KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

Assessment of the prevalence of Palm Oil adulteration with Sudan IV dye in the Greater Accra Region

BY

Jacob Amoako-Mensah

A thesis submitted to the Department of Food Science and Technology, College of Science, in partial fulfilment of the requirements for the degree of Master of Science

JULY, 2016

ASSESSMENT OF THE PREVALENCE OF PALM OIL ADULTERATION WITH SUDAN IV DYE IN THE GREATER ACCRA REGION

BY

JACOB AMOAKO-MENSAH

SUPERVISOR

DR. FRANCIS ALEMAWOR

A THESIS PRESENTED TO THE DEPARTMENT OF

FOOD SCIENCE AND TECHNOLOGY, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

GHANA, IN PARTIAL FULFILLMENT FOR THE

DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, GHANA

JULY, 2016

DECLARATION

I declare that the work recorded in this thesis is my own, unless otherwise stated and that it is of my own composition. No part of this thesis has been submitted for another degree.

…….

JACOB AMOAKO-MENSAH

(STUDENT)

……..

DR. FRANCIS ALEMAWOR

(SUPERVISOR)

……..

PROFESSOR MRS. IBOK ODURO

(HEAD OF DEPARTMENT)

DEDICATION

This work is dedicated to my wife, Afua Amoako-Mensah and the entire Amoako-Mensah family.

ACKNOWLEDGEMENT

Thanks be to the Almighty God for the gift of life and His abounding grace which has brought me this far. His mercy during my studies has been overwhelming. Your Word is indeed a lamp onto my feet and a light onto my path.

I express my profound gratitude to my academic supervisor Dr. Francis Alemawor, without whose support I would not have achieved this end. The lessons you have taught me will be my guide from this day forward. God bless you.

A special appreciation to all my lecturers for your patience kindness and support, the lessons I have learnt are invaluable. Your friendship during my studies has made me a better person. It is my hope and prayer that this relationship will not end when we leave Kwame Nkrumah University of Science and Technology (KNUST).

A express my profound gratitude to Mr. Hudu Mogtari, Mr. John Odame-Darkwah, Ms Lovelace Joshnson, Mr. Chetam Mingle Mr. Lesley Owusu-Ansah, Beatrice Aberdey Mensah, Mr. Benjamin Osei Tutu, Edward Archer, Cynthia Hushie, Jane Hayford and Osman Amatu Subuuru, all of the Food and Drugs Authority.

Without your patience, love, care, understanding and sacrifices this would not have been possible. It has been the most challenging moment in our life by far but your relentlessness has paid off. May the good Lord restore whatever you have lost and make the relationship we share even stronger. Afua, Reginald, Jason, Lemuel and Ivan Amoako-Mensah, these are your husband and father's words of appreciation for your support.

ABSTRACT

Sudan IV dye, a category 3 carcinogen used as an industrial dye for the manufacture of plastics and textiles to impact colour was detected in palm oil originating from Ghana in 2004 by the European Union Authorities. To ascertain the current status of Sudan IV adulteration of palm oil consumed in the Greater Accra Metropolis, fifty-five (55) samples obtained from ten (10) markets and four (4) supermarkets were analyzed using High Performance Liquid Chromatography (HPLC) to assess the prevalence of Sudan IV adulteration of crude palm oil. The results showed Sudan IV adulteration of palm oil to be prevalent in all the markets where samples were taken. Eight (8) out of the ten (10) markets surveyed recorded 100% adulteration, whilst the remaining two recorded 80% adulteration of crude palm oil. Ninety-six (96) percent of all samples (without FDA approval) picked from the local markets tested positive for Sudan IV dye. Sixty (60) percent of samples (with FDA approval) picked from supermarkets tested positive for Sudan IV dye. Sudan I, Sudan II and Sudan III dyes were also detected in the samples (without FDA approval) picked from the local markets. Studies have shown that the dye is used to enhance the colour of the crude palm oil which is lost due to the use of poor quality palm fruits, and production practices that result in a loss of the characteristic orange red colour of the crude palm oil. The Ghana Standard for Animal and Vegetable Fats and Oils Specifications for Edible Palm Oil (GS 223:2001) does not permit the use of colourants, thus its use is considered as adulteration of the product as per the provisions of the Public Health Act 2012, Act 851. Considering the health implications reportedly associated with Sudan IV the FDA and the relevant stakeholders should work together to curb the practice in line with the recommendations given.

TABLE OF CONTENTS

TABLES

FIGURES

APPENDIX

ABBREVIATIONS

USP	United States Pharmacopeia
EMA	Economically Motivated Adulteration
USFDA	United State Food and Drug Administration
MSU	Michigan State University
EFSA	European Food Safety Authority
FSSAI	Food Safety Standard Authority of India
CAC	Codex Alimentarius Commission
R&D	Research and Development
GMP	Good Manufacturing Practices
GMA	Grocery Manufacturers Association
QA	Quality Assurance
QC	Quality Control
HACCP	Hazard Analysis Critical Control Point
RASFF	Rapid Alert System for Food and Feed
IARC	International Agency for Research on Cancer
EU	European Union
FSMA	Food Safety Modernization Act
FFB	Fresh Fruit Bunches

CHAPTER ONE

1.0 BACKGROUND

Adulteration of food is a global phenomenon that can have serious consequences on public health and safety. It is an unacceptable practice that is designated as illegal in food safety regulations globally. The spectra of food products adulterated vary from country to country and may include dairy products, fruit juices, confectionery, oils, and flour and meat products. It has not been assessed conclusively how wide spread food adulteration is worldwide. Adulteration is the addition of adulterants to food. The word adulteration is appropriate under conditions when the substance being added is unwanted by the consumer. Otherwise the expression would be food additive. Food is adulterated mainly to make it attractive, mask the effects of the use of unwholesome ingredients, improve organoleptic properties and safeguard nutritional properties; thus food adulteration brings economic benefit to those who engage in it.

Food adulterants are additives which are not permitted for use in a given food and in the event that a severe health hazard is eminent due to the adulteration, urgent regulatory measures must be instituted to protect public health and safety. Adulterants in food may have adverse health consequences ranging from acute symptoms such as abdominal pain, asthma, vomiting, headache, mental retardation, cardiac arrest and chronic effects such as cancers (Alauddin, 2012). Below are some incidents of food adulteration:

- Beech-Nut, a baby food company in 1987 paid in fines $2.2 million for violating the Federal Food, Drug, and Cosmetic Act by selling apple juice containing sugar, water and artificial flavouring.
- In 1997 one of the units of ConAgra Foods, an American packaged foods company, was found guilty of increasing the weight and value of stored grains by spraying water on the grains.
- In 2007, melamine (a white crystalline compound made by heating cyanamide and used in making plastics) was found to have been added to wheat gluten to produce artificially inflated protein content during testing. This was detected in the human and animal food supply chain; several U.S. pet food brands were implicated. The melamine adulterated gluten was produced by a China-based Company.
- In 2008, melamine adulterated milk was used in the production of infant formula, resulting in the death of at least six children. Several other children were reported to have been hospitalized following their consumption of the implicated product. Investigations confirmed widespread melamine contamination of China's milk supply. (Laksmi et al., 2012)

Analysis of data from the US Pharmacopeial Convention (USP) on food adulteration indicates that seven food ingredients are mainly the target for intentional or economically motivated adulteration (EMA) of food. A study by the Food Safety Standard Authority of India (FSSAI) in 2012 revealed that 100% synthetic milk was produced completely without cow or buffalo milk. Detergents, urea, cheap oil, etc., were employed in the manufacture of the synthetic milk which competes with genuine milk in the market. There are reports of fruits, especially mangoes being

ripened with calcium carbide and fish being made to appear fresh using formalin (Lakshmi et al., 2012). These adulterants are known to be toxic with some being classified as carcinogens and as such are banned for use in food items. In 1991 random samples from 33 states in India tested, only 31.5% conformed to the FSSAI standards while the remaining 68.4% failed (Lakshmi et al., 2012).

The above incidents are indications of how adulteration can result in the introduction of chemical hazards into food, thus compromising its safety for economic gains. In the case of melamine in food products, the intent was to increase the nitrogen content which is the target during protein content test. The fraudulent practice was economically motivated without considering the effect of the unsafe ingredient on consumer's health. (Moore et al., 2010).

