yields fruit all year round, with two significant peak dates noted (April-May and September-October) (Hamid, 2008).

According to Pan et al. (2012) in recent years an emergence of palm oil as a non-traditional export good has been observed. Ghana primarily exports two types of oil: "zomi," which is salted and spiced, and "virgin" or "crude oil," which is the natural, unprocessed form. (Essuman et al., 2022). The addition of spices, salt, and heating gives the 'zomi' a richer color, while the virgin palm oil takes on an orange-red hue. The Eastern and Central regions of Ghana are the main producing regions. The Volta region is where most 'zomi' is produced (Amoako-Mensah, 2016).

Based on the complexity and throughput of processing into edible oil, palm fruits may be divided into three classes (Sharma, 2013). The three categories are large industrial mills, medium-sized mills, and small-scale processors. Up to two tons of Fresh Fruit Bunches (FFB) may frequently be processed by small-scale systems in an hour (Nwalieji & Ojike, 2018). Large-scale mills process more than 10 tons of fresh fruit bunches (FFB) per hour, whereas medium-sized processing mills usually have infrastructures that can process 3 to 8 tons of FFB per hour (Mensah, 1999).

Palm oil processing generally includes the following steps: At the mill, new fruit bunches are harvested and received. They are then sterilized and threshed before being mashed and pressed to extract crude palm oil (Nde & Foncha, 2020). Prior to storage, crude palm oil may be refined further. Small-scale processors bring FFB to processing facilities in a basket carried on their heads, whereas large-scale processors transport it in trucks. Threshing is accomplished by removing the fruit-laden spikelets from the bunch stem using an axe or machete, then manually separating the fruit from the spikelets (Nwalieji & Ojike, 2018). Large-scale processors employ an automated

technique to separate the fruit from the bunch and the spikelets. Prior to the threshing step, large-scale facilities sterilize at 120 C for 90 minutes. The goal of sterilization is to cease enzymatic activity and soften the pulp so that the fibrous material may be readily detached (Balakrishnan et al., 2021). Mechanical pressure is used to the digested mash during the extraction process to separate the oil, moisture, fiber, and nuts. For big scale processors, the oil is separated from contaminants during the clarifying step and filtered using a centrifuge (Balakrishnan et al., 2021). Before extracting the oil, small-scale processors heat a spindle press to lower the water content. The press residue is composed of fiber and palm nuts. The nut is subsequently removed from the fiber by hand. After further crushing the fiber with a spindle press, a second-grade oil is obtained, which is commonly utilized in soap manufacture. The approach varies for most small-scale processors (Mensah, 1999).

The next phase of this process is to extract the oil. As a result, the fruits are subjected to steam-treatment. This is an important step because of its bactericidal activity and stops the activity of any enzymes (Genualdi et al., 2016). This prevents the degradation of the palm fruit. Simultaneously, the steamer tenderizes the oil palm fruits, allowing the organic oil to be released. Following the steam treatment, the fruit aggregates are separated into their constituent elements, which comprise water, oil, fibers, and palm kernel (Verheye, 2010). High pressure is then used to extract the oil and water from this naturally occurring fruit. As a result, a combination of water and oil is formed. Palm oil will ultimately create a suspended layer of oil since water is denser oil. The oil can then be extracted from the water using this approach. The ultimate product are the first droplets of pure crude palm oil which necessitate greater refinery processing (Balakrishnan et al., 2021).

New processing technologies are being used to enhance palm oil processing. Currently used techniques include bioconversion (which uses microbial cells and enzymes to produce useful

chemicals) biomodification (which uses lipase to modify palm oil and its fractions through interesterification) and molecular distillation. (Mensah, 1999).

2.2.1 Processing and Extraction of Palm Oil

Palm oil extraction is constantly checked to ensure that the completed product is extremely attractive (Basiron & Weng, 2004). The harvest of the fruit is frequently the first stage in the production of palm oil. This is the physical extraction method for palm oil (screw pressing), as well as the factors that influence the standard of the palm oil released. Palm oil manufacturing process is preceded by the harvest of fresh fruit bunches and maintenance until the next phase of palm oil production (Sharma, 2013). Following the harvesting of fresh fruit bunches, the threshing stage follows. Before commencing the threshing process, separate the fruit from the bunches (MacArthur et al., 2021). This method is normally done by hand; however, a mechanical thresher that produces a superior end product can be employed on occasion. This is because the thresher vibrates continuously and rotates the fruit as it pulls it from the bunch making it well known for this capacity (Balakrishnan et al., 2021). Most small-scale processors lack the ability to provide steam for sterilization. After threshing, the fruits are boiled in water. When cooked, spikelet-containing entire bunches absorb a lot of water. Using high pressure steam, bundles may be heated more effectively and with less water loss. Unlike most small-scale procedures, high-pressure sterilization threshes bunches after heating to liberate the fruits (Verheye, 2010). Small-scale producers use empty bunches as household fuel; larger mills burn the waste. The ash is high in potassium and therefore repurposed in the plantation as fertilizer (Andoh et al., 2020).

The digestion of the palm fruit is the next step in the extraction process. In a palm oil processing factory, digestion is used to crush the fruit's oil-containing cells and releases the palm oil (Sharma, 2013). A steam-heated cylindrical vessel serves as a digester. The fruit is pounded in the digester

by several beater arms rotating around a central shaft. The digesting process is carried out in a heated atmosphere under high pressure to remove the exocarp of the palm fruit, lyse the oil cells, and lessen the thickness of the oil (Mensah, 1999). However, to save expenses, many small-scale palm oil mills lack heat insulation and steam injections by design. When metal wear in the digester of a palm oil processing mill exceeds the maximum rate, the risk of oil oxidation and spoilage increases (Nwalieji & Ojike, 2018). The weight and size of the fruits generally dictate the fresh fruit bunch extraction rates. Fruits weighing more than 20 kilograms extract oil at a rate of 19 to 21%, but fruits weighing less than 10 kilograms extract oil at a rate of 15 to 16% (Hamid, 2008).

During the pressing step, oil is recovered from the digested material using one of two methods. The "dry" method which employs mechanical presses and the "wet" method which drains the oil using hot water (Nde & Foncha, 2020). The extraction step works by mechanically pressing the digested mash in the "dry" process to separate oil from a combination of oil, moisture, fiber, and nuts. There are many different types of presses, but they all work on the same basic principles (Nde & Foncha, 2020). The presses can be set up to run continuously or in batches (for short-term, low-volume operations on material).

After the oil has been removed, the next step is to clarify it. The fundamental purpose of clarifying is to remove suspended contaminants from the oil (Kasim, 2009). The fluid that comes out of the press contains palm oil, water, cell debris, fibrous material, and "non-oily substances." Because of the non-oily particles, the mixture is exceptionally thick (viscous). Consequently, the fluid from the press is thinned using the hot water. The heat used to break the emulsion by adding water, acts as a barrier, resulting in the lighter droplets of oil rising to the surface of the container via the watery mixture (oil in water with gums and resins) and the denser solids settle at the bottom. A 3:1 ratio is attained by adding water (Basiron & Weng, 2004).

To get rid of the coarse fiber, the diluted liquid is filtered through a screen. For the palm oil to separate and rise to the top of the mixture after boiling for one to two hours, the mixture must first be screened since it is lighter than water (Kaya & Hung, 2021). The poured out translucent oil is supplied into a receiving tank. Traces of debris and water-droplets remain in the clarified oil. Moisture content in the oil needs to be lowered to 0.15 to 0.25 percent to prevent the oil's autocatalytic hydrolysis from producing FFA (Mensah, 1999).

After carefully removing any imbedded dirt from the dry oil, any leftover moisture is eliminated by warming the decanted oil in a frying pot. Continuous clarifiers have three compartments: one for the crude mixture, one for drying the decanted oil, and one for storing the finished oil and acting as a heat exchanger in the outer shell (Kasim, 2009). The nearby sludge pits were dug specifically to contain the clarifier's effluent. Small mills do not process the sludge any further. In most cases, accumulated sludge in the processing area is collected in buckets and utilized to control weeds (Akinola et al., 2010).

2.3 NUTRITIONAL AND HEALTH BENEFITS OF PALM OIL

People all around the world have been fed with palm oil for many centuries. The primary components of palm oil include palmitic, oleic, and linoleic acids, which are the most common fatty acids present in plant species (Mensah, 1999; Gee, 2007). Consuming palm oil has no effect on blood pressure but improves coronary blood flow (Heber et al., 1992). In addition, vitamin E included in palm oil acts as a dietary antioxidant and helps lessen the harm that free radicals—These are generated either by the body's oxidative energy metabolism or through exposure to environmental pollutants and harmful chemicals—resulting in damage to cells (Rao, 2000).

According to Gizaw (2019), free radicals are connected to induction of cancer, chronic degenerative diseases, and the aging process.

High levels of beta-carotene, purported to have to health benefits, exist in palm oil (Absalome et al., 2020). Unrefined palm oil is the most abundant natural source of beta-carotene, a provitamin A pigment. Crude palm oil is eaten in various Southeast Asian countries as well as West Africa, even though it completely loses its beta-carotene content after processing (Teye et al., 2019). Lung cancer risk has been associated with a diet low in vegetables high in carotenoid content in several studies (Wolf, 1982). In 1995, an estimated three million children were affected by Vitamin A deficiency, resulting in xerophthalmia accompanied by an increased risk of vision loss, even though the average daily requirement for the nutrient is just one milligram (Pirie, 1983).

Moreover, it has been predicted that 250 million children worldwide are subclinical Vitamin A deficient, making them susceptible to severe morbidity and early mortality (Howson et al, 1998). Thus, the promise of palm oil should be realized as an affordable way to reduce disease and death among children in developing nations like Ghana (Ross et al., 1993). This is particularly relevant considering Pirie's (1983) claim that of all the vegetable oils commonly used, carotenoids are present in high concentrations in palm oil produced by agriculture.

Palm oil lacks the same isomers of trans-fatty acids as hydrogenated fats (Goggin & Murphy, 2018). Because of their low melting points and sensitivity to oxidative degradation, highly unsaturated oils, such as those found in many fish and seed oils, are not ideal for numerous culinary applications (Machado et al., 2023). However, the hydrogenation process broadens the culinary possibilities of polyunsaturated oils, which would ordinarily have much lower melting points. Hydrogenation reduces unsaturation by converting cis-double bonds in unsaturated fatty acids to trans-double bonds (Alonso et al., 2000). Excessive trans-fatty acid consumption can produce

metabolic and dietary problems; one consequence is the reduced levels of free fatty acids (FFA), which could potentially impair platelet aggregation and cardiovascular health (Heber et al., 1992). Palm oil, on the other hand, does not need to be hydrogenated to improve its use and stability. As a result, it is devoid of all the drawbacks associated with trans-fatty acids (Heber et al., 1992). Antioxidants included in palm oil are utilized to minimize oxidative stress and inflammation produced by free radicals. When used sparingly, palm oil promotes healthier living. The mixing of palm oil with other oils and fats in food items determines the total fatty acid amount and functionality of its final product, making it flexible and functional (Hartley, 1977). Palm oil outperforms other oils and fats at high temperatures due to its stability at high cooking temperatures (Amira et al., 2014). Even at high temperatures, palm oil retains its properties. Palm oil-containing goods keep their tastes and textures, such as crispiness or crunch, for a longer length of time, making them an excellent choice for products with a long shelf life (Edem, 2002). Margarine with no saturated fat, for example, would be liquid at normal temperature, but palm oil, which has a higher solid content, is often solid or semi-solid at ambient temperature (Hartley, 1977). The physicochemical properties of various food items are classified in terms of smoothness as well as the presence of saturated fat; palm oil's creamy texture is an excellent solution for increasing solidity and improving product uniformity (Imoisi et al., 2015). Furthermore, palm oil-based meals have an excellent tongue feel and fulfil special specifications for several items. Palm oil, for example, contributes to the smooth, creamy, spreadable texture of margarine and chocolate spreads, as well as their crisp and crunch (Ofosu-Budu & Sarpong, 2013).

2.4 SUDAN DYES USED IN FOOD ADULTERATION

According to the legislation, adulterants are chemical contaminants that shouldn't be found in food or other substances (Cristina, 2014). The use of adulterants is more common and unrestrained in

nations with few regulatory structures and few laws. When German scientist Frederict Accum investigated the use of adulterants in food and drink in 1820, he found numerous dangerous food colorings (Charnley-Berris, 2008). In the early 1850s, Arthur Hill Hassall detailed his comprehensive research on food contamination in *The Lancet*. As a result, several legislations, like the Food Adulteration Act of 1860, were passed (Charnley-Berris, 2008).

According to Genualdi et al. (2016) and Li et al. (2018), Sudan dyes are azo-dyes, industrial dyes applied for its color altering effect in the manufacturing of plastics and other materials. Of all organic colorants, azo colorants make up 60–80%. They are the most widely used synthetic dyes and pigments (Li et al., 2018). Textile fibersleather, hair, waxes, mineral oils, cosmetics, papers, and plastics are among the materials that frequently include them (Puntener & Page, 2004). Sudan dyes are used to boost and preserve color in food, which poses a significant risk to public health (Rahman et al., 2015). May 2003 saw the discovery of Sudan dye being used illegally in food throughout the European Union. The coloring was found in meals that included chili powder and chili powder itself (Sciuto et al., 2017). Since then, various EU member states have utilized the RASFF to inform of the assessment of Sudan IV and Sudan I in palm oil, as well as in curry powder, chili powder, sumac, curcuma, and chili-flavored products. Occasionally, the same category of products has been found to contain Sudan II and Sudan III (Sciuto et al., 2017). A food product that contains industrial dye is contaminated since such dyes are not permitted as food colors under European Council Directive 94/36/EC on the use of colours in food products (Abdulmumeen et al., 2012).