Ghana is no exception to the menace of food adulteration. Food products adulterated include but not limited to powdered pepper, groundnut paste and milled melon seeds (Agushie). There is however no empirical data on the range of food products adulterated in Ghana. This may be due to inadequate laboratory testing systems and equipment and under resourced regulatory institutions. One major food product which is adulterated in Ghana is palm oil. Palm oil has been reported to be adulterated with Sudan dyes (I II III and IV). In the year 2004 and 2005, Ghana received 57 and 35 notifications respectively from the European Union (EU) through its Rapid Alert System for Food and Feed Safety (RASFF) on adulteration of palm oil with Sudan IV dyes. In view of these notifications the Food and Drugs Authority implemented export control measures to prevent a possible ban of palm oil exportation to the European Union and protect the fledgling palm oil industry. The measure instituted by the regulatory institution (FDA) with

technical support from the EU, ensured that every consignment of palm oil destined for the EU is tested for Sudan dyes and a certificate of compliance issued prior to exportation. The measure instituted by the FDA, however did not impact on the safety of palm oil consumed locally by Ghanaians. Assessment of the prevalence of the adulteration of palm oil with Sudan dyes in the Greater Accra Region which is the hub of trade in Ghana is vital in protecting public health and safety.

In 2007, the FDA conducted a survey on palm oil adulteration with Sudan IV dye in the following regions of Ghana, Greater Accra, Eastern, Central, Ashanti, Northern and Volta Regions. The survey indicated that palm oil was adulterated with Sudan IV dye and some regulatory measures were undertaken. However, Ghana continued to receive alerts after this survey and whatever intervention measures that was instituted. Between the year 2007 and 2010, eleven (11) food safety alerts relating to Sudan IV dye adulteration was received through the RASFF, an indication of the persistence of the practice.

Sudan dyes (I, II, III and IV) are colouring solvent used in industrial colouring of oils, waxes, petrol, shoe and floor polishes. Sudan dyes are not authorized food colours which are added to foods to enhance the natural colour lost due to processing, to reduce batch-to-batch variation, and maintain consistency of appearance to promote products with consumer appeal where no natural colour exists.

The International Agency for Research on Cancer (IARC) has classified Sudan I, Sudan II, Sudan III and Sudan IV as category 3 carcinogens. This implies there is inadequate evidence in humans of the carcinogenicity of the dyes.

1.1. PROBLEM STATEMENT

Palm oil is widely used as food and for food preparation in Ghana and exported especially to Europe. In Ghana palm oil has been found to have been adulterated with Sudan IV dye. From July 2004 to May 2005 the European Commission received 83 Sudan IV dye adulteration notifications relating to palm oil from Ghana through the RASFF.

In view of the problem, the Food and Drugs Authority in Ghana has since 2005 instituted a protocol to ensure that palm oil exported to the EU is free of Sudan IV dye. The protocol ensured that every consignment of palm bound for the EU is tested and a Certificate of Analysis issued before export. However, palm oil produced for the local market is not screened for the presence of Sudan IV dye, a situation which meant that the safety of palm oil sold on the local market and consumed by the Ghanaian population could not be ascertained. This research therefore seeks to assess the prevalence of Sudan IV dye adulteration of palm oil from selected markets in the Greater Accra Region.

1.2. JUSTIFICATION

Palm oil is widely consumed as food in Ghana. Almost every household in Ghana use palm oil for one food dish or the other. The common food dishes include beans and gari and fried plantain (red red), palava sauce (Kontomre stew) and mashed yam (Etoo).

Unfortunately, the regulatory protocol which was instituted when Sudan dyes were detected in palm oil exported from Ghana to the EU did not take into consideration palm oil sold on the

local market and consumed by Ghanaians. This means that the safety of palm oil on the Ghanaian market cannot be guaranteed, thus a determination of palm oil safety in respect to its adulteration with Sudan dyes on the Ghanaian markets is important. This will help in the development of intervention strategies and policies to safeguard public health and safety to curb the menace for both the local and international trade. Assessment of the prevalence of the practice, the source of the dye and the point along the palm oil value chain where the adulteration is carried out is critical in curtailing the practice.

1.3. AIM OF RESEARCH

The aim of this study was to assess palm oil sold on the local markets in Accra for the presence of Sudan IV dye; determine the point along the palm oil value chain where the adulteration is done.

1.4. SPECIFIC OBJECTIVES

1. To ascertain the prevalence of Sudan IV adulteration of palm oil in ten markets in Greater Accra.
2. To ascertain the sources of supply of palm oil and Sudan dyes.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. FOOD ADULTERATION AN ASPECT OF FOOD FRAUD

Food supply chain has increasingly become global and complex and has resulted in the emergence of new and challenging risks (Spink 2010). A food safety risk gaining the attention of industry, governments and standard setting agencies is food fraud carried out for economic gains by food manufacturers, processors, distributors and retailers. A report by the Department of Homeland Security of the United State of America defines food fraud as the thoughtful substitution, addition, modification, or false or misleading proclamations made about a product for economic gain (Spink, 2011b). The US Pharmacopeia Expert on Food Ingredients asserts that economically motivated adulteration of food ingredient is a fraudulent practice since in most instances it involves the removal or replacement of authentic substance or addition of non-authentic substances without adequate information to the customer or purchaser for monetary gain for the vendor (DeVries, 2009). Other terminologies normally used to describe food fraud include economic adulteration, economically motivated adulteration and food counterfeiting.

Food fraud is often measured in economic terms and of less concern for the traditional food defense intervention and response system. However, food fraud alters the true nature and purity of the unique ingredient or product by substitution, dilution or modification of its biological, physical and chemical characteristics. By the nature of such adulteration, the criminal and fraudulent intent is to evade existing quality assurance (QA) and quality Control

(QC) systems which include Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP) plans. In food fraud, only the perpetrator knows how the food raw material has been altered but does not have the requisite knowledge to determine whether there is any toxicological effect or hygienic risk to the consumer due to the alteration of the raw material. (Moore *et al*, 2011)

There are concerns that traditional threats to food supply which has to do with contamination along the value chain may be less risky than food fraud. This is because unconventional adulterants are often used in such activity. Melamine, for example before 2007 was neither considered a potential adulterant nor contaminant in the food supply chain and therefore was not considered in QA and QC analysis protocol. In addition, the rudiments of modern food safety protocols are not designed to assess the countless number of possible adulterants which may be present in the food supply (Spink 2011a; Moore *et al*, 2011).

Food raw material and additives constitute pose a major risk because they have a wide array of use in food products and in most instances do not have functional properties which are visible to promote easy differentiation from other comparable raw material or adulterants along the supply chain. It is challenging to distinguish glycerin, a sweet, clear, colourless, viscous liquid, organoleptically from other liquid syrups with same properties like diethylene glycol which in previous years has been substituted for glycerin with fatal consequences (Schier *et al* 2009).

Prevention, intervention, and response are the three main features of a traditional food protection system with a process cycle back to prevention (Fig. 2.1; Acheson, 2007). Some have

suggested that food fraud protection should start with emphasis on understanding the novel risk at the intervention step (Spink *et al*, 2011b).

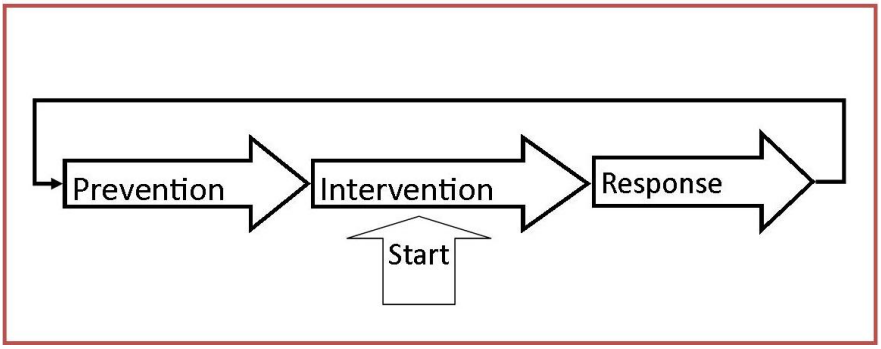


Figure 2.1 Food protection plan progression (Spink and Moyer 2011b)

2.2. FOOD PROTECTION

Food fraud is economically motivated. Food defense is considered to be food fraud and the motivation includes the intention to inflict public harm or threaten consumer. Perpetuators of food fraud are mainly actors involved in the food chain. Problems associated with food defense are usually perpetuated by people who are not normally involved in the production of the food; they are mainly outsiders and terrorist. The harm associated with food fraud on public health is generally negligible, however sometimes mistakes and unintended health consequences occur (Johnson, 2014). Table 2.1 outlines the public health risk associated with various food risk types.

Table 2.1: Food protection risk: Examples, Cause and Effects

Risk Type	Example	Cause and Motivation	Effect	Public Health Risk Type	Secondary Effect
Food Quality	Bruising of fruits accidentally	Mishandling	Possible additional contamination	None, or possible food safety	Brand equity or food safety incident
Food Fraud	Adulteration of milk with melamine internationally	Increased profit margins	Toxic poisonings	Food safety	Public fear and possible lower prices industry wide
Food Safety	Contamination of raw vegetables unintentionally with E. coli	Protection and control during harvesting and processing is limited	Illness and/or death	Food safety	Damaged industry, recall expense, and public fear
Food Defense	Contamination of ground beef with nicotine intentionally	Revenge against the manager	Nonlethal poisonings	Food defense	Adulterated product, damaged industry, recall expense, public fear

Source: Spink *et al*, (2011)

2.3. FOOD FRAUD/ADULTERATION CATEGORIES

As at 2012 identified database records for food ingredient fraud after a review of 660 scholarly, media and other publically available reports was 1305. The type of food fraud for each record in the database was categorized using three (3) broad categories: replacement, addition and removal (Table 2.2; Moore *et al*, 2011).

Table 2.2 Categories and explanations for type of fraud field

Type of fraud	**Definition**	**Subtypes included**	**Examples**
Replacement	Complete or partial replacement of a food ingredient or valuable authentic constituent with a less expensive substitute without the purchasers' knowledge	Dilution, addition, or extension of an genuine ingredient with an adulterant False declaration of geographic species, botanical or varietal origin False declaration of the raw material origin or production process used to manufacture and ingredient. False declaration of origin to evade taxes or tariffs	Artificially increasing the apparent protein content measured by total nitrogen by the addition of melamine to milk. Addition of water and citric acid to lemon juice to fraudulently increase the titratable acidity of the final juice product. Over treating frozen fish with extra water (ice) Substitution of cow's milk for sheep or goat's milk Substitution of common wheat for durum wheat Substitution of synthetically produced vanillin for botanically derived (natural) vanillin
Addition	The addition of non-authentic substance to mask inferior quality ingredient without the purchasers' knowledge	Color enhancement Taste enhancement	Enhancing the colour of poor-quality paprika by the addition of Sudan dyes Masking the astringent taste of poor-quality pomegranate juice by the addition of sugar
Removal	Removing without the purchasers knowledge a genuine and valuable constituent	NA	Sale of the defatted paprika lacking valuable flavoring compounds

According to records in database, 95% of food fraud fall within the replacement category; the addition category accounts for less than 5%; removal accounts for less than 1%. Replacement describes the substitution of authentic material with a less expensive alternative for the sellers' economic gain without the knowledge of the purchaser. An example of replacement fraud type is the use of hazelnut oil as an ingredient to substitute in part or whole for olive oil. (Moore *et al.* 2011).

The replacement type of food fraud is the most common among the three because current analytical testing strategies are most appropriate to detect this type of adulteration. In the detection of adulteration two analytical strategies can be used. The first strategy determines the presence or absence of known adulterant(s). By its nature it cannot detect unknown adulterant; it seeks the particular adulterant of interest and not others. The approach is ideal for identifying adulterants at very low concentrations. The second strategy ascertains the authenticity, purity and identity of a food or ingredient. This is known as compendium strategy. With this approach the identity of the adulterant(s) are not required to establish adulteration. However, relatively high levels of adulteration get detected and not lower levels as with the first approach. (Moore *et al*, 2011).

2.4. FOOD ADULTERATION AND FOOD ADDITIVES

Food additives are mainly chemical substances added to processed food to enhance or maintain quality features which include but not limited to texture, physical properties, taste and flavours. Additives are also used to enhance shelf-life of processed foods and control spoilage. Some food additive categories are: Antioxidants, Emulsifiers or stabilizers, Preservatives, Anti-caking agents, artificial sweeteners, bulking agents, acid regulators, leavening agents, flavor enhancers and glazing agents. Additives are not adulterants when used in accordance to maximum limits and Good Manufacturing Practices (GMP). When the prescribed limit is exceeded, the additive is considered as an adulterant and may pose a health hazard to consumers. Food additives are considered as adulterants only when there is a reported incidence of an outbreak of foodborne illness which is attributed to the additive in question. (Vasireddi, 2013).

Additives which pose serious food safety risk to consumers are the unauthorized and illegal ones which are often used with impunity. The European Union (EU) rules that all food additives need to be authorized and listed in the EU positive list for food additives with conditions of use after safety assessment, technological need and the fact that its use should not mislead consumers.

Azo dyes are not included in the Codex General Standard for Food Additives (EC, 2015b) and the positive additive list for the EU (Annex II of Regulation (EC) No 1333/2008); the use of these dyes is not permitted in food. Ghana being a member of the Codex Alimentarius Commission

aligns its national standard to that of the commission, where there are no national standards the Codex Standards are adopted, as is the case for the General Standard for Food Additives.

2.5. FOOD ADULTERATION ANALYTICAL DETECTION METHODS

In detecting food ingredient adulteration, the most important defense mechanism is through the use of analytical detection methods. There are sixteen (16) approaches used and they are all instrumental based. The list is as follows:

1. High Performance Liquid Chromatography
2. Infrared spectroscopy
3. Gas chromatography
4. Isotope ratio mass spectrometry
5. Hyphenated mass spectroscopy method
6. Near infrared spectroscopy
7. Polymerase chain reaction
8. Capillary electrophoresis
9. Enzyme linked immunosorbent assay
10. Thin-layer chromatography
11. Site-specific natural isotope fractionation
12. Mid-infrared spectroscopy
13. Raman spectroscopy (Raman)
14. Nuclear magnetic resonance spectroscopy

15. High-performance anion exchange Chromatography
16. Differential scanning calorimetry

2.6. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a technique of separation that involves small volumes of liquid samples being injected into tubes packed with tiny particles, 3 to 5 micron in diameter called the stationary phase. Individual components of the sample are moved down the packed tube (column) with a liquid forced through the column by high pressure delivered by a pump, this constitute a Mobile phase. The column packing that involves various chemical and physical interactions between their molecules and packing particles separates the components from one another. At the exit of the tube (column) the separated components are detected by a flow-through device (detector) that measures their amount. The output from this detector is called a “liquid chromatogram”. (Agilent Technologies)

HPLC is used for the identification of individual compounds in a sample. The retention time which is the time it takes for that specific compound to elute from the column after injection is the most common parameter for compound identification. Compound identification could also be based on the chemical structure, molecular weight or some other molecular parameter depending on the detector used. HPLC has five main components as follows:

1. Pump: The pump forces a liquid (called the mobile phase) through the liquid chromatograph at a specific flow rate, expressed in milliliters per min (mL/min).

2. Injector: The injector serves to introduce the liquid sample into the flow stream of the mobile phase.
3. Column: It is considered the “heart of the chromatograph” the column’s stationary phase separates the sample components of interest using various physical and chemical parameters.
4. Detector: The detector can see (detect) the individual molecules that come out (elute) from the column.
5. Computer: Frequently called the data system, the computer not only controls all the modules of the HPLC instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis). (Agilent Technologies)

2.7. SUDAN DYES USED IN FOOD ADULTERATION

For legal purposes adulterants are chemical substances and should not be contained in food or other substances. In societies with few legal controls and inadequate regulatory systems, the usage of adulterants is more rampant and done with impunity. In 1820, the German Chemist Frederict Accum investigated into adulterant usage and identified many toxic colouring in foods and drinks (Charnley, 2008). Arthur Hill Hassall in the early 1850s published his extensive research findings on food adulteration in the Lancet. This led to the enactment of the Food Adulteration Act in the 1860 and subsequently additional legislation (Charnley, 2008).

Sudan dyes are in the family of azo-dyes which are industrial dyes used in the manufacture of plastics and other materials to impact colour. Azo colorants are the most important class of synthetic dyes and pigments, representing 60 - 80% of all organic colorants. They are used widely in substrates such as textile fibres, leather, plastics, papers, hair, mineral oils, waxes, and cosmetics (Püntener *et al*, 2014; Hayenga, 2011). The use of Sudan dyes in food to enhance and maintain its colour constitutes a major public health concern.