The European Food Safety Authority (EFSA) analyzed toxicologically several colors discovered illegally in food in the European Union in 2005. EFSA stated that there was high evidence that Sudan I was carcinogenic and genotoxic to humans (Pan et al., 2012). Given the structural

similarities between Sudan I and other Sudan dyes, it is assumed that all Sudan dyes would have the same harmful effects (Selvaraj et al., 2021).

2.5 THE TOXICOLOGY OF SUDAN DYES

Sudan dyes are rated as Category 3 carcinogens by the International Agency for Research on Cancer (IARC) and their addition to food is not permitted in any part of the world (Loprieno 1975). It suggests that using Sudan dyes as food additives is prohibited, a view firmly supported by the USFDA and the EU.

2.5.1 Sudan I

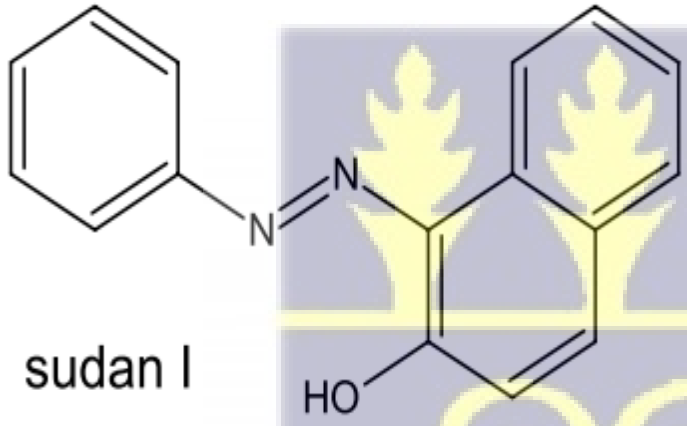


Figure 2.1 Structure of Sudan I dye

Sudan-I dye has been shown to have genotoxic effects and capable of triggering metabolic activation in Ex vivo and in-vivo experiments (Johnson et al., 2010). Sudan I's genotoxic and carcinogenic effects have been related to its products of decomposition 1-amino-2-naphthol and aniline, as well as the subsequent metabolic activation (Stiborova et al., 2014). In vitro experiments employing rat and human microsomes revealed comparable mechanisms for DNA interaction and metabolism (Johnson et al., 2010).

2.5.2Sudan II

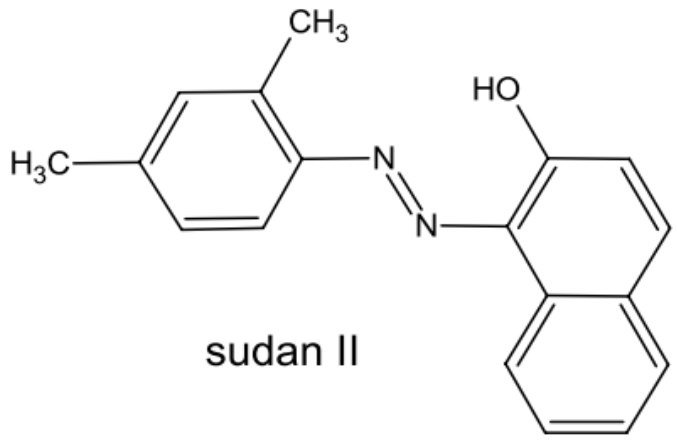


Figure 2.2 Structure of Sudan II dye

Sudan II is genotoxic in bacterial tests, according to limited data from in vitro research, and there is considerable evidence which implies that this happens after bioactivation (Bienstock et al., 2022). According to the EFSA, the dye should be considered potentially genotoxic at this time, even though there is no available in vivo data, and a single mammalian cell tested negatively in vitro (Johnson et al., 2010); however, there is inadequate information to infer and therefore make judgments regarding the carcinogenic effects of Sudan II after subcutaneous injection or ingestion. Conversely, after implantation of pellets impregnated with Sudan II, a considerable predominance of bladder tumors was seen, which was sufficient to classify the dye as a potential carcinogen unless shown differently (Stiborova et al. 2014).

2.5.3 Sudan III

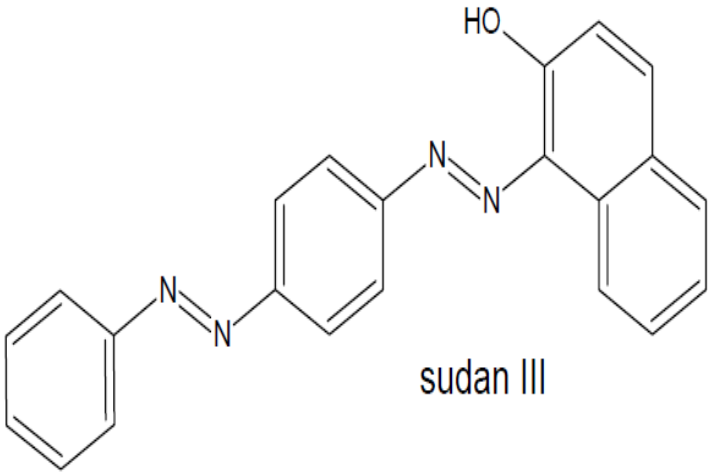


Figure 2.1: Structure of Sudan III dye

Relatively little research has been done on Sudan III's genotoxicity, and several of such studies have been Inconclusive because of the absence of an effective incentive system (Mohamed et al. 2016). With the limited breadth of investigations, there is no confirmation of Sudan III's carcinogenicity, nor is there any indication that it has the potential to be carcinogenic. Sudan III's structural relationship with Sudan I facilitates the production of Sudan I-like metabolites (Mohamed et al., 2016). Without any evidence to support the distinction between Sudan I and Sudan III metabolism, it may be safe to assume that it is carcinogenic and maybe genotoxic (Stiborova et al., 2014).

2.5.4 Sudan IV

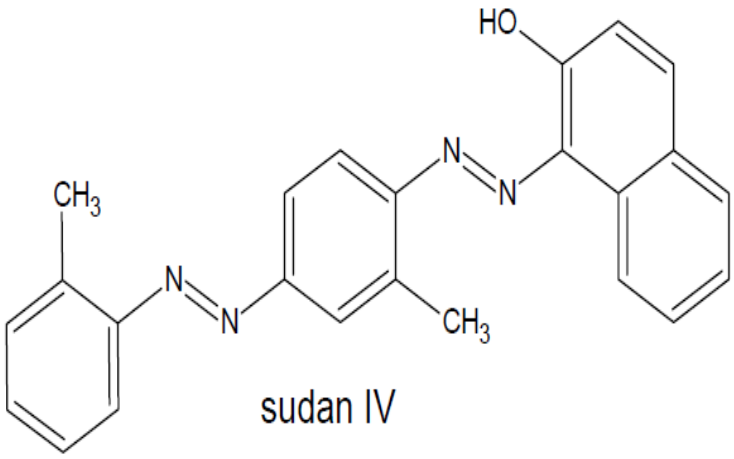


Figure 2.2: Structure of Sudan IV dye

Sudan IV's potential genotoxicity is undeniable, as the pattern of consistency with respect to the advantageous effects that follow metabolic activation has been shown to be consistent with other similar dyes (Li et al., 2018).

Sudan IV was discovered to have the capacity to induce epithelial proliferation while being employed as a dressing addition in wound healing. Because of this feature of the dye, as well as the observed impact of structurally related dyes, highlights its possible carcinogenic properties (Puntener & Page, 2004).

2.6 CARCINOGENICITY OF AZO DYES

Sudan dyes' toxicity is linked to their chemical structures, specifically the presence of aromatic amines. Sudan dyes have azo groups, which are made up of two nitrogen atoms joined by a double bond (N=N) within an aromatic ring system. Azo dyes can be metabolically transformed in the body, resulting in the production of aromatic amines, with some potential products being

carcinogenic. A single or multiple nitrogen-nitrogen double bonds, or "azo groups," are present in azo dyes (Pan et al., 2012).

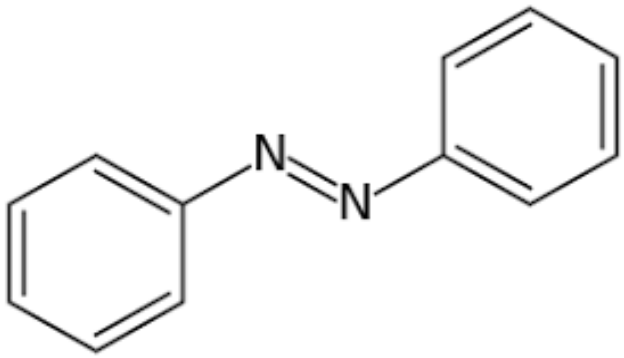
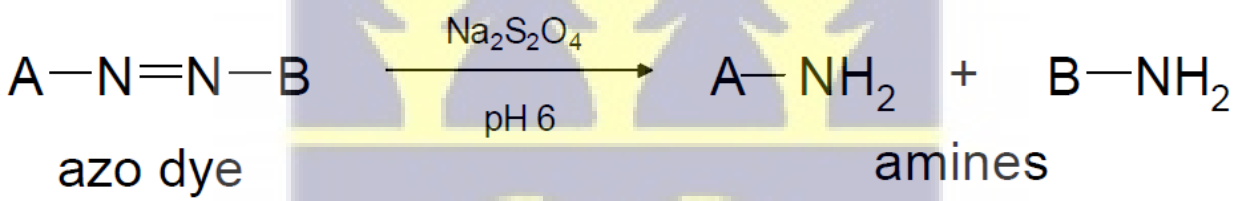


Figure 2. 3:The Azo group

When sodium dithionite is used under reducing conditions, the azo groups are cleaved to generate two amines.



Amine formation by reductive cleavage of azo dye

Cleavage of the azo group yields aromatic amines, some of which have been shown to have possible carcinogenic effects on humans. However, not all azo dyes may release these amines during reductive cleavage; only a handful are impacted (Puntener & Page, 2004).

The mutagenic potential of azo dyes is determined by the molecule's capacity to form active aromatic amines. This is affected by structure-activity interactions, which cause the azo linkage to break, resulting in the oxidation of the freed primary aromatic amine. The liver and gastrointestinal system are two primary places where the azo connection might be broken (Selvaraj et al., 2021).

Sudan I is genotoxic and carcinogenic, according to EFSA experimental findings (Pan et al., 2012). The data for Sudan II, III, and IV is equivocal, but regarding the structural similarities to Sudan I, the potential for genotoxicity and carcinogenicity cannot be dismissed. (Stiborova et al., 2014; Bienstock et al., 2022).

2.7 OVERVIEW OF FOOD ADULTERATION IN PALM OIL

Food adulterants are substances added to a product that are not authorized for use in that item. If adulteration poses a major health risk, prompt regulatory action must be taken to safeguard public health and safety (Choudhary et al., 2020). Palm oil adulterants can produce acute symptoms which include bronchial asthma, stomach-ache, vomiting, headache, cardiac arrest, cognitive impairment and long-term consequences like cancer (Tomar & Alka, 2022). The following are examples of instances of food adulteration:

- Beech-Nut, a manufacturer that sold infant food, was fined $2.2 million in 1987 for selling apple juice that was tainted with sugar, water, and artificial flavoring in violation of the Federal Food, Drug, and Cosmetic Act (Pal & Mahinder, 2020).
- In 1997, it was discovered that ConAgra Foods, an American packaged food company, had sprayed water on grains to increase their weight and value (Tsagkaris et al., 2017).
- It was found in 2007 that wheat gluten had been infused with melamine, a white, crystalline substance that is produced by boiling cyanamide and is used to make plastics, to offer tests with an artificially high protein level. This was found in the food supply networks for humans and animals, and it affected several US pet food firms. The gluten contaminated with melamine was produced by a Chinese manufacturer. In 2008, baby formula that included melamine was manufactured, which led to the deaths of at least six children.

Reports state that after ingesting the related product, several children were admitted to the hospital. Research has shown that China's milk supply is contaminated with melamine (Gossner et al., 2009).

- Seven food items are the main targets of intentional or commercially driven food adulteration (EMA), according to an analysis of data on food adulteration from the US Pharmacopeial Convention (USP) (Everstine et al., 2013). The Food Safety Standard Authority of India (FSSAI) research in 2012 discovered an absence of cow or buffalo milk in the production of 100% synthetic milk. Synthetic milk is a product that is sold alongside real milk and is made with detergents, urea, cheap oil, and other substances (Poonia et al., 2017). There have been claims that formalin is used to make fish appear fresh and that calcium carbide is used to ripen fruits, especially mangoes (Tsagkaris et al., 2017). These adulterants are recognized as hazardous; several have been identified as carcinogens and are therefore prohibited from use in food. Only 31.5% of randomly selected samples from 33 Indian states in 1991 satisfied the FSSAI requirements; the remaining 68.4% did not (Tsagkaris et al., 2017).

These examples above demonstrate how adulteration introduces harmful chemicals into food, compromising its safety and quality to gain profit. Enhancing nitrogen content—the goal of protein content testing—was the aim in reference to the case of melamine addition in food products. Profit took precedence above the harmful substance's potential ability to impact the health of consumers in this dishonest behavior (Gossner et al., 2009).