In May 2003, the illegal presence of Sudan dye was reported in foods in the European Union. Chilli powder and foods containing chilli powder were the foods in which the dye was found. Since then several EU member states have issued notifications on the presence of Sudan IV and Sudan I in curry powder, chilli powder, sumac, curcuma, processed products containing chilli and palm oil via the RASFF. Sudan II and Sudan III have also been detected occasionally in the same range of products. (RASFF, 2005).

A food product containing industrial dye constitutes adulteration because these dyes according to the European Council Directive 94/36/EC on colours for use in foodstuff are not authorized food colours.

In 2005 various dyes found in food illegally in the European Union were reviewed toxicologically by the European Food Safety Authority (EFSA). For Sudan I EFSA concluded that there was strong evidence for it being carcinogenic and genotoxic to humans. It is therefore presumed that all Sudan dyes will have the same deleterious consequences due to similarities in the structure of Sudan I and the other Sudan dyes (Hayenga, 2011).

2.8. REVIEW OF TOXICOLOGY OF SUDAN DYES

The International Agency for Research on Cancer (IARC) has classified Sudan dyes as Group 3 carcinogens and are not permitted world-wide as food additives (IARC, 1987). It implies that the use of Sudan dyes as food additives is illegal, a position which has been strongly affirmed by the USFDA and EU.

2.7.1. Sudan I

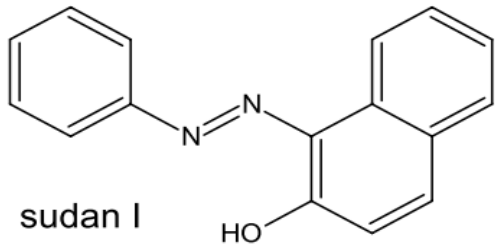


Figure 2.2 Molecular structure of Sudan I dye

Source: EFSA (2005)

Both *in vitro* and *in vivo* studies have shown Sudan I dye to be genotoxic and to cause metabolic activation. In rats, Sudan I has been found to be carcinogenic but not in mice. The genotoxic and carcinogenic effect of Sudan I was attributed to its breakdown products, aniline and 1-amino-2-naphthol and their ensuing metabolic activation (BfR Opinion 2003). DNA interaction and metabolism showed similar pathways as in the *in vitro* studies using microsomes of rat and humans (EFSA, 2005).

2.7.2. Sudan II

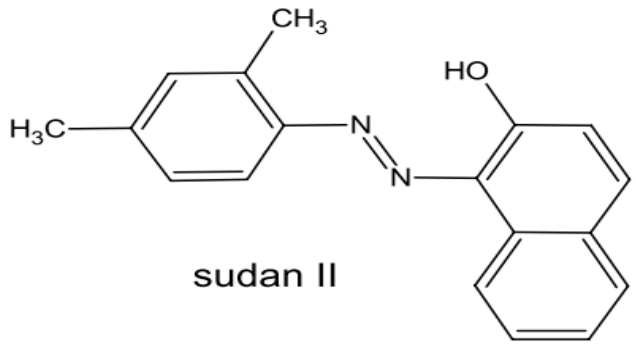


Figure 2.3 Molecular structure of Sudan II dye

Source: EFSA (2005)

Sudan II according to limited data from *in vitro* studies reveals genotoxicity in bacterial tests and there is sufficient evidence to show that this happens after metabolic activation. According to EFSA at present the dye should be considered potentially genotoxic though in vitro test on a single mammalian cell was negative and there are no in vivo data. There is however insufficient data to draw conclusion on the carcinogenic effect of Sudan II following subcutaneous administration or ingestion. However, following implantation of impregnated pellets of Sudan II, high incidence of bladder tumours were recorded and was sufficient until proved otherwise to consider the dye as a possible carcinogen (EFSA, 2005).

2.7.3. Sudan III

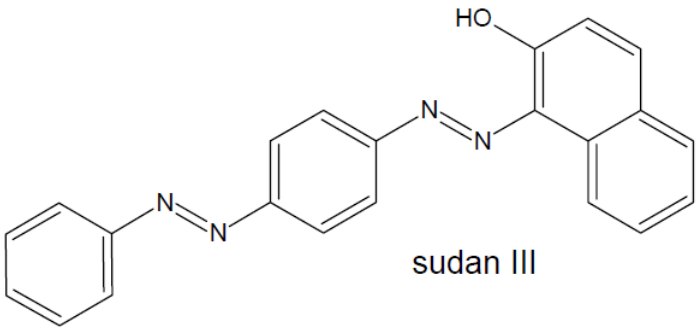


Figure 2.4: Molecular structure of Sudan III dye

Source: EFSA (2005)

There has been very limited study on the genotoxicity of Sudan III and these studies have not been conclusive due to the absence of suitable motivation system in many of the studies. With the limited range of research, there has not been any evidence of carcinogenicity of Sudan III and there is no indication of the potential to be carcinogenic. There is however the possibility of the formation of some metabolites which are identical to that of Sudan I due to the structural relationship of Sudan III with Sudan I. It may be prudent to accept in the absence of data to explain the difference between the metabolism of Sudan I and Sudan III that it is possibly carcinogenic and potentially genotoxic.

2.7.4. Sudan IV

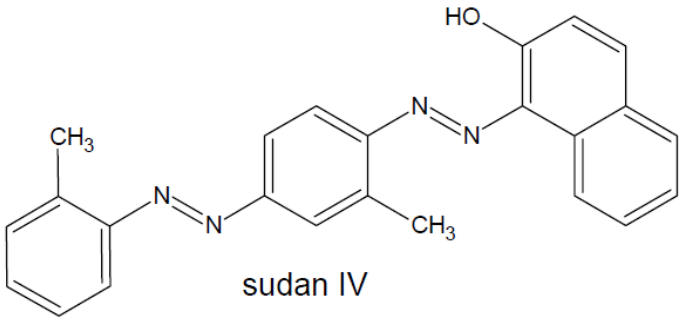


Figure 2.5: Molecular structure of Sudan IV dye

Source: EFSA (2005)

The potentially genotoxic effect of Sudan IV is not in doubt because it has been established that the pattern of consistency in relation to the positive consequences following metabolic activation is in tandem with other related dyes.

Sudan IV has been used as a dressing additive in wound healing and was found to have the ability to initiate epithelial proliferation. This property of the dye coupled with the known effect of structurally similar dyes makes it a potential carcinogen (Püntener *et al*, 2014).

2.9. CARCINOGENICITY OF AZO DYES

Azo dyes comprise one or more nitrogen-nitrogen double bonds which are referred to as azo groups. (Figure 2.5).

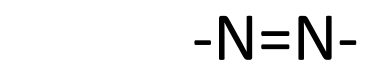


Figure 2.6 Azo group

Using sodium dithionite under reductive conditions will result in the cleavage of these azo groups to form two amines as illustrated in figure 2.6

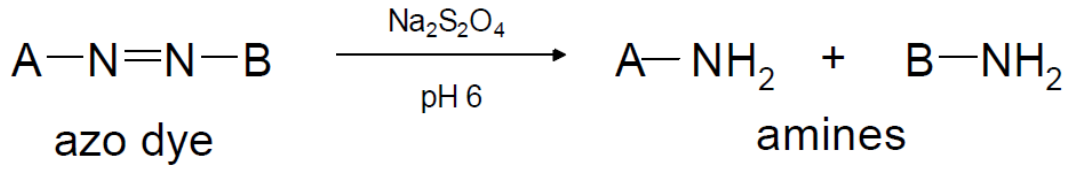


Figure 2.7: Reductive cleavage of the azo dye to form amines

The azo group when cleaved results in aromatic amines of which a small number have been classified as being potentially carcinogenic to humans. However, upon reductive cleavage it is not all the azo dyes which can liberate these amines, those that are affected are just a few (Püntener *et al*, 2014)

The potential for mutagenicity of azo dyes depends on the ability of the molecule to produce aromatic amines which are active. This is influenced by the structure-activity relationships which results in the breakage of the azo linkage leading to oxidation of the primary aromatic amine which has been liberated. The Liver and gastrointestinal tract are major sites where the breaking of the azo linkage can occur (Püntener *et al*, 2014)

Experimental evidence by EFSA indicates genotoxicity and carcinogenicity for Sudan I. In the case of Sudan II, III, and IV, the evidences is inconclusive, however due to structural similarities to Sudan I, the potential for genotocity and carcinogenicity cannot ber ruled out. (Püntener *et al*, 2014).

2.10. PALM OIL-IMPORTANT VEGETABLE OIL

Oil palm (*Elaeis guineensis*) is the crop with the highest oil yield per unit area planted (Jalani *et al* 1997). Therefore earning hard currency from palm oil is a workable possibility for Ghana, since the environmental conditions for the cultivation of the oil palm tree favours Ghana (Hartley, 1984). Palm oil and palm kernel oils have become growth leaders in the whole field of oils and fats since the 1970s (World Growth Report, 2011).

The palm tree produces the fruit from which palm oil is derived, it is used for both food and non-food consumption. Total global production of palm oil is estimated at over 45 million tonnes and the major producers and exporters in the world are Malaysia and Indonesia. India, China and Europe are the major importers (World Growth Report, 2011).

Globally, approximately 80% of palm oil produced is used for food purposes including as cooking oil, in margarines, noodles, baked goods etc. In addition, palm oil is used as an ingredient in the production of soaps, detergents, cosmetics, surfactants, bio-fuel, pharmaceuticals which are non-edible product (World Growth Report, 2011).

The current annual production for Ghana is put at 109,000 metric tonnes down from 121,000 in 2006. The domestic consumption level is estimated to be around 230,000 tonnes. Importation takes care of the shortfall (Ayodele, 2010).

2.11. NUTRITION AND HEALTH BENEFITS OF PALM OIL CONSUMPTION

For centuries palm oil has served as food for humans all over the world. The main components of palm oil are palmitic, oleic and linoleic acids, the commonest fatty acids in the plant kingdom (Mensah, 1999).

Palm oil feeding improves coronary blood flow and has no effect on blood pressure (Hornstra, 1987). Palm oil also contains Vitamin E, which acts as nutritional antioxidants and helps to reduce cellular damage due to free radicals arising from the body's hemal oxidative energy metabolism or from the action of toxic chemicals and pollutants found in the environment. Free radicals have been implicated in the process of ageing, chronic degenerative diseases and cancer (Ciba Foundation Symposium, 1983).

Palm oil contains high amounts of beta-carotene, which has been found to have beneficial properties. Unrefined palm oil is the richest known natural source of the pro-vitamin A pigment, beta-carotene. Though processing removes all the beta-carotene, crude palm oil is eaten in West Africa and some South East Asian countries. Studies have shown that inadequate intake of carotenoid-containing vegetables were associated with the risk of lung cancer (Wolf, 1982). Though the required daily average of Vitamin A is one milligram, it was estimated in

1995 that three million children clinically exhibited Vitamin A-deficiency, xerophthalmia and were at risk of blindness. In addition, children who are sub-clinically Vitamin A-deficient and at risk of severe morbidities and premature death were estimated to be 250 million (Howson *et* ,1998). The potential of palm oil, therefore, should be harnessed as a cost-effective way of reducing morbidity and mortality amongst children in developing countries including Ghana (Chandrasekharan *et al*, 1995). It is all the more so, since Choo (1995), has expressed the view that of the vegetable oils that are widely consumed, the concentration of agriculturally derived carotenoids is highest in palm oil.

Palm oil does not contain trans-fatty acids isomers as in hydrogenated fats. Highly unsaturated oils such as in many seeds and in fish oils, are unsuitable for many food uses because they have very low melting points and also because they are more susceptible to oxidative deterioration. The process of hydrogenation, however, extends the food uses of the polyunsaturated oils whose melting points would otherwise be much lower. Hydrogenation reduces the level of unsaturation and transforms the cis-double bonds in unsaturated fatty acids to the trans-form. Consumption of excessive amounts of trans-fatty acids can result in metabolic and nutritional disturbances; one effect is to limit the availability of Free Fatty Acid FFA affecting possibly, platelet aggregation and cardiovascular functions (Gurr, 1984). Palm oil however, does not have to undergo hydrogenation to increase its suitability for use and stability. It is thus free from all the disadvantages associated with trans-fatty acids (Gurr, 1984).

2.12. PALM OIL PRODUCTION

General it is assertion that the origin of the palm tree *(Elaeis guineensis)* is the West African tropical rain forest. For thousands of years, the processing of the fruit of the palm oil for crude and refined oil has been a practice in Africa. Palm oil derives its deep red colour from carotenoids, a naturally occurring red pigment. A major component of its glycerides is the saturated fatty acid palmitic acid. At tropical ambient temperature it is viscous semi-solid and solid fat under temperate climates DG SANCO, 2005).

The palm tree due to its economic importance as source of edible oil is grown in tropical climate and in areas with high rainfall (≥ 1,200 mm per annum). The fruit of the palm which is in bunches various from 10 to 40 kg. The individual fruits are made up of an outer skin (the exocarp) and a pulp (mesocarp) and ranges from 6 to 20gm. It also has a nut in the centre made up of a shell (endocarp); and the kernel which in itself contain oil which is different to palm oil. It is estimated that 100 tonnes of fresh fruit bunches (FFBs) processed yields 20 to 24 tonnes of crude palm oil in a well-run palm oil mill. Harvesting of the palm oil oil tree is distributed throughout the whole year but mainly two peak periods are known (April-May and September-October) DG SANCO, 2005).

In recent years palm oil has become a non-traditional export commodity. Mainly, the variants exported from Ghana are two, the “virgin” or “crude oil”, which is the natural oil; and the salted and spiced variant, “zomi”. The zomi presents a darker colour which is as a result of the added spices, salt and cooking while the virgin palm oil has an orange-red colour. The main producing

regions in Ghana are the Eastern region and Central region. Zomi is produced mainly from the Volta region (DG SANCO, 2005).

According to the degree of complexity and their throughput, palm fruits can be grouped into three categories based on the scale of processing into edible oil. The three categories are small-scale processors, medium-scale mills and large industrial mills. Small scale processing units generally handles a maximum of 2 tonnes of Fresh Fruit Bunches (FFB) per hour. Medium-scale processing mills are normally facilities which have installations for processing between 3 and 8 tonnes of Fresh Fruit Bunches (FFB) per hour whereas mills that process more than 10 tonnes of Fresh Fruit Bunches (FFB) fall within the large-scale category (Mensah, 1999).

The processing of palm oil generally involves the following: Fresh Fruit Bunches harvesting and reception at the mill, the fruit bunches are freed after sterilization and threshing, the crude palm oil is derived after mashing and pressing the fruit. Further processing to refine the crude palm oil may be carried out prior to storage. For small scale processors the FFB are transported in basket carried on the head and in vehicles in the case of large-scale processors to the processing sites. Removal of the fruit from the bunches, threshing is done by cutting the fruit-laden spikelets with an axe or machete from the bunch stem and then by hand the fruit is separated from the spikelets. A mechanized system which detaches the fruit from the bunch and the spikelets is used by large-scale processors. In the large scale facilities, Sterilization at 120 ^{o}C for 90 minutes is done prior to the threshing stage. Sterilization is aimed at stopping enzymatic activities and softening the pulp in order to easily detach the fibrous material. In the extraction process, mechanical pressure is applied on the digested mash to separate the oil,

moisture, fibre and nuts. The separation of the oil from impurities is carried out at the clarification stage and purified using a centrifuge for large scale processors. A spindle press is heated to reduce the content of water prior to the obtaining of the oil for small-scale processors. Fibre and palm nut constitute the residue from the press. The hand is then used to separate the nut from the fibre. A second grade oil normally used in soap making is obtained after pressing the fibre again with a spindle press. For most small scale processors the process varies. (Mensah, 1999).

New methods of processing are being harnessed to improve the processing of palm oil. Current techniques include biomodification (use of lipase for modifying palm oil and its fractions by interesterification), bioconversion (the use of enzymes and microbial cells in manufacturing useful products), and molecular distillation (Mensah, 1999).

2.13. IMPACT OF FOOD SAFETY MODERNIZATION ACT ON FOOD ADULTERATION

In April 2009, the USFDA conducted a public meeting to create awareness on FDA-regulated product and solicit for public input regarding economically motivated adulteration. The move which was part internal activities aimed at addressing the problem of intentional adulteration also resulted in the establishment of an internal workgroup which is made up of FDA product centers employees, spanning food and non-food products regulated by FDA's Office of Regulatory Affairs (ORA) and the office of the commissioner. A multidisciplinary collaborative

approach is used by the workgroup to capitalize on commonalities among FDA's product centers. The Food Safety Modernization Act (FSMA) employs a variety of strategies and well-resourced regulatory authorities to prevent both intentional and unintentional contamination of foods. By the FSMA, companies are required to identify and implement mechanisms for increased food risk protection to prevent the sale of adulterated products and then ensure compliance with Good Manufacturing Practices (GMP). The companies are expected to share their plan with the USFDA. The companies are therefore empowered by such controls to consider their responsibility of ensuring that potentially adulterated foods involving the "absence, substitution, or addition of constituents" do not end up on the market (Johnson, 2014).