Food adulteration is a threat that does not exempt Ghana. Contaminated food items include groundnut paste, pepper powder, and milled melon seeds (Agushie) (Banti, 2020). On the other hand, there are no actual statistics about the kinds of food products that are faked in Ghana. This

could be brought on by inadequate equipment and methods for laboratory testing, as well as inadequate funding for regulatory bodies. One important food item that gets tainted in Ghana is palm oil. It has been documented to contain Sudan dyes I, II, III, and IV (Andoh et al., 2020). Through its Rapid Alert System for Food and Feed Safety (RASFF), the European Union (EU) sent Ghana 57 and 35 alerts in 2004 and 2005, respectively, for contamination of palm oil containing Sudan IV dyes. Food and Drugs Authority (Ghana) created procedures to control exportation in response to these warnings to protect the developing palm oil industry and prevent a possible ban on palm oil shipments to the European Union (Ofosu-Budu & Sarpong, 2013). With technical support from the EU, the FDA enforces a step that guarantees every cargo of palm oil on export to Europe is tested for the presence of these dyes and a documentation of conformity given before its departure. On the other hand, Ghanaians' local consumption of palm oil remained unaffected by the FDA regulation. In order to maintain public health and safety, it is imperative that the Greater Accra Region, Ghana's commercial hub, reduce the frequency of adulteration of palm oil with Sudan dyes (Ofosu-Budu & Sarpong, 2013; Gizaw, 2019).

In Ghana's Ashanti, Central, Eastern, Greater Accra, Northern, and Volta Regions, the FDA conducted an inspection of palm oil contamination using Sudan IV color in 2007 (Amoako-Mensah, 2016). Following the survey's discovery that palm oil samples were contaminated with Sudan IV dye, regulatory action was taken. Ghana, however, kept receiving notifications after the survey and any corrective measures that were put in place. The frequency of Sudan IV dye adulteration was demonstrated by the eleven (11) food safety notifications the RASFF received between 2007 and 2010 (Amoako-Mensah, 2016). West Africa, which was once recognized for producing genuine palm oil and topping the global production list, is reportedly facing a significant problem with palm oil adulteration using Sudan dyes (Okogeri & Otika, 2011).

A study by the Eastern Regional Secretariat of the Food and Drugs Authority (FDA) found that over 42% of palm oil samples from local markets tested positive for carcinogenic Sudan dyes (Business Day Ghana, 2015). In the United States when Genualdi et al. (2016) evaluated the contamination of palm oil with Sudan dye I–IV, they found that samples of palm oil produced in West Africa included Sudan colors, and that one sample from the Ivory Coast was highly contaminated. Amoako-Mensah (2016) also found Sudan dyes (I–IV) in samples of palm oil they took from Ghanaian local markets. The authorities tasked with combatting Sudan dye contamination in the palm oil-producing countries have not been quick to apprehend the perpetrators. For instance, since 2005, Ghana has been dealing with the problem of adulteration of Sudanese dyes in palm oil. Most importantly, authorities have not done a good job of gathering information about current adulteration of palm oil before it reaches consumers. This could be because the wet chemistry procedures that were used were expensive and time-consuming (Essuman et al., 2022). These methods need sophisticated laboratory procedures, careful sample preparation, and personnel with the necessary training. Moreover, only a small number of samples can be analyzed at a considerable expense due to the gradual and laborious nature of the detection techniques employed, suggesting that the market's safety of palm oil cannot be guaranteed (Amoako-Mensah 2016).

2.8 FOOD ADULTERATION IN PALM OIL, AN ASPECT OF FOOD FRAUD

Due to the globalization and complexity of the food supply chain, new and serious risks have emerged (Spink, 2010). Standards-setting organizations, governments, and the food business are all interested in food fraud that is committed by food growers, processors, distributors, and retailers for financial gain Food fraud is defined as the deliberate act of substituting, adding, altering, or making false or inaccurate claims about a product to gain financial benefit, according to research

conducted by the US Department of Homeland Security (Spink, 2012). According to the US Pharmacopeia Expert on Food Ingredients, adulterating food ingredients for economic gain is considered a fraudulent practice because it typically entails the omission or substitution of genuine substances or the addition of imitation substances without providing the buyer or customer with sufficient information to benefit the vendor financially (Everstine et al., 2013). Other names that are frequently used to classify food fraud include food counterfeiting, or economic adulteration (Alim-Un-Nisa & Akhlaq, 2015).

According to Okogeri. (2013), food fraud is often measured in monetary terms, which is not as significant for the conventional food protection intervention and response system. Contrarily, food fraud alters the nature and integrity of a particular ingredient by substituting it with another or changing its chemical, physical, or biological characteristics (Choudhary et al., 2020). Such seeks to circumvent established quality assurance (QA) and quality control (QC) systems, including Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP) protocols, through illegal and deceptive means. The only person who is aware of how the food raw material has been changed in a food fraud case is the perpetrator, but they lack the knowledge and experience to determine whether the altered raw material poses a health risk to the public or has any toxicological effects (Moore et al, 2011).

An increased awareness and concern have emerged about food theft which may pose a greater threat to the food supply than more established hazards like contamination along the value chain. This is because uncommon adulterants are commonly used in such conduct. According to Gossner et al. (2009), melamine was not regarded as a possible adulterant or contaminant in the food supply chain before 2007, thereby excluded from QA and QC analytical techniques. Moreover, the

principles of contemporary food safety procedures are not intended to detect the various potential adulterants in the supply chain. (Spink, 2011; Moore et al, 2011).

Owing to their extensive range of applications, in food products and, for the most part, their lack of functional properties that facilitate clear distinction from other similar raw materials or adulterants along the supply chain, food additives and raw materials present a significant risk (Pal & Mahinder, 2020). Organoleptically, glycerin is a sweet, transparent, colorless, viscous liquid that is hard to distinguish from other liquid syrups that share similar characteristics, including diethylene glycol, which has been fatally substituted for glycerin in the past (Amoako-Mensah, 2016).

The three main components of a traditional food safety system are reaction, prevention and intervention, with a cycle of operation that ends with prevention (Acheson, 2007). According to some, identifying the specific risk at the intervention stage should be the first step in preventing food fraud (Spink, 2011).

2.8.1 FOOD ADDITIVES

Chemical substances known as food additives are added to processed meals to enhance or maintain certain quality attributes, including texture, flavor, taste, and physical characteristics (Abdulmumeen et al., 2012). Additionally, additives are utilized to prolong the validity period of processed foods and prevent spoilage. Among the several kinds of food additives include antioxidants, preservatives, emulsifiers or stabilizers, acid regulators, leavening agents, taste enhancers, artificial sweeteners, bulking agents, anti-caking agents, and glazing agents (Adhikari, 2021). Additives are not regarded as contaminants if they are utilized in accordance with legal

restrictions and Good Manufacturing Practices (GMP). The additive is considered a contaminant and may incur a health risk to consumers when the permitted limit is exceeded (Sciuto et al., 2017). In cases when an outspread of foodborne illness is traced to a specific component, then food additives involved in the outbreak may be deemed adulterants (Oti, 2021). Consumers are seriously put at risk for food safety when unapproved and illegal additives are used often and with impunity. Regulations from the European Union (EU) declare that every food additive needs to be authorized and included in the EU's positive list of approved food additives, which has usage guidelines based on technical necessity, safety assessment, and the idea that using them shouldn't deceive customers (Adhikari, 2021).

Azo dyes are prohibited from being used in food products.; this is stated in both the EU's positive additive list (Annex II of Regulation (EC) No 1333/2008) and the Codex General Standard for Food Additives (EC, 2015b) (Mohamed et al., 2016). The General Standard for Food Additives is one example of a Codex Standard that is accepted and used when there are no national standards; Ghana, as a member of the Codex Alimentarius Commission, ensures conformity of its national standards with the commission's standards.

2.9 FOOD PROTECTION

Food fraud is driven by money (Everstine et al., 2013). Food defense is considered food fraud, with the purpose of causing public harm or threatening consumers. Food fraud perpetrators are mostly players in the supply chain. Terrorists and foreigners are the main groups who commit crimes against food defence methods because they are not often involved in the food manufacturing process. The public health impact of food fraud is normally small, although errors and unforeseen health implications do occur (Johnson, 2014). Table 2.1 summarizes the risk associated with public health which is connected to several forms of dietary risk.

Table 2. 1: Public health risk linked with several forms of dietary risk

TYPE OF RISK	CASE STUDY	CAUSE AND MOTIVATION	PRIMARY EFFECT	RISK TO PUBLIC HEALTH	REMOTE EFFECT
Food Quality	Accidental fruit bruising	Mishandling	Additional contamination is possible.	Food safety is not guaranteed, but it is attainable.	Food safety incident or brand equity
Food Fraud	International melamine contamination of milk	Increased Profit margins.	Toxic poisonings	Food safety	Fear among the general public and the possibility of decreased industry-wide prices
Food Safety	Unintentional E. coli contamination of raw veggies	Protection and control are restricted during harvesting and processing.	Disease or mortality	Food safety	Industry damage, recall costs Fear and panic among customers
Food Defense	Intentional nicotine contamination of ground beef	Revenge on the management	Poisonings that are not fatal	Food defense	Product contamination, industry harm, recall costs, and public panic

2.10.1 FOOD FRAUD CATEGORIES

A survey of 660 scientific, journalistic, and other publicly available sources in 2012 yielded 1305 recognized database entries for food component fraud (Johnson, 2014). For each entry in the database, the type of food fraud was classified into three (3) major forms: addition, replacement, and removal (Moore et al., 2011).

Table 2. 2: Types of food fraud

TYPE OF FRAUD	DEFINITION	SUBTYPES INCLUDED	EXAMPLES
Replacement	A food product or valuable authentic substance is substituted entirely or partially with a cheaper alternative without the consumers' knowledge.	Adulterant dilution, addition, or extension of a genuine substance False statement of geographical species, botanical or varietal origin false information about the source of raw material or the process of production utilized to make an ingredient. falsifying a claim of origin to evade paying customs or taxes	To artificially raise the apparent protein level in milk as measured by total nitrogen, melamine was injected. To artificially increase the end juice product's titratable acidity, lemon juice is mixed with water and citric acid. Adding more water (ice) to frozen fish Cow's milk can be substituted for sheep or goat's milk. Common wheat is exchanged for durum wheat. Natural, botanically derived vanillin is replaced by synthetic vanillin.
Addition	Without the consumers' knowledge, the inclusion of non-authentic substances to disguise poor quality ingredients.	Color improvement Enhancement of flavor	Sudan dyes used to inferior paprika to enhance its color Cheap pomegranate juice has an astringent flavor that is covered up with sugar.
Removal	removing a real and valuable element without the purchaser's knowledge	NA	Sale of defatted paprika devoid of important tasting ingredients

Johnson. (2014) states that replacement accounts for 95% of food fraud, while addition and removal account for less than 5% and 1%, respectively. When genuine material is substituted for a cheaper version for the seller's profit without the purchaser's awareness, this is referred to as replacement. One instance of replacement fraud is the complete or partial substitution of hazelnut oil as a food component partially in place of olive oil (Moore et al., 2011).

Of the three, replacement food fraud is the most widespread since existing analytical testing processes are best adapted to detect this kind of adulteration. Adulteration can be detected using two different analytical approaches. The first method determines if acknowledged adulterants are present or not (Lohumi et al., 2015). By nature, it is unable to identify unknown adulterants; it only looks for the adulterant of interest. The technique works well for finding adulterants even in very small amounts. The second technique verifies a food's or ingredient's authenticity, purity, and identification (Goggin & Murphy, 2018). This is referred to as compendium strategy. The identification of the adulterant(s) is not necessary in this technique to demonstrate adulteration. However, rather than lower levels of adulteration as with the previous strategy, rather significant amounts of adulteration are found (Lohumi et al., 2015).

2.10.2 Adulteration of Palm Oil in Ghana and West Africa: A Regional Context

While the adulteration of foodstuffs is a global challenge, its manifestations are highly region-specific, driven by local economic pressures, regulatory environments, and agricultural practices. In West Africa, and particularly in Ghana, the adulteration of palm oil with Sudan dyes has been a persistent and well-documented public health issue, though a significant gap remains in understanding its current prevalence in the post-ban era.

The historical context of this problem is crucial. The European Union's Rapid Alert System for Food and Feed (RASFF) issued numerous notifications between 2004 and 2005 concerning Sudan IV contamination in palm oil from Ghana, triggering the implementation of the FDA's export testing regime (Amoako-Mensah, 2016; Essuman et al., 2022). This regulatory response was primarily designed to protect international trade. However, subsequent studies have consistently indicated that the domestic market remained vulnerable. A significant study by the Eastern Regional Secretariat of the FDA found that over 42% of palm oil samples from local markets tested positive for carcinogenic Sudan dyes (as reported in Business Day Ghana, 2015).

Research by Amoako-Mensah (2016) further confirmed the presence of Sudan I–IV in samples collected from Ghanaian markets, highlighting the ongoing nature of the threat and the ineffectiveness of a policy focused solely on exports. This aligns with broader regional concerns. Studies from neighbouring countries echo this trend; for instance, research from Nigeria has also identified Sudan dye adulteration as a common fraudulent practice, often attributed to the desire to mimic the deep red colour of high-quality oil and command a higher price (Okogeri & Otika, 2011; Andoh et al., 2020).

The motivations for adulteration within the Ghanaian context are deeply economic. Small-scale processors and retailers, operating within a highly competitive market, may resort to using inexpensive industrial dyes like Sudan IV to mask the inferior quality of poorly processed or diluted oil, thereby appealing to consumer preferences for a vibrant red colour associated with freshness and high carotenoid content (Teye et al., 2019; MacArthur et al., 2021). This economic incentive exists within a regulatory environment where surveillance of the vast and fragmented domestic supply chain remains a formidable challenge.