2.14. SUMMARY

Food adulteration is an aspect of food fraud and it is considered as an economic issue. Adulteration however results in the alteration of the identity and purity of the original product. Sudan dyes (I, II, III, and IV) are unauthorized food additives used in the adulteration of many food products. The dye has been found in palm oil exported to the EU.

Sudan dyes are known as azo dyes due to the presence of one or more nitrogen-nitrogen double bonds in their chemical structure. The azo group under reductive conditions undergoes

cleavage which results in the formation of aromatic amines. In humans some aromatic amines such as o-toluidine have been categorized as potentially carcinogenic. It implies that the mutagenicity potential of azo dyes corresponds to the structure-activity relationships of the generated aromatic amine product from the molecule during metabolism by breaking the azo linkage. This may occur in the gastrointestinal tract and in the liver. There is a strong evidence to conclude that Sudan I is both genotoxic and carcinogenic. There is however a great structural similarities between Sudan I and the other dyes. Sudan IV is therefore presumed to have the same deleterious effect.

It is evident that an immediate innovative counter measures and analytical methods to detect adulteration and protect the food supply chain are instituted in Ghana and Africa to safeguard public health and safety.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted mainly in the Greater Accra Metropolitan and Tema Metropolitan Areas. Samples were collected from ten main food markets namely Madina, Dome, Dansoman, Agbogbloshie, Kaneshie, Mallam, Mallam Atta, Adenta, Ashaiman and Tema Community 1 markets. FDA registered brands were sampled from three supermarkets in Accra namely Shoprite, Koala and Maxmart.

According to the Statistical Service of Ghana, Greater Accra Region occupies a total land surface of 3,245 square kilometres, which is 1.4% of the total land area of Ghana. Greater Accra Region is the anchor of a larger metropolitan area, the Greater Accra Metropolitan Area (GAMA), having the same boundaries or extent in space and time. The Region has a population of about 4 million people, making it the largest metropolitan assembly in Ghana by population. The Greater Accra Region accounts for 13% of the total population of Ghana.

The Ghana Housing and population census in the year 2000, estimated that the region had a population of 2,905,726. Accra is the most populous region of Ghana in terms of the number of people, behind the Ashanti Region. The region has the highest population density of 1,236 sq km in the country and it is mainly due to immigration and high population growth. Greater Accra had a population growth rate of 38% which is more than the national average of 30.4 % (Ghana Statistical Service, 2012).

3.2 Sampling of Palm Oil Products

A total of fifty five (55) palm oil samples were picked from ten food markets and three (3) supermarkets in the Greater Accra Region. Five samples (5) were randomly drawn from five vendors from each market and five (5) prepackaged samples from Supermarkets for analysis. The samples were collected in PET bottles and stored under ambient temperature. Table 3.1 below shows the various places where samples were taken from.

Table 3.1: Sampling Markets

Name of Market	**Location**	**Number of Samples collected**
Madina Market	Madina	5
Dome Market	Dome	5
Dansoman Market	Dansoman	5
Agbogbloshie Market	Agbogbloshie	5
Kaneshie Market	Kaneshie	5
Mallam Market	Mallam Market	5
Mallam Atta Market	Mallam Atta	5
Ashaiman Market	Ashaiman	5
Tema Community 1 Market	Tema	5
Makola Market	Makola	5

3.3 Administering of Questionnaire

As part of the sample collection process questionnaires were administered to palm oil vendors to ascertain the source of palm oil and Sudan dyes to determine the point along the value chain where the product is adulterated. Questions in the questionnaire will bring to the fore the points along the value chain where the adulteration is carried out.

3.4 LABORATORY ANALYSIS

3.4.1 Sample Preparation

Five grams (5g) of palm oil were weighed into 250ml conical flasks and 20 ml of methanol added for extraction of Sudan dye. The samples were mechanically shaken for 20 minutes and stored in the dark for 20 minutes. The samples were then filtered into 1mL amber vials for HPLC analysis.

3.4.2 HPLC Method Analysis of Sudan Dyes

For each test sample was run in duplicates. Characteristics of HPLC set-up and conditions for the analysis include the following:

Column: Zorbax SB-C8; 4.6mm x 250mm; 5um; S/N USSE012888

Mobile Phase: MeOH : H_20 (85: 15)

Injection Volume: 10.00mic

Wavelength; 505 nm

Temperature; 35^{o}C

Flow Rate; 1.5 ml/min

3.4.3 HPLC Detection of Sudan Dyes in Samples

For each test sample analysis, a blank test was run using methanol to determine the baseline for the chromatographs; to identify and ignore peaks that may result from the blank.

A standard Sudan dyes, (I, II, III, IV) test was carried out to determine the time at which the dyes were detected. The time of detection for the dyes varied for each standard analysis. Generally, the four (4) dyes were detected within 2 to 14 minutes. The run time for each sample analysis is therefore 16 minutes.

3.4.4 Data Analysis

Microsoft excel was used for analysis of the results. The percentage adulteration of Sudan IV dye for the ten (10) markets was calculated and bar charts drawn to compare the results. Pie chart was used to show the percentage of regulatory approved product from supermarkets adulterated with Sudan IV dye.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 PALM OIL SAMPLES FROM THE OPEN MARKET

4.1.1 Sudan IV Adulteration

From the results forty eight (48) samples representing 96% of the samples drawn from the open market tested positive for Sudan IV dye. For both Madina and Kaneshie markets, four (4) out of the five (5) samples collected representing 80% of the samples analyzed, tested positive for Sudan IV dye. All five 5 samples representing 100% from the other eight (8) markets, namely Dome, Dansoman, Agbogbloshie, Makola Number 2, Mallam, Mallam Atta, Tema Community 1 and Ashiaman markets tested positive for Sudan IV dye as shown in Figure 4.1.

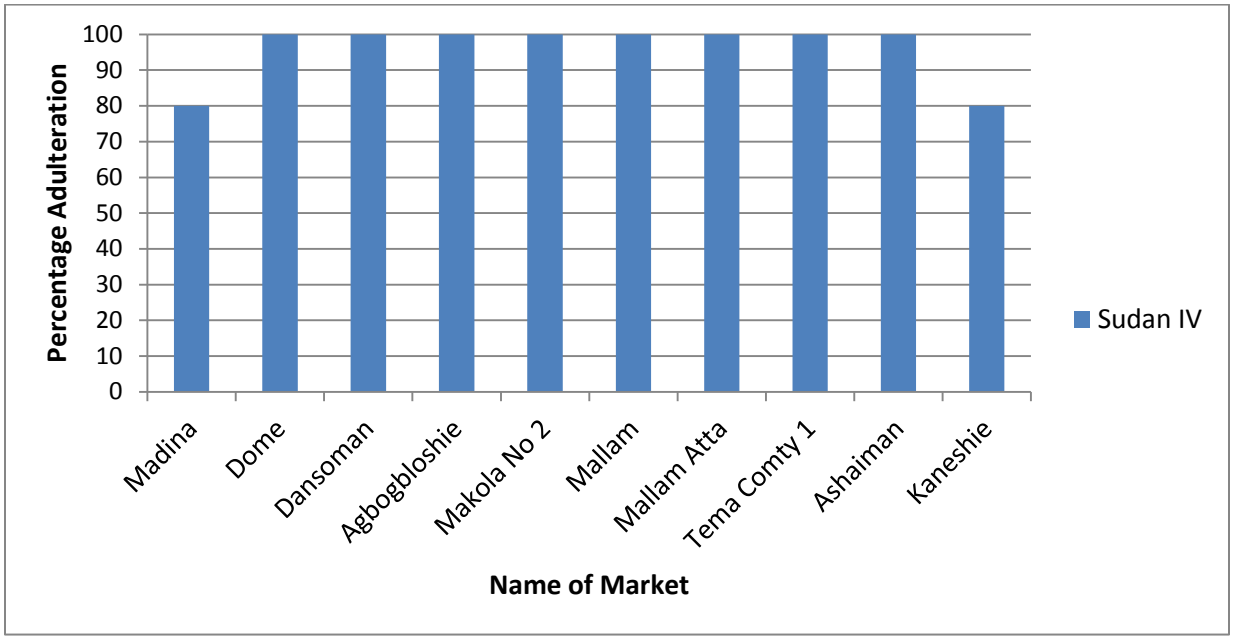


Figure 4.1: Sudan IV dye adulteration of Palm Oil from Markets in the Accra Tema Metropolis.

As indicated in the figure 4.1, two (2) markets (Madina and Kaneshie markets) representing 20% of the markets sampled recorded 80% of sampled palm oil adulterated with Sudan IV dye. The remaining eight markets representing 80% of the markets surveyed, recorded 100% of sampled crude palm oil, adulterated with Sudan IV dye.

4.1.2 Adulteration with Sudan I, II & III

Figure 4.2 shows the prevalence of Sudan I, Sudan II and Sudan III in addition to Sudan IV dye in the fifty (50) samples drawn from the open market. Sudan I dye was detected in 20% of samples from Makola No 2 market - representing 10% of the markets surveyed. Sudan II dye was detected in 40% of samples from Kaneshie market; 80% of samples from each of the following markets: Madina market, Dome market, Mallam Atta market and Tema Community 1 market; and 100% of samples from Makola number 2 market and Mallam Atta market. These represent 80% of the markets sampled from. Sudan III dye was detected in 20% of samples from Mallam Atta Market and represent 10% of the markets surveyed.

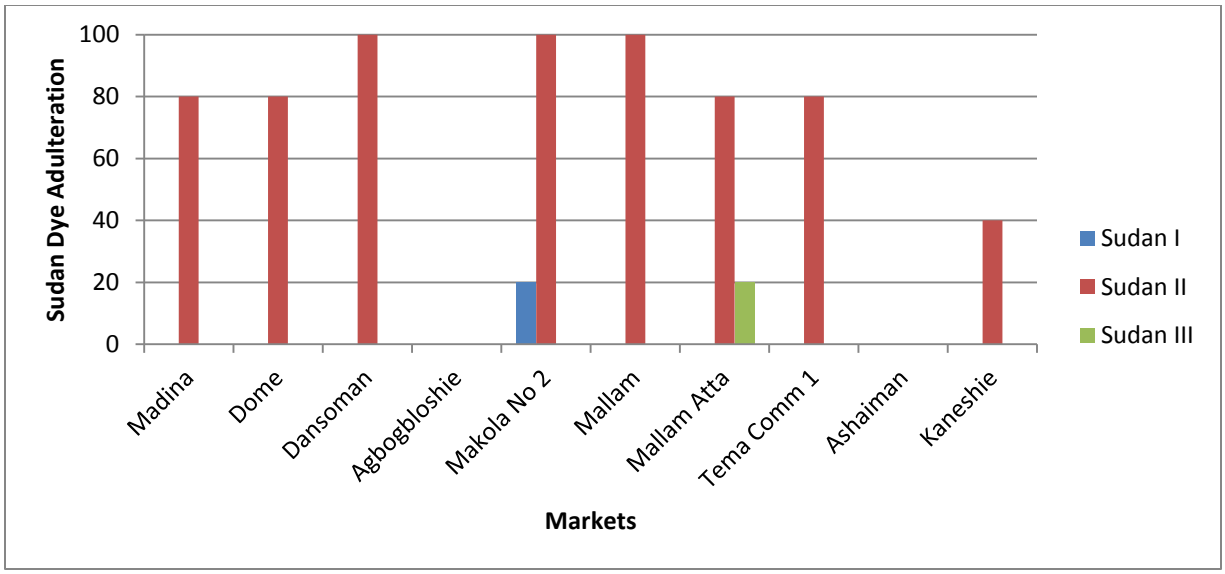


Figure 4.2: Prevalence of Sudan I, II and III in Palm Oil in Accra and Tema Metropolis.

From Figure 4.1 and 4.2 the predominant adulterant used is Sudan IV for palm oil sampled in all the markets. Eighty percent (80%) of the markets sampled from tested positive for Sudan II. Palm oil samples from Ashaiman and Agbogbloshie markets were not adulterated with the other Sudan dyes. Makola and Mallam Atta markets both recorded 20% of samples adulterated with Sudan I and Sudan III respectively.

4.2 BRANDED PREPACKAGED PALM OIL SAMPLES FROM SUPERMARKETS IN ACCRA

Figure 4.3 shows that 60% of the branded prepackaged products sampled tested positive for Sudan IV dye. Sudan I, II and III dyes were not detected in the branded prepackaged products.

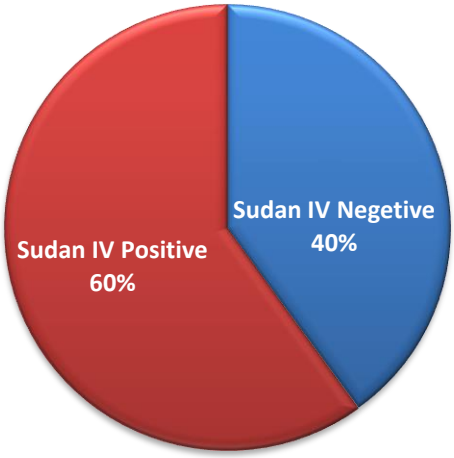


Figure 4.3: Prevalence of Sudan IV adulteration of Palm Oil from Supermarkets

4.3 RESULTS OF QUESTIONNAIRE

Table 4.1 shows the source of palm oil and Sudan dyes from the ten (10) markets. Palm oil retailed at all markets sampled from was sourced from Kade and kwae in the Eastern Region of Ghana. Sudan dyes were sourced from Nigeria

Table 4.1: Source of Palm oil and Sudan dye to the ten markets

Name of Market	Source of Palm Oil	Source of Sudan Dye
Madina Market	Kade	Nigeria
Dome Market	Akroso	Nigeria
Dansoman Market	Kwae	Nigeria
Agbogbloshie Market	Akroso	Nigeria
Kaneshie Market	Asamankese	Nigeria
Mallam Market	Kwae	Nigeria
Mallam Atta Market	Asamankese	Nigeria
Ashaiman Market	Kwae	Nigeria
Tema Community 1 Market	Kade	Nigeria
Makola Market	Kade	Nigeria

4.4 DISCUSSION

From the results, it was evident that Sudan IV dye was predominantly used in the adulteration of palm oil due to its deep red colour. The dye was used in the adulteration of crude palm oil in all the markets sampled from with impunity. The Ghana Standard for Animal and Vegetable Fats and Oils Specifications for Edible Palm Oil (GS 223:2001) does not permit the modification of the colour of the product with any additive. The food legislation in Ghana therefore does not accept the use of Sudan IV dye. Permitted food colours and additives are added to food in

accordance to required specifications and the principles of Good Manufacturing Practices (GMP) which ensure that the additive is within levels which will not pose health risk to the consumer for a lifetime. However, Sudan IV dye, an unauthorized food additive was used in the adulteration of crude palm oil regardless of GMP protocols.

Adulteration of palm oil with Sudan IV dye was observed in all markets. This suggests the endemic nature of the practice. One of the markets which represent 10% of the markets had oil samples containing Sudan I in association with other dyes. Sudan II was used in eight (8) markets representing 80% of the markets. Sudan III was used in one market which represents 10% of the markets under study. This shows that in the open market all the four (4) Sudan dyes were used in the adulteration of palm oil with the predominant dyes being Sudan IV and Sudan II. The reasons behind the use of other Sudan dyes in combination with Sudan IV could not be ascertained. It could however be envisaged that since all the Sudan dyes I, II, III and IV have characteristic red colour, the perpetrators of the adulteration practice are not able to differentiate between them.