Despite this established history, a clear gap exists. Most existing studies are several years old, and there is a lack of recent, comprehensive data mapping the *current* prevalence and concentration levels of Sudan dyes I-IV across major markets in Greater Accra following the FDA's public reiteration of the ban. This study seeks to fill this gap by providing an updated, empirical assessment of the situation, determining whether regulatory messaging and interventions have been effective in protecting local consumers or if the significant public health risk previously identified persists unabated.

2.10.3 THEORETICAL/CONCEPTUAL FRAMEWORK

This study is guided by the Food Fraud Mitigation Framework, as conceptualized by food safety experts (Spink & Moyer, 2011; Everstine et al., 2013). This framework posits that economically motivated adulteration (EMA) occurs when two factors converge: motivation (the potential for economic gain) and opportunity (a weakness in the system that makes the fraud possible).

- Motivation: In this context, the motivation is economic. Adulterating palm oil with inexpensive Sudan dyes enhances its visual appeal (mimicking the natural red colour of high-quality, carotenoid-rich oil), allowing sellers to command a higher price for an inferior or fraudulent product.
- Opportunity: The primary opportunity for this fraud stems from an enforcement gap. The Food and Drugs Authority (FDA) of Ghana has established a prohibition on Sudan dyes and implemented a rigorous testing regime for the *export* market to comply with international regulations (Food and Drugs Authority, 2022). However, this study operates on the premise that a corresponding level of enforcement is absent in

the *domestic* market. This regulatory asymmetry creates a significant opportunity for adulterators to target products destined for local consumption with a low perceived risk of detection or penalty.

- Control Measures: The framework's third element involves interventions designed to reduce opportunity. This research directly assesses the effectiveness of the existing control measure—the FDA ban—by measuring its outcome (the prevalence of dyes) in the domestic market.

Therefore, this study utilizes this framework to investigate the hypothesis that the opportunity for fraud created by the domestic enforcement gap has led to a high prevalence of Sudan dye adulteration in palm oil sold in Greater Accra markets. The findings will be interpreted as a measure of the current control measures' failure and will inform recommendations for stronger, more holistic risk mitigation strategies.

2.11 ANALYTICAL METHODS FOR DETECTION OF FOOD ADULTERATION

The most essential defensive mechanism in identifying food ingredient adulteration is the employment of analytical detection technologies (Banti, 2020). There are several ways employed, all of which are instrumental in nature. Some include:

- Polymerase chain reaction
- Infrared spectroscopy
- Enzyme-linked immunosorbent assay
- Gas chromatography
- Capillary electrophoresis
- High Performance Liquid Chromatography
- Thin-layer chromatography
- Near infrared spectroscopy
- Raman spectroscopy
- Nuclear magnetic resonance spectroscopy
- Mid-infrared spectroscopy
- Isotope ratio mass spectrometry
- Differential scanning calorimetry (Rebane et al., 2010).
- High-performance anion exchange Chromatography

2.11.1 Liquid Chromatography (LC) coupled to Mass Spectrometry

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) is a powerful analytical technique that combines the physical separation capabilities of liquid chromatography (LC) with the mass analysis capabilities of mass spectrometry (MS). This method is widely used for the detection, quantification, and characterization of complex mixtures of compounds, particularly in the fields of pharmaceuticals, environmental analysis, and proteomics. (Chen & White, 2016)

Liquid Chromatography (LC)

Liquid chromatography is the initial step in LC-MS/MS, where a liquid sample is separated into its components influenced by their interaction with the stationary phase (usually a column packed with a solid material) and the mobile phase (a liquid that flows through the column). The principle of separation process is centered on different characteristics of the molecules, such as polarity, size, and charge. As the sample moves through the column, constituents with a higher propensity for binding to the stationary phase migrate slower than substances with a lower affinity leading to separation. (Chen & White, 2016)

Mass Spectrometry (MS)

After separation by LC, the sample enters the mass spectrometer, where it undergoes ionization. Atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) are the two most widely used ionization methods in LC-MS/MS. These methods convert the molecules into charged particles (ions) that can be detected by the mass spectrometer (Glish & Vachet, 2003).

Mass Spectrometry (MS/MS)

Mass spectrometry consists of multiple rounds of measuring masses. In LC-MS/MS, the first mass analyzer (MS1) selects ions which have a definite mass-to-charge ratio (m/e), followed by subsequent fragmentation in a collision cell. The resulting fragments generated are interpreted by a second mass analyzer (MS2), providing elaborate and precise structural information about the molecule. The dual-step process of separate mass analysis enables accurate identification and measurement of compounds, even in complex mixtures (Saito et al., 2018).

Applications

LC-MS/MS is commonly used in various fields:

- Pharmaceuticals: To identify and measure the amount of drug metabolites in biological samples.

- Proteomics: For protein identification and quantification, as well as post-translational modification analysis.

- Environmental Analysis: For detecting pollutants and other chemicals in environmental samples.

- Clinical Diagnostics: For the quantification of biomarkers and drugs in biological fluids.

Advantages

-High Sensitivity and Specificity: LC-MS/MS can detect low concentrations of compounds with high specificity.

- Structural Information: Provides detailed structural information about the analytes.

-Versatility: Can analyze a broad spectrum of compounds, from small molecules to large biomolecules.

Challenges

- Complexity and Cost: The equipment is expensive and requires skilled operators.

- Sample Preparation: Often requires extensive sample preparation to remove impurities that can interfere with analysis.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 STUDY AREA

Greater Accra Region was the primary study site. Its major districts sampled are ten in number namely: Accra Metropolitan Assembly (Agbogbloshie), La Nkwantanang District (Madina), Ashaiman Municipal District (Ashaiman), Dangme West District (Dodowa), Ada East (Ada Foah), Ledzokuku Krowor Municipal (Nungua), Ga-East Municipal District (Kwabenya), Lower Manya Krobo (Odumase), Tema Municipal District (Tema) and Ga South Municipal District (Galilea). Palm oil samples were collected from eleven local markets respectively. A part of Ghana's total geographical area approximately 3,245 square kilometres, is made up of the Greater Accra Region, according to the country's statistical service. Due to immigration and the country's rapid increase in population, this region, which accounts for 13% of the total population, has the greatest population density in the nation—1,236 persons per square kilometre. Urban centres like Accra and Tema serve as major distribution nodes for palm oil from both the smallholder and industrial sectors. Smallholder producers transport crude palm oil to these cities for sale, and refined oil also enters the market via industrial processors. This is documented in studies of Ghana's palm oil marketing systems. For example, FAO's value-chain study shows marketers from Pramkese selling palm oil in Accra and Tema (Ofosu-Budu & Sarpong, 2013). Thus, these districts were chosen due to their notable role as primary hubs for the distribution of crude palm oil, which is extensively transported from these regions to markets throughout the country.

3.2 SAMPLE COLLECTION

Palm oil samples had been sourced out of local markets across the study areas mentioned above. The samples were procured using sterile universal aseptic bags, and stringent measures were implemented to prevent contamination throughout the collection process and subsequent handling. The samples from each market were stored under ambient temperature and transported to the laboratory for Sudan dye analysis.

Table 3. 1: Sampling Locations

MARKET NAME	LOCATION	NUMBER OF SAMPLES
Ashaiman Market	Ashaiman Municipal	10
Agbogbloshie	Accra Metropolitan	10
Tema Comm. 1 Market	Tema Municipal	10
Kwabenya Market	Ga East	10
Ada Afoah Market	Dangme East	10
Dodowa Market	Dangme West	10
Galilea Market	Ga South	10
Odumase Market	Ga West	10
Madina Market	La Nkwantanang	10
Nungua Market	Ledzokuku-Krowor	14

3.2.1 SAMPLING QUESTIONNAIRE

During the sample collection, with the aid of questionnaires, palm oil retailers were asked to indicate the suppliers or points of purchase of the palm oil they sold, as well as the sources of any Sudan dyes involved, with the aim of pinpointing where in the value chain the adulteration occurs. The questions in the questionnaire are designed to reveal the stages in the value chain at which adulteration takes place.

3.3 MATERIALS & REAGENTS

All reagents were of analytical grade:

Chemical compounds studied in this thesis were Sudan I, II, III, and IV. Solid standards of the following were obtained from Sigma-Aldrich (St. Louis, MO): Sudan I, 1-(phenylazo)-2-naphthol (purity 96%); Sudan II, 1-[(2,4-dimethylphenylazo]-2-naphthol (purity 96%); Sudan III, 1-(4-phenylazophenylazo)-2-naphthol (purity 96%); and Sudan IV, o-tolyazo-o-tolylazo-2-naphthol (purity 96%). All chemicals and solvents used for the analysis, such as n-hexane, ethyl acetate, acetone, acetonitrile, and formic acid were supplied by Sigma-Aldrich; the calibration standard (Sudan dye), used for Mass spectrometry was purchased from Agilent Technologies (Santa Clara, CA). All solvents were MS grade (purity 99.9%) and solid standard high-performance liquid chromatography tested. All glassware, containers, and storage bottles were subjected to an acid-washing protocol prior to analysis to remove residual inorganic contaminants and ensure reliable, interference-free results for trace-level analysis (APHA et al., 2017; US EPA, 1996).

3.4 CALIBRATION STANDARD PREPARATION

Individual stock standard solutions (1 mg/L and 10 mg/L) of Sudan I, II, III, and IV were prepared by dissolving the appropriate mass of reference standard, adjusted for purity, in ethyl acetate. A seven-point calibration curve was prepared in palm oil matrix at concentrations of 10, 20, 50, 100, 200, 500, and 1000 µg/kg (ppb). This range encompasses the EU's maximum permissible residue level of 500-1000 µg/kg for Sudan dyes. Method recovery was evaluated at two spiking levels, 20 ppb and 50 ppb, by comparing the measured concentrations with the known fortification levels.

The required volume of the standard to be aliquoted from the (1 ppm and 10 ppm) stock solution to reach each fortification level was calculated with the relation:

$$Volume\ of\ standard = \frac{fortification\ level \times Weight\ of\ sample\ to\ be\ fortified}{standard\ Concentration}$$

3.4.1 SAMPLE FORTIFICATION

To validate the accuracy of the analytical method, recovery experiments were performed by fortifying blank palm oil samples of known endogenous Sudan dye concentrations. Samples (5 g each) were fortified with appropriate volumes of the standard solutions to achieve final concentrations of 20 µg/kg and 50 µg/kg for each Sudan dye (I-IV).

An aliquot of 2 mL of acetonitrile was added to each sample to facilitate the extraction process. The corresponding volume of acetonitrile added was adjusted to account for the volume of the standard already added, using the relation: [2 mL acetonitrile - volume of standard added]. The fortified samples were then vortexed and proceeded through the extraction protocol detailed in Section 3.5. Linearity was checked by calculating the linear correlation coefficient (R^2).

3.5 SAMPLE EXTRACTION PROCEDURE

The extraction of Sudan dyes from palm oil samples was performed using a solvent extraction technique with acetonitrile. The procedure was applied to all collected samples, calibration standards, and blank controls as follows:

1. **Weighing:** Exactly 5.0 g of each homogenized palm oil sample was accurately weighed into a 15 mL centrifuge tube.
2. **Solvent Addition and Extraction:** 2.0 mL of acetonitrile was added to the tube. The mixture was vigorously vortexed for 2 minutes to ensure complete homogenization and facilitate the partitioning of the Sudan dyes into the acetonitrile phase.
3. **Phase Separation:** The tubes were then centrifuged at 2160 rpm for 15 minutes to achieve complete phase separation. This resulted in a lower palm oil layer and an upper acetonitrile layer containing the extracted analytes.
4. **Collection:** Following centrifugation, 1.0 mL of the upper acetonitrile supernatant (extract) was carefully collected using a micropipette to avoid disturbing the lower oil layer.
5. **Storage and Analysis:** The extract was transferred into a 2 mL amber glass vial to prevent photodegradation of the analytes. The vials were sealed and stored at 4°C prior to analysis by LC-MS/MS.

To ensure analytical accuracy and compensate for matrix effects, matrix-matched calibration standards (prepared as in Section 3.4) and procedural blanks (prepared without palm oil) were processed simultaneously with each batch of samples using the identical extraction protocol.

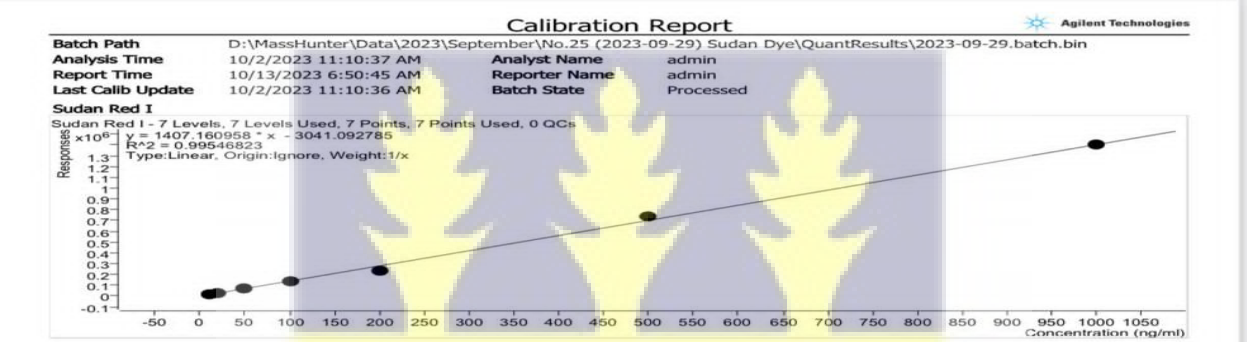
CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 RESULTS

Linearity and accuracy

To verify method performance and ensure accuracy of results, a calibration using Sudan dye standards in samples of known concentration was used. Below is the diagram showing the result of the calibration and the measure of linearity.



Calibration STD Path	Cal Type	Level	Enabled	Resp.	Exp. Conc	Resp. Factor
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\10ppb sudan.d	Calibration	1	x	12275	10.0000	1227.5004
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\20ppb sudan.d	Calibration	2	x	24510	20.0000	1225.4903
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\50ppb sudan.d	Calibration	3	x	69253	50.0000	1385.0548
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\100ppb sudan.d	Calibration	4	x	139964	100.0000	1399.6406
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\200ppb sudan.d	Calibration	5	x	230850	200.0000	1154.2503
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\1000ppb sudan.d	Calibration	6	x	739142	500.0000	1478.2832
D:\MassHunter\Data\2023\September\No.25 (2023-09-29) Sudan Dye\500ppb sudan.d	Calibration	7	x	1408182	1000.0000	1408.1817

Figure 4. 1: A diagram showing the calibration graph derived from fortified palm oil samples at seven fortification levels.

In the figure above the linear correlation coefficient R^2 is 0.9954. The equation of the line

y = 1407.160958x – 30401.092785 can be used for further extrapolation of concentration of Sudan I dyes in samples. Identical graphs were generated for the same application on the other Sudan dyes.

Recoveries were set at 20 ppb and 50 ppb for known samples spiked with Sudan standards. The table below shows the results of the recovery for each dye at the set concentrations.

Table 4. 1: Mean percentage recovery (n=4) for each Sudan dye at 20 ppb and 50 ppb fortification levels.

Compound Name	Mean Recovery at 20 ppb (%)	Mean Recovery at 50 ppb (%)
Sudan I	118.0	123.6
Sudan II	105.1	104.3
Sudan III	98.6	83.8
Sudan IV	88.1	73.2

The method's accuracy was assessed by calculating the mean percentage recovery for each analyte, as detailed in Table 4.1. Recoveries for Sudan I and II were satisfactory, exceeding 100% at both fortification levels. However, recoveries for Sudan III and IV were lower, particularly at the 50 ppb level, indicating a potential matrix effect or loss of these specific analytes during extraction. The wide range of recoveries across the different dyes underscores the importance of evaluating each compound individually rather than as a group.

Quantification of Sudan dyes in palm oil samples

The detection of Sudan dyes (I to IV) in 104 palm oil samples was recorded in mg/kg. The various concentrations detected for each dye in each sample were recorded and shown separately. Below are the tables representing the various concentrations of Sudan dyes (I to IV) detected in each sample (1 to 104).

Table 4. 1: Concentrations of Sudan I to IV dyes detected in palm oil samples from Agbogbloshie

Sample number	Dye Concentration (mg/kg) (Agbogbloshie)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	140.78
2	ND	ND	ND	139.30
3	ND	ND	ND	139.91
4	ND	ND	ND	141.95
5	ND	ND	ND	112.42
6	ND	ND	ND	ND
7	ND	ND	ND	ND
8	ND	ND	ND	126.03
9	ND	ND	147.79	15,638.95
10	ND	ND	64.23	11,421

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, eight (8) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 15,638mg/kg and 112.42 respectively. Sudan III was present in two (2) samples, and two (2) samples had no dyes in them.

Table 4. 2: Concentrations of Sudan I to IV dyes detected in palm oil samples from Madina.

Sample number	Dye Concentration (mg/kg) (Madina)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	72.63
2	ND	ND	ND	68.08
3	ND	ND	ND	64.77
4	ND	ND	ND	64.88
5	ND	ND	ND	63.34
6	ND	ND	ND	111.29
7	ND	ND	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	ND
10	ND	ND	ND	ND

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, six (6) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 111.29 mg/kg and 63.34 respectively. Sudan I to III were absent.

Table 4. 3: Concentrations of Sudan I to IV dyes detected in palm oil samples from Ashaiman.

Sample number	Dye Concentration (mg/kg) (Ashaiman)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	ND
2	ND	ND	ND	ND
2	ND	ND	ND	ND
4	ND	ND	ND	ND
5	ND	ND	ND	ND
6	ND	ND	ND	121.20
7	ND	ND	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	110.53
10	ND	ND	ND	109.62

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, three (3) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 109.62 mg/kg and 121.20mg/kg respectively. Sudan I to III were absent.

Table 4. 4: Concentrations of Sudan I to IV dyes detected in palm oil samples from Tema Comm. 1 market.

Sample number	Dye Concentration (mg/kg) (Tema Comm. 1 market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	72.22
2	ND	ND	ND	67.61
2	ND	ND	ND	63.57
4	ND	ND	ND	65.22
5	ND	ND	ND	64.87
6	ND	ND	ND	112.46
7	ND	ND	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	ND
10	ND	ND	ND	ND

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, six (6) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 112.46 mg/kg and 63.57mg/kg respectively. Sudan I to III were absent.

Table 4.5: Concentrations of Sudan I to IV dyes detected in palm oil samples from Nungua

Sample number	Dye Concentration (mg/kg) (Nungua Market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	0.042
2	ND	ND	ND	0.04
3	ND	ND	ND	ND
4	ND	ND	ND	ND
5	ND	ND	ND	ND
6	ND	ND	ND	ND
7	ND	ND	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	ND
10	ND	ND	ND	1.82
11	0.4	ND	0.1	1.85
12	0.4	ND	0.1	7.69
13	ND	ND	ND	7.18
14	ND	ND	ND	ND

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, six (6) out of 14 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 0.04mg/kg and 7.69mg/kg respectively. Sudan II was absent.

Table 4.7: Concentrations of Sudan I to IV dyes detected in palm oil samples from Ada Foah.

Sample number	Dye Concentration (mg/kg) (Ada Foah Market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	ND
2	ND	ND	ND	2,631.55
3	ND	ND	ND	2,273.45
4	ND	ND	ND	ND
5	ND	ND	ND	2,166.24
6	ND	ND	14.57	1,980.57
7	67.06	ND	12.13	9,092.48
8	66.39	ND	11.3	10,231.33
9	88.7	ND	11.2	11,346.55
10	ND	ND	ND	213.23

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, eight (8) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 11,346.55mg/kg and 213.23mg/kg respectively.

Table 4.8: Concentrations of Sudan I to IV dyes detected in palm oil samples from Dodowa.

Sample number	Dye Concentration (mg/kg) (Dodowa Market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	17.15	9,506
2	ND	ND	16.79	9,250.49
3	ND	ND	18.85	10,120.26
4	ND	ND	16.98	10,290.80
5	ND	ND	18.68	10,144.79
6	ND	ND	ND	11,629.17
7	ND	ND	ND	ND
8	55.0	ND	ND	6,788.87
9	ND	ND	ND	7,200
10	ND	ND	ND	3,854

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, nine (9) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 11,629.17 mg/kg and 3,854mg/kg respectively. Sudan III was present in five (5) samples, Sudan I and II was present in one sample each and one (1) sample had no dyes in them.

Table 4.9: Concentrations of Sudan I to IV dyes detected in palm oil samples from Galilea market.

Sample number	Dye Concentration (mg/kg) (Galilea Market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	ND
2	ND	ND	ND	2,358
3	ND	ND	ND	5,265
4	ND	ND	ND	11,582
5	ND	ND	ND	4,226
6	ND	ND	ND	0
7	ND	ND	ND	5,255
8	ND	ND	ND	559
9	ND	ND	ND	ND
10	ND	ND	ND	2,565

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, seven (7) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 11,582mg/kg and 559mg/kg respectively. Sudan I to III were absent.

Table 4.10: Concentrations of Sudan I to IV dyes detected in palm oil samples from Kwabenya market.

Sample number	Dye Concentration (mg/kg) (Kwabenya Market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	ND
2	ND	ND	ND	ND
3	ND	ND	ND	3,559
4	ND	ND	ND	8,366
5	ND	ND	ND	7,666
6	ND	ND	ND	ND
7	ND	ND	ND	3,696
8	ND	ND	ND	ND
9	ND	ND	ND	2,452
10	ND	ND	ND	652

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, six (6) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 8,366 mg/kg and 652 mg/kg respectively. Sudan I to III.

Table 4.11: Concentrations of Sudan I to IV dyes detected in palm oil samples from Odumase market.

Sample number	Dye Concentration (mg/kg) (Odumase Market)			
	Sudan I	Sudan II	Sudan III	Sudan IV
1	ND	ND	ND	658
2	ND	ND	ND	ND
3	ND	ND	ND	ND
4	ND	ND	ND	11,524
5	ND	ND	ND	5,462
6	ND	ND	ND	2,265
7	ND	ND	ND	ND
8	ND	ND	ND	ND
9	ND	ND	ND	2,266
10	ND	ND	ND	ND

ND: Not Detected

In the screening of Sudan I to IV dye in the palm oil samples, five (5) out of 10 samples indicated the presence of the Sudan dye IV with highest and lowest concentrations detected at 11,524 mg/kg and 658 respectively.

A summary of the data of Sudan dye concentrations

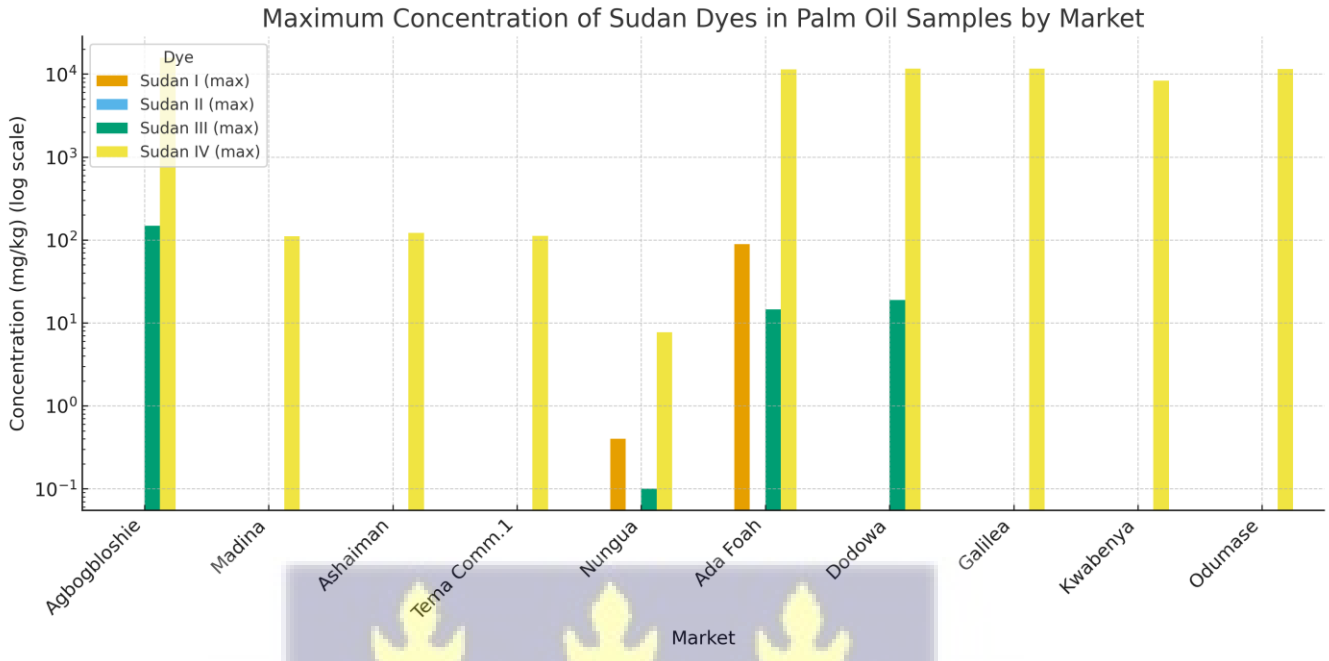


Figure 4. 2 Variation in Adulteration Severity Across Markets

The maximum concentration of Sudan dyes detected in palm oil samples varied considerably across the different markets surveyed in the Greater Accra Region. Figure 4.2 illustrates this variation, plotting the maximum concentration (mg/kg) for each market on a logarithmic scale due to the wide range of values.

Markets including Agbogbloshie, Ada Foah, and Dodowa recorded the highest maximum concentrations of Sudan IV, with values exceeding 10,000 mg/kg. Conversely, markets such as Madina, Ashaiman, and Tema Community 1 exhibited maximum concentrations that were several orders of magnitude lower. The markets of Nungua, Galilea, Kwabenya, and Odumase showed intermediate maximum concentrations. A complete dataset of concentrations for all samples is provided in Tables 4.2 through 4.11.

Percentage Occurrence of Sudan dyes

Table 4.12: summarizes the prevalence and concentration range of each Sudan dye across all 104 samples. Sudan IV was the most frequently detected adulterant, found in 61.5% (64 of 104) of the samples. It was also found at the highest concentrations, with a maximum of 15,638.95 mg/kg, far exceeding international safety limits. The mean concentration for Sudan IV was 3,565.73 mg/kg (± 4,308.78 SD), with a median of 2,073.40 mg/kg, indicating a highly variable and right-skewed distribution. Sudan III was detected in 12.5% of samples (n=13), with a mean concentration of 26.91 mg/kg (± 39.51 SD). Sudan I was present in 5.8% of samples (n=6), with a mean concentration of 46.32 mg/kg (± 37.21 SD). Sudan II was not detected (ND) in any of the samples analysed.

Table 4.12: Summary of Sudan dye prevalence and concentration in palm oil samples (n=104)

Dye	n	Occurrence (%)	Mean (mg/kg)	Standard Deviation (SD)	Median (mg/kg)	Min (mg/kg)	Max (mg/kg)
Sudan I	6	5.8%	46.32	± 37.21	60.70	0.40	88.70
Sudan II	ND	0%	ND	ND	ND	ND	ND
Sudan III	13	12.5%	26.91	± 39.51	16.76	0.10	147.79
Sudan IV	64	61.5%	3565.73	± 4,308.78	2073.40	0.040	15,638.95

ND: Not Detected.

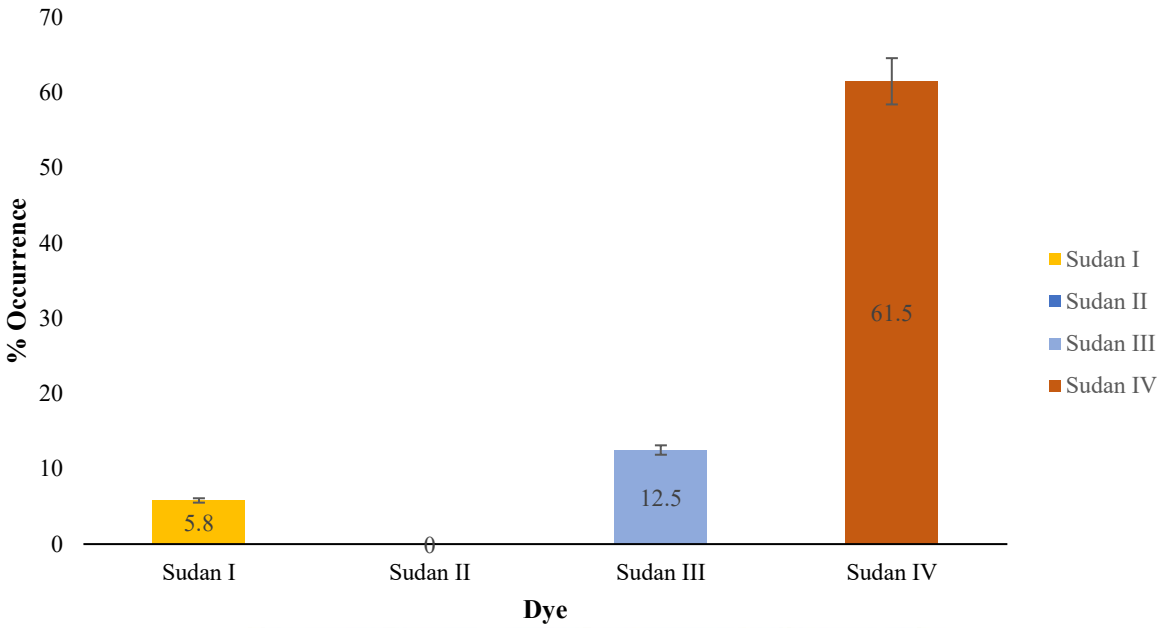


Figure 4. 3 Occurrence of Sudan dyes

The prevalence of each Sudan dye, defined as the percentage of samples in which the dye was detected, is presented in Figure 4.3. Sudan IV was by far the most prevalent adulterant, found in 61.5% of all samples tested. Sudan III was detected in 12.5% of samples, and Sudan I in 5.8%. Sudan II was not detected in any of the 104 samples analyzed

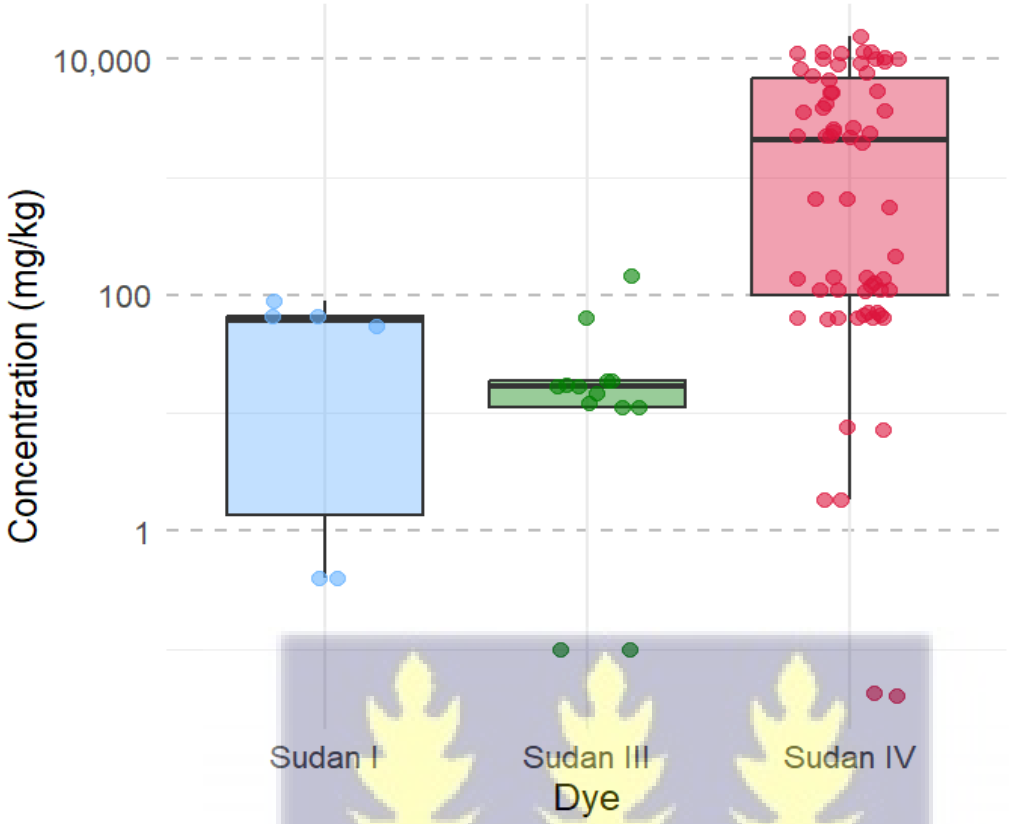


Figure 4. 4 Distribution of Sudan Dye Concentrations in Contaminated Palm Oil Samples

The box plots show the distribution of concentrations for Sudan I, III, and IV in the samples where each dye was detected. The line within the box represents the median concentration, the box extends to the 1st and 3rd quartiles, and the whiskers show the data range within 1.5 times the interquartile range. Individual points represent outliers. Note the logarithmic scale on the y-axis. For the samples in which dyes were detected, the concentration ranges were notably high. The descriptive statistics for these concentrations are presented in Table 4.12. The data for Sudan IV shows a particularly wide range. This distribution is visually confirmed by the box plot in Figure 4.2, which reveals two key findings. First, the median concentration for Sudan IV is substantially higher than that of Sudan I and III, indicating that when adulteration occurs, it typically involves a much greater quantity of Sudan IV. Second, the plot shows a significant spread in the data for

Sudan IV, with concentrations spanning several orders of magnitude. The presence of numerous data points located far above the main box represents extreme outliers. These outliers are indicative of severe, deliberate adulteration in specific samples, far exceeding the typical levels of contamination.

Statistical Analysis of Geographic Variation

To determine if the concentration of Sudan dyes varied significantly between the different markets in the Greater Accra Region, a one-way Analysis of Variance (ANOVA) was performed for each dye. The results of this analysis are summarized in Table 4.13 and supported by Figures 4.5, 4.6, and 4.7.

Table 4.13Results of one-way ANOVA testing for differences in Sudan dye concentrations across markets.

Dye	*F-Statistic*	*p-Value*	*Statistical Significance*
Sudan I	3.18	0.0021	Significant ($p < 0.01$)
Sudan III	1.98	0.0505	Marginally Significant ($p = 0.05$)
Sudan IV	6.40	5.02×10^{-7}	Highly Significant ($p < 0.001$)

The ANOVA for **Sudan I** revealed a statistically significant difference in mean concentrations across the markets ($F = 3.18$, $p = 0.0021$). While Sudan I was detected less frequently, its presence was not random and was concentrated in specific locations.

For **Sudan III**, the analysis indicated a marginally significant difference across markets ($F = 1.98$, $p = 0.0505$). This suggests a trend of geographic variation, but the pattern is less distinct than for the other dyes.

Most notably, the ANOVA for **Sudan IV** showed a highly statistically significant difference in concentrations across the sampled markets ($F = 6.40$, $p = 5.02 \times 10^{-7}$). This strong result confirms that the level of Sudan IV adulteration is not uniform across the Greater Accra Region but is instead heavily dependent on the specific market.

These figures illustrate the distribution of concentrations within each market, highlighting the specific markets that are outliers with exceptionally high levels of adulteration. The results confirm that the adulteration problem is not homogenous but is clustered in specific geographic hotspots within the region.

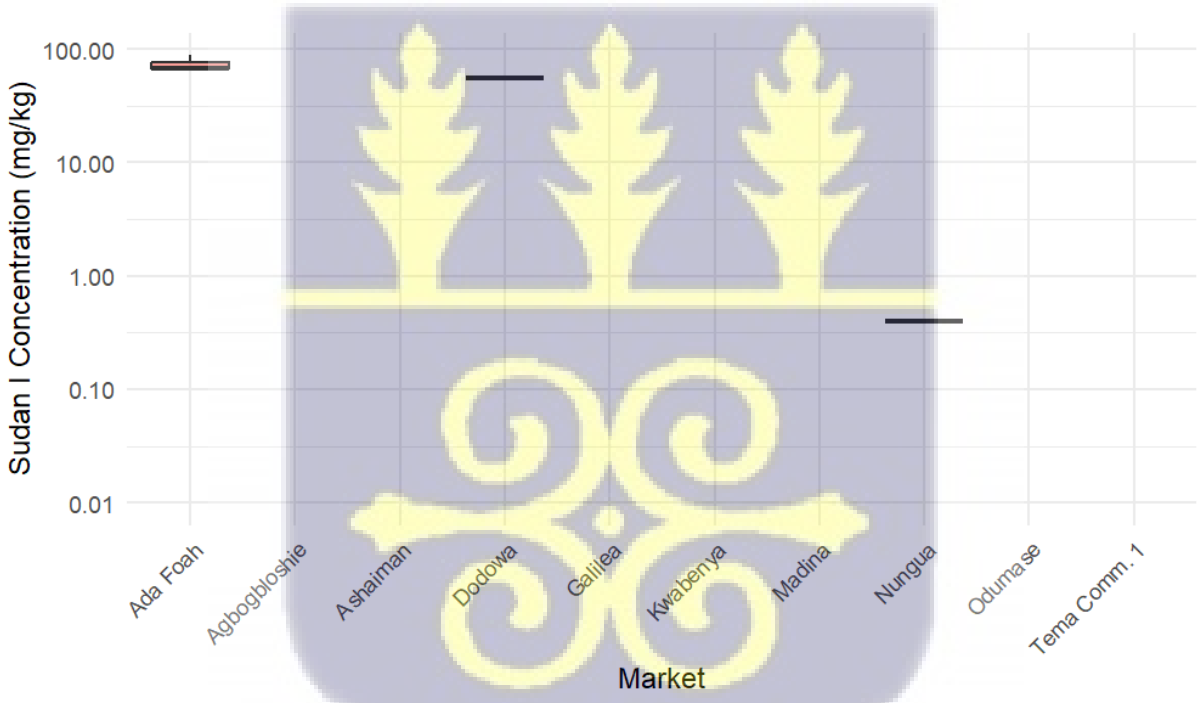


Figure 4. 5 ANOVA visualization of Sudan I concentration by market

Figure 4.5 presents a boxplot showing the distribution of Sudan I concentrations across the sampled markets. The analysis revealed a statistically significant difference in mean concentrations ($F = 3.18$, $p = 0.0021$). The plot shows that while most markets had no detectable

Sudan I (with values at zero), a few key markets, notably **Ada Foah**, exhibited clear outliers with substantially higher concentrations. This indicates that the presence of Sudan I is not random but is concentrated in specific locations.

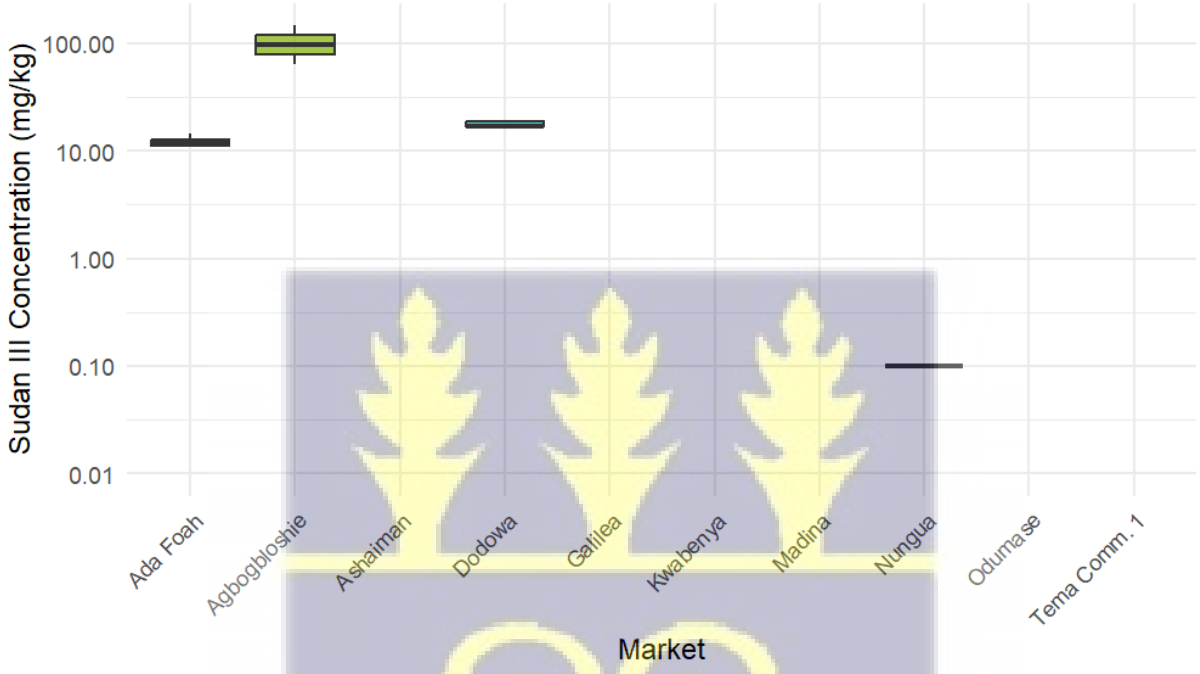


Figure 4. 6 ANOVA visualization of Sudan III concentration by market

Figure 4.6 shows the distribution of Sudan III concentrations via a boxplot. The ANOVA result indicated a marginally significant difference across markets ($F = 1.98$, $p = 0.0505$). The plot visualizes this trend, showing that the interquartile ranges for most markets overlap considerably, suggesting similarity. However, markets such as **Agbogbloshie** and **Ada Foah** show distinct median concentrations and the presence of high-value outliers, contributing to the observed marginal significance and pointing to localized points of contamination.

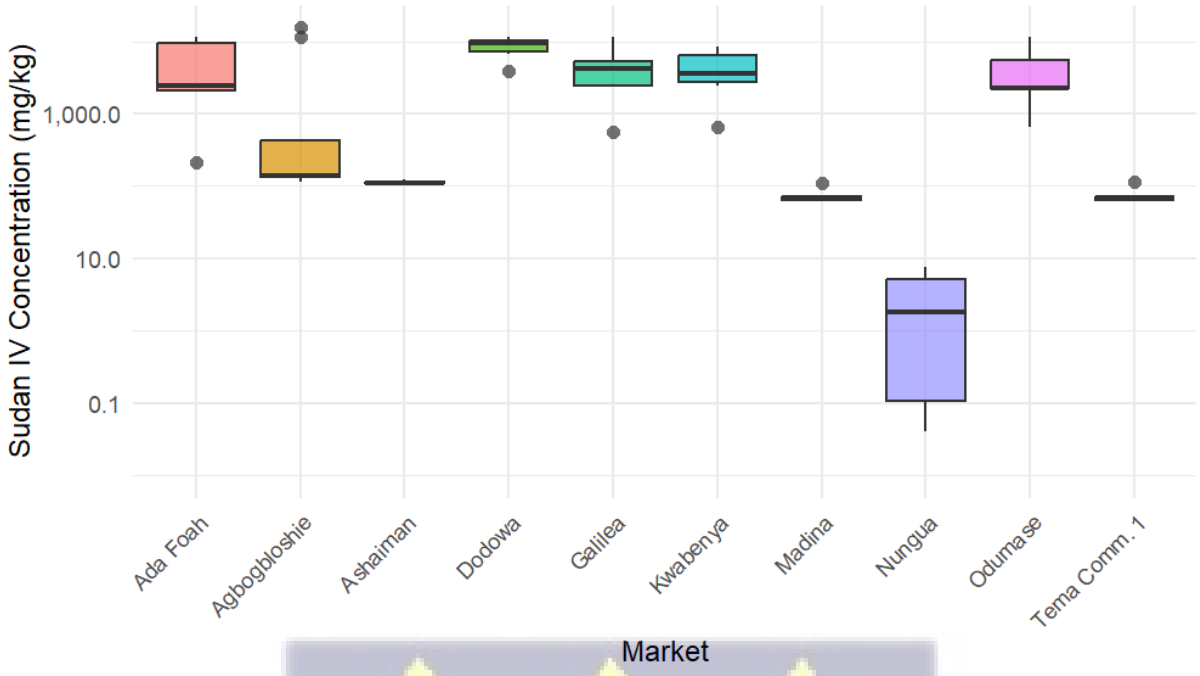


Figure 4. 7 ANOVA visualization of Sudan IV concentration by market.

Figure 4.7 illustrates the highly significant variation in Sudan IV concentrations across markets ($F = 6.40$, $p < 0.001$). The boxplot clearly demonstrates stark differences in the central tendency and spread of concentrations. Markets like **Ashaiman** and **Tema Community 1** show relatively low median concentrations, while **Agbogbloshie, Ada Foah, and Dodowa** are standout hotspots with dramatically higher median concentrations and extensive ranges. This visualization powerfully confirms that the adulteration of palm oil with Sudan IV is a severe and geographically concentrated problem within the region.

Table 4.14: Summary of the result.

	AGBO	MADINA	ASHAIMAN	TEMA	NUNGUA	DODOWA	GALILEA	KWABENYA	ODUMASE
SUDAN I	0%	0%	0%	0%	0%	10%	0%	0%	0%
SUDAN II	0%	0%	0%	0%	0%	0%	0%	0%	0%
SUDAN III	20%	0%	0%	0%	0%	50%	0%	0%	0%
SUDAN IV	80%	60%	30%	60%	43%	70%	70%	60%	50%
NONE	20%	40%	70%	40%	57%	30%	30%	40%	50%
HIGHEST	15,378mg/kg	111mg/kg	121mg/kg	112mg/kg	121mg/kg	11,629mg/kg	11,582mg/kg	8,366mg/kg	11,524mg/kg
LOWEST	112mg/kg	63mg/kg	109mg/kg	64mg/kg	108mg/kg	6,789mg/kg	559mg/kg	652mg/kg	658mg/kg

Table 4.15: Summary of vendors suppliers' location from the questionnaire.

AGBO	TEMA	ODUMASE	KWABENYA	GALILEA	MADINA	ASHAIMAN	ADA FOAH	DODOWA	NUNGUA
KADE 80%	ADEISO 70%	KADE 80%	KADE 80%	BEKWAI 70%	KADE 90%	ADEISO 60%	BEKWAI 70%	KADE 90%	
REFUSE 20%	KADE 30%	ASAMANKESE 10%	ASSIN FOSU 10%	VOLTA 30%	RESUSE 10%	BEKWAI 30%	VOLTA 20%	REFUSE 10%	
		ASSIN FOSU 10%				VOLTA 10%	REFUSE 10%		

4.2 DISCUSSION

Sudan dyes are azo dyes that dissolve in oil and are utilized in the textile and plastic industries to produce colors ranging from amber to reddish - orange to rich red hue (Butt, 2016). This dye was originally intended to provide a rich-red, yellow, or red-orange color to fabrics, but it has since been misapplied to contaminate tomato puree, spices, palm oil, and other foods with the sole intention of improving consumers' color perception (Basri et al., 2017).

This study aimed to quantify the presence of Sudan dyes in palm oil from Greater Accra markets following the FDA's prohibitions. The results reveal a critical public health issue: Sudan IV was detected in 61.5% of samples, with concentrations reaching an extreme of 15,638.95 mg/kg. This chapter interprets these findings within the Food Fraud Mitigation Framework. It demonstrates that the high prevalence is not random but shows statistically significant geographic clustering, exposing a severe regulatory enforcement gap for the domestic market. The discussion links these patterns to supply chain vulnerabilities and quantifies the resulting inequitable health risks, concluding with the urgent need for an evidence-based policy shift.

4.2.1Widespread Adulteration Confirms a Systemic Failure

The high prevalence of Sudan dyes, particularly Sudan IV in 61.5% of samples, indicates that adulteration is a normalized practice within the domestic palm oil supply chain. This figure is higher than the 42% reported by the Eastern Regional FDA in 2015 (Business Day Ghana, 2015), suggesting the problem has intensified in the capital region. The extreme concentrations, often exceeding 10,000 mg/kg, are unequivocal evidence of deliberate, large-scale fraud, not minor contamination. This points to a systemic failure where economic motivation to enhance oil colour meets a permissive environment devoid of effective controls.

4.2.2 Geographic Hotspots: Statistical Evidence of Targeted Adulteration

The ANOVA results transform our understanding from a generalized problem to a mappable one with clear hotspots. The highly significant variation in Sudan IV concentrations across markets ($F = 6.40$, $p < 0.001$) confirms that adulteration is geographically concentrated. Figure 4.6 identifies Agbogbloshie, Ada Foah, and Dodowa as epicentres of contamination, with median concentrations drastically higher than in markets like Ashaiman and Tema Community 1. This clustering is statistically robust and indicates that the source of the most severely adulterated oil is likely specific suppliers or processors whose products flow into these distinct distribution networks. The significant variation for Sudan I ($F = 3.18$, $p = 0.0021$), concentrated in Ada Foah, further supports the conclusion of geographically distinct adulteration practices.

4.2.3Regulatory Failure in Practice: The Two-Tier System and the Hotspot Paradox

The geographic heterogeneity underscores a profound regulatory failure. A functional monitoring system would target high-risk hotspots. The fact that markets like Agbogbloshie, Ada Foah, and Dodowa operate with impunity demonstrates a vacuum of enforcement for the domestic market. This creates a "two-tier" system: a stringent, effective regime for exports versus a near-total lack of oversight for local consumers. The statistically significant clustering ($p < 0.001$ for Sudan IV) presents a paradox: the data needed for targeted enforcement is obtainable, yet it is not utilized. The FDA's restriction exists in law but fails in practice, protecting international trade while externalizing health risks to Ghanaian citizens.

4.2.4 Supply Chain Opacity and the Source of Contamination

The vendor questionnaire revealed an opaque supply chain, with frequent reluctance to disclose sources. The geographic clustering of adulteration provides a crucial clue to interpret this opacity. The systematic high contamination in specific markets like Ada Foah and Dodowa strongly

suggests adulteration occurs upstream —at the level of processors or major aggregators supplying these areas—rather than by individual retailers. This pattern points to organized activity within specific supply networks. The disparity in dye types (Sudan I vs. IV) across hotspots further suggests multiple adulteration sources. This opacity is a major barrier to enforcement and highlights the need for traceability systems from mill to market.

4.2.5 Public Health Implications: Quantified and Inequitable Exposure:

The public health risk is severe and quantifiable. Chronic intake of Sudan dyes, which metabolize into carcinogenic aromatic amines (Stiborova et al., 2014), poses a significant threat. A consumer using oil adulterated at the mean Sudan IV concentration (3,565 mg/kg) could ingest approximately 71 mg of the dye daily, a dose orders of magnitude above any safe threshold. Crucially, the ANOVA results show this risk is not equitable. Consumers in identified hotspot markets face exponentially higher exposure than those in lower-risk areas. This creates a disproportionate health burden on specific communities, demanding geographically targeted public health interventions.

4.2.6 Limitations of the Study:

This study has limitations. Its focus on Greater Accra may not reflect situations in other regions. As a cross-sectional analysis, it cannot capture seasonal fluctuations or the long-term persistence of hotspots. The vendor questionnaire was basic; deeper ethnographic research in identified hotspots could provide richer insights into the mechanisms of fraud. Nevertheless, the findings provide a robust, statistically powered baseline that clearly identifies the scale and specific locations of the problem.

Finally, this discussion demonstrates that palm oil adulteration with Sudan dyes is a widespread, geographically concentrated, and severe public health crisis in Greater Accra. The highly

significant ANOVA results (e.g., $F=6.40$, $p<0.001$ for Sudan IV) empirically identify specific high-risk markets, proving that the FDA's domestic ban is ineffective. The problem stems from organized adulteration upstream in the supply chain, facilitated by a stark enforcement gap. This research provides the evidence base for a fundamental policy shift: from a blanket, ineffective prohibition to a targeted, risk-based enforcement strategy focused on the supply chains feeding the statistically identified hotspots of Agbogbloshie, Ada Foah, and Dodowa. Protecting the health of Ghanaian consumers requires action as precise and evidence based as the data presented here.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

This study successfully achieved its aim of identifying and quantifying Sudan dyes (I-IV) in palm oil from Greater Accra's local markets in the post-ban era. The application of a robust LC-MS/MS method yielded unequivocal evidence of a significant public health crisis. The data revealed that Sudan IV was the predominant adulterant, contaminating 61.5% of the 104 samples analysed, with concentrations reaching an alarming maximum of 15,638.95 mg/kg—a level that profoundly exceeds international safety limits. Crucially, statistical analysis confirmed that this adulteration was not random but was geographically concentrated in specific market hotspots, with highly significant variation across the region ($F = 6.40$, $p < 0.001$).

A central finding of this research is the definitive assessment of the FDA's regulatory prohibition. The results lead to an unambiguous conclusion: the ban has been fundamentally ineffective in protecting the domestic market. The high prevalence and extreme concentrations of Sudan dyes, particularly the statistically identified clusters in markets like Agbogbloshie and Ada Foah, expose a critical enforcement gap. This failure creates a de facto two-tier food safety system: one that effectively safeguards exports for economic reasons, and another that neglects the health of local consumers. The prohibition, therefore, exists as policy on paper but not in practice for the Ghanaian public.

Beyond the primary threat of Sudan IV, the detection of Sudan I and III in 5.8% and 12.5% of samples, respectively, alongside the non-detection of Sudan II, suggests varied adulteration

practices within the supply chain. This further underscores the complexity of the issue and the need for monitoring that can detect multiple contaminants.

Therefore, this thesis concludes that the adulteration of palm oil with carcinogenic Sudan dyes remains a severe, widespread, and geographically structured problem in Greater Accra. It demonstrates a systemic failure in food safety governance, where economic adulteration flourishes due to a lack of credible enforcement. The study underscores the urgent imperative for regulatory bodies to bridge the chasm between policy and practice. Moving forward, a paradigm shift is required—from a blanket, ineffective prohibition to a targeted, evidence-based enforcement strategy that prioritizes consumer safety universally, ensuring that the same vigour applied to protecting Ghana's trade interests is extended to protecting the health of its citizens.

5.2 RECOMMENDATION

Based on the empirical findings of this study, the following evidence-informed recommendations are proposed to address the critical public health risk of Sudan dye adulteration in palm oil.

1. Implement Targeted, Risk-Based Market Surveillance: Regulatory efforts should be immediately prioritized towards the statistically identified high-risk hotspots, specifically the markets of Agbogbloshie, Ada Foah, and Dodowa. The FDA should initiate a program of frequent, unannounced sampling and LC-MS/MS analysis in these areas, with swift and publicized enforcement actions against violators. This data-driven approach ensures efficient use of limited resources for maximum public health impact.

2. Develop and Deploy Rapid Screening Tools for Field Use: To overcome the barrier of costly and slow laboratory testing, investment is urgently needed in the development and validation of low-cost, rapid screening kits (e.g., immunoassays or portable spectrometers)

specific to Sudan IV. These kits should be distributed to regulatory field inspectors at the municipal level, enabling real-time, preliminary screening at market points and creating a credible deterrent.

3. Establish a Pilot Traceability System for High-Risk Supply Chains: Given the evidence of upstream adulteration, a traceability pilot program should be launched focusing on the supply chains feeding the high-risk markets. This could involve simple batch documentation or digital tracking for larger aggregators, allowing regulators to trace adulterated oil back to its source processor, moving enforcement beyond the retail level to where the fraud originates.

4. Launch a Geographically Targeted Public Awareness Campaign: Public education campaigns should be initially concentrated in the high-prevalence districts identified in this study. Campaign materials should clearly state the carcinogenic risks of Sudan dyes and provide consumers with visual guides to identify suspiciously coloured oil. Publicizing the names of sanctioned vendors would empower consumer choice and enhance the deterrent effect of enforcement actions.

5. Strengthen Inter-Agency Collaboration on Dye Import Control: The FDA should formalize collaboration with the Environmental Protection Agency (EPA) and Customs Division to monitor and restrict the diversion of industrial Sudan dyes into the food supply. This should include stricter licensing for importers, tracking of dye quantities, and joint investigations into suspicious sales, addressing the problem at its source.

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APPENDICES

Appendix A: Questionnaire to determine the source of Palm oil and Sudan IV Dye

PALM OIL TRADERS SURVEY

(INFORMAL SECTOR)

1. Age Group

   a) 20 -30yrs b) 31-40yrs c)41-50yrs

2. Gender

   a) Male b) Female

3. Where do you often buy your purchase?

   ……

   ……

4. Why do you buy from the particular supplier?

   a) Pricing b) Quality

5. When you purchase oil, what factors do you pay attention to?

   ……

   ……

SAMPLE SIZE

THE GREATER ACCRA REGION

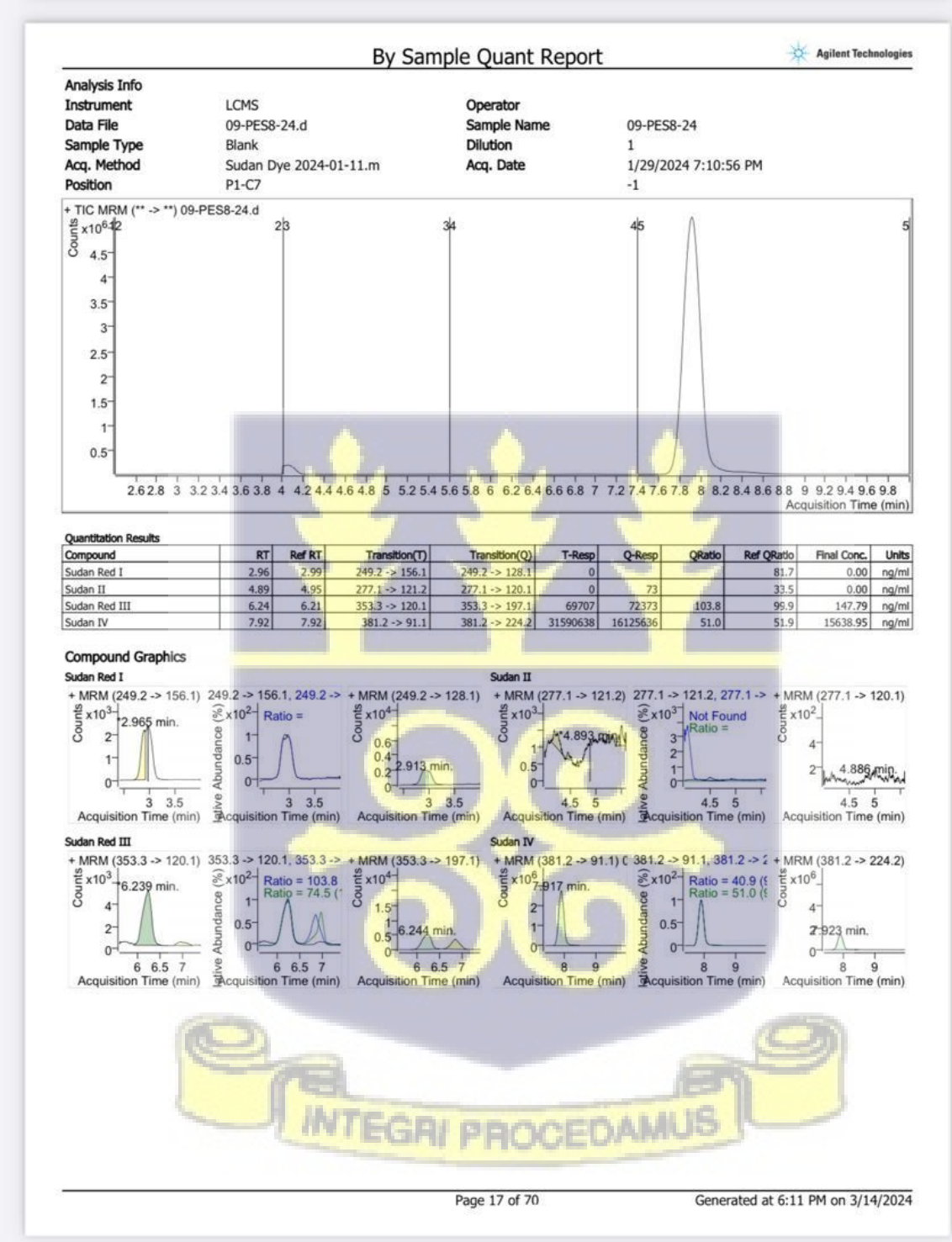
104 samples were taken from the region. There are 10 District Assemblies in the region and 10 samples were taken randomly from each of their major markets and 14 samples from the Nungua market as follows:

1. Accra metropolitan assembly - Agbogbloshie
2. Tema metropolitan assembly – Tema
3. Ledzokuku Krowor assembly - Nungua
4. Ga east metropolitan assembly – Kwabenya
5. Ga south metropolitan assembly – Galilea
6. La Nkwantanang assembly – Madina/Adenta
7. Ashaiman Municipal assembly - Ashaiman
8. Lower Manya Krobo Municipal - Odumase
9. Ada East District assembly-Ada Foah
10. Dangme west metropolitan assembly- Dodowa

A tally for market survey

Market	Source	Dye Cause
Agbogbloshie	Kade/refuse to disclose	Manufacturer
Tema	Adeiso/ Kade	Manufacturer
Kwabenya	Kade/Assin Fosu	Manufacturer
Ga Odumase	Kade/Asamankes/Assin Fosu	Manufacturer
Galilea	Volta/Bekwai	Manufacturer
Madina	Kade/ refused to disclose	Manufacturer
Ashaiman	Volta/Adeiso/Bekwai	Manufacturer
Dodowa	Kade	Manufacturer
Ada Foah	Volta /Bekwai/refused to disclose	Manufacturer
Nungua	Kade/refused to disclose	Manufacturer

Appendix A: Some results from palm oil samples and calibration using Sudan I, II, III, IV standards



By Sample Quant Report

Agilent Technologies

Analysis Info

Instrument	LCMS	**Operator**	
Data File	09-PES8-24.d	**Sample Name**	09-PES8-24
Sample Type	Blank	**Dilution**	1
Acq. Method	Sudan Dye 2024-01-11.m	**Acq. Date**	1/29/2024 7:10:56 PM
Position	P1-C7		-1

Quantitation Results

Compound	RT	Ref RT	Transition(T)	Transition(Q)	T-Resp	Q-Resp	QRatio	Ref QRatio	Final Conc.	Units
Sudan Red I	2.96	2.99	249.2 -> 156.1	249.2 -> 128.1	0			81.7	0.00	ng/ml
Sudan II	4.89	4.95	277.1 -> 121.2	277.1 -> 120.1	0	73		33.5	0.00	ng/ml
Sudan Red III	6.24	6.21	353.3 -> 120.1	353.3 -> 197.1	69707	72373	103.8	95.9	147.79	ng/ml
Sudan IV	7.92	7.92	381.2 -> 91.1	381.2 -> 224.2	31590638	16125636	51.0	51.9	15638.95	ng/ml

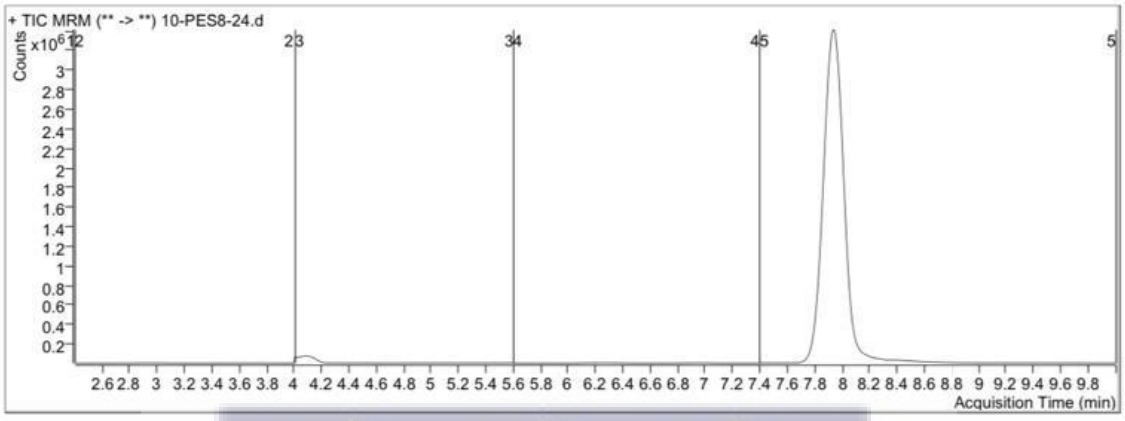
Compound Graphics

By Sample Quant Report

Agilent Technologies

Analysis Info

Instrument	LCMS	**Operator**	
Data File	10-PES8-24.d	**Sample Name**	10-PES8-24
Sample Type	Blank	**Dilution**	1
Acq. Method	Sudan Dye 2024-01-11.m	**Acq. Date**	1/29/2024 7:24:00 PM
Position	P1-C8		-1

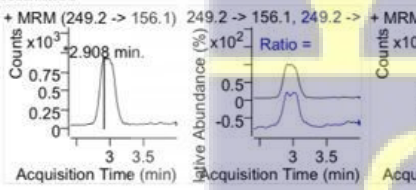


Quantitation Results

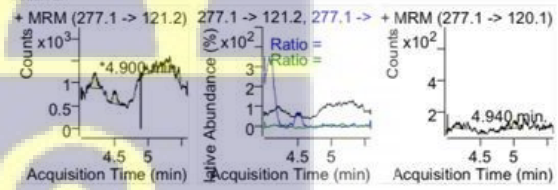
Compound	RT	Ref RT	Transition(T)	Transition(Q)	T-Resp	Q-Resp	QRatio	Ref QRatio	Final Conc.	Units
Sudan Red I	2.91	2.99	249.2 -> 156.1	249.2 -> 128.1	0	539		81.7	0.00	ng/ml
Sudan II	4.90	4.95	277.1 -> 121.2	277.1 -> 120.1	0	116		33.5	0.00	ng/ml
Sudan Red III	6.26	6.21	353.3 -> 120.1	353.3 -> 197.1	29074	29033	99.9	99.9	64.23	ng/ml
Sudan IV	7.94	7.92	381.2 -> 91.1	381.2 -> 224.2	18647610	9521722	51.1	51.9	11421.13	ng/ml

Compound Graphics

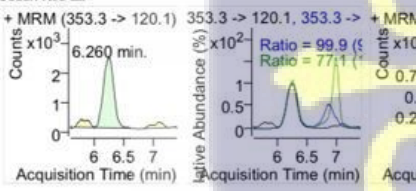
Sudan Red I



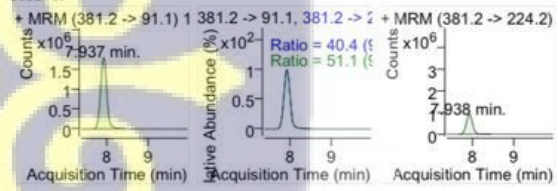
Sudan II



Sudan Red III



Sudan IV



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