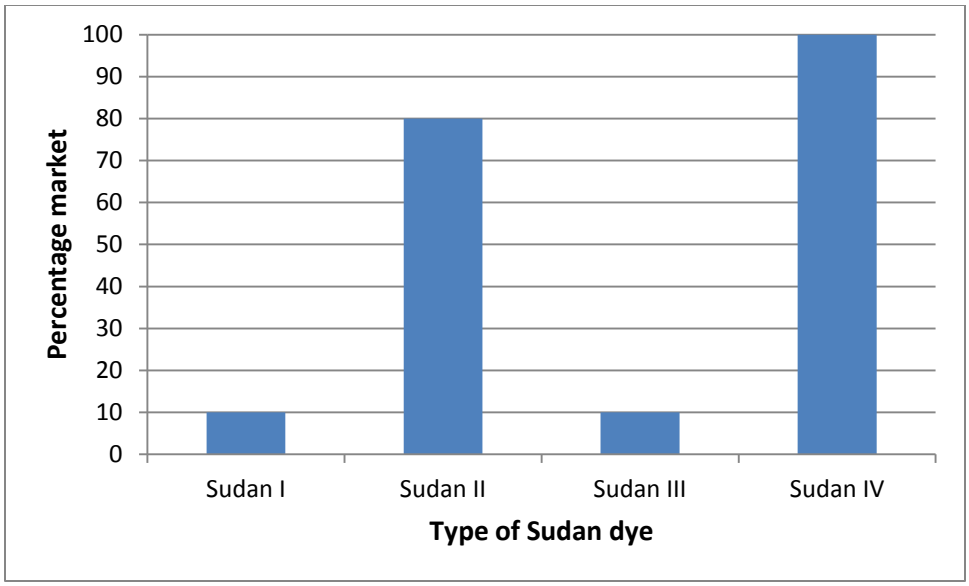


Figure 4.4: Use of Sudan dyes in palm oil sampled from the open market

In the case of the branded prepackaged products sampled from Supermarkets, Sudan IV was the only dye detected. Sudan I, Sudan II and Sudan III were completely absent in all the samples analyzed. Sudan IV was found in three (3) out of the five (5) branded prepackaged samples from Supermarkets, representing 60% of the samples. Adulteration of palm oil with Sudan IV dye was observed in products sampled from both the open markets and in supermarkets. However, in the open market other dyes were used together with Sudan IV dye. Two (2) out of the five (5) branded prepackaged products which represent 40% of the samples were not adulterated with Sudan dyes. A follow-up on the companies which produced the products in question revealed that the companies had their own farms, milling and processing plants. The companies were also involved in palm oil export to Europe and as a practice had Quality Assurance (QA) schemes in place to promote the production of quality and consistent product. The three (3) products which failed and constituted 60% of branded prepackaged products were produced from companies which purchased palm oil from different suppliers to blend prior to packaging.

Contamination of the raw material (crude palm oil) from one supplier will end up contaminating the entire consignment during blending. An interesting revelation is the fact that no other Sudan dyes were found in these branded prepackaged products apart from Sudan IV. It could therefore be inferred that the Sudan IV dye was added deliberately to improve the colour of the product.

4.5 SOURCE OF PALM OIL AND SUDAN DYES

The source of palm oil for all the ten (10) markets surveyed was Kade, Akroso, Asamankese and Kwae all in the Eastern Region of Ghana. All the markets have distributors who bring the consignment in drums to retailors at the various the markets.

Investigations revealed that Sudan dyes are imported into the country and retailed in 5g portions by Nigerians. These small scale Nigerian retailers of the dye move from market to market to sell the dyes to the vendors and distributors of palm oil. According to the vendors, adulteration of palm oil was done at the markets by the vendors and distributors. Some of the vendors purchased the dye and carry out the mixing right at the market. For the distributors, the dye is poured into the distribution drum prior to filling. Homogeneous mixing of the dye and the oil is achieved during the transportation of the consignment to the markets.

The palm oil vendors displayed gross ignorance with regard to the consequences of palm oil adulteration with Sudan dyes on public health and safety and explained that the widespread adulteration was as a result of customers demand for red palm oil. According to the vendors,

customers did not patronize products without the dye because it was bright orange red, an indication of inferior quality.

A visit to twenty (20) palm oil processing factories (Craman centers), in the Eastern Region revealed that Sudan dyes were not added to palm oil consignments at the processing site. Seventy five percent (75%) of the processors indicated that they had not seen the dye before and do not know what it is but have heard of it. Twenty five percent (25%) of the manufacturers indicated that some distributors have shown the dye to them and wanted them to add it but they refused because it has never been a practice to add dyes to palm oil. According to the processors of palm oil they are not involved in the use of Sudan dyes to adulterate the palm oil.

4.6 COLOUR OF CRUDE PALM OIL

The reason for adulterating crude palm oil with Sudan IV dye was to enhance the colour to meet customer expectation and mask the effect of inferior quality oil feature due to poor processing practices. The palm fruit has carotenoids which are responsible for the bright orange red colour of the crude palm oil. Therefore any factor which affects the carotene content of the fruit most invariably influences the colour of crude palm oil. The variety and maturity of palm fruit coupled with the processing conditions influence the colour of the product. The mesocarp of the palm fruit content has chlorophyll and carotenoid; the ratio of the two in the mesocarp influences the colour of crude palm oil (Sundram, n.d.).

Carotenoid content of matured palm fruits are more than its chlorophyll content than for immature palm fruits. This implies that the quantity of carotenoids in the mesocarp is influenced by the time of harvesting and consequently the colour of crude palm oil. During lipid oxidation, hydroperoxides generated accelerate carotene oxidation resulting in bleaching and discolouration of crude palm oil (Sundram, n.d.).

For most small scale processors palm fruits are harvested and stored for weeks before processing. This result in an increase in the free fatty acid content of crude palm oil which according to Osei Amponsah (2012) results in the generation of hydroperoxides during lipid oxidation which breakdown the carotenoids and consequently deteriorate the bright orange-red colour of crude palm oil. Leaching of iron into the product during processing due to the use of equipment fabricated from cast iron and mild steel catalyzes the formation of hydroperoxides during storage (Sundram, n.d.).

The above factors degrade the bright orange-red colour of the crude palm oil and drive the consumer demand for brightly coloured red palm oil; which is achieved by adulteration of crude palm oil with Sudan IV dye.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

In considering adulteration of palm oil with Sudan IV dye, an assessment of how widespread the practice was in the Greater Accra Region was done. This involved sampling palm oil products from ten (10) open market and three (3) supermarkets. The level of adulteration of palm oil with Sudan IV dye on the open market in the Greater Accra region was determined to be 96%. This implied that almost all the palm oil products on the open market was adulterated with Sudan IV dye. The level of adulteration of branded prepackaged palm oil products with Sudan IV dye was determined to be 60%. Analysis of the samples from the open market revealed three other Sudan dyes, Sudan I, Sudan II and Sudan III. These dyes were however not found in branded prepackaged products.

The detection of crude palm oil adulterated with Sudan IV dye is an indication of inadequate surveillance and testing protocols by the Ghanaian regulatory institutions. It is important for these institutions to be adequately resourced to discharge their mandate of safeguarding public health and safety.

5.2. RECOMMENDATIONS

Based on the above findings and the need to remedy the situation I recommend the following:

1. Regulatory market surveillance and testing system which involve randomly sampling crude palm oil from markets should be strengthened. The regulatory institutions must be adequately resourced to undertake this exercise since the core mandate of the institution is to protect public health and safety.

2. There is the need to foster a closer relationship with the various Palm Oil Vending Associations in the various markets for educational visits aimed at curbing the practice entirely. It is envisaged that the collaboration will not only promote the volunteering of information but will also ensure a watchdog activity on the markets.
3. An intensive public education on the need to abstain from food fraud and the dangers of food adulteration. The public must also be made aware of punitive measures against the practice.
4. Development of cost effective processing technology and equipment and education of stakeholders within the palm oil value chain on the role of how processing practices and conditions end up compromising the quality and colour of crude palm oil.
5. Reorganization of the crude palm oil value chain to promote traceability to facilitate the implementation of regulatory and market surveillance mechanisms. This will promote effective regulation of the informal sector involved in the production and retailing of crude palm oil.

6. Development of rapid detection test kits for Sudan dyes in general and specifically for Sudan IV dye. These rapid detection kits could be used by retailers and regulatory institutions for quick detection.
7. The importation of Sudan dyes into the country should be monitored and used solely for the purposes imported into the country. The Environmental Protection Agency (EPA) should monitor the use of these dyes and the effect on the environment.

REFERENCES

1. A Report by World Growth, (2011): World Growth, Palm Oil Growth Development. The Economic Benefit of Palm Oil to Indonesia. http://worldgrowth.org/site/wp-content/uploads/2012/06/WG_Indonesian_Palm_Oil_Benefits_Report-2_11.pdf
2. Acheson D. (2007): Food Protection, Food Safety and Food Defense. Paper presented at the Association of Food and Drug Officials (AFDO) Annual Conference, USA.
3. Agilent Technologies, Inc., HPLC Basic, Fundamentals of Liquid Chromatography (HPLC)
4. Alauddin, S. (2012): Food adulteration and society, JCIRA 1(7): 3-5
5. Ayodele T., (2010): African Case Study: Palm Oil and Economic Development in Nigeria and Ghana; Recommendations for the World Bank's 2010 Palm Oil Strategy
6. BfR Opinion, (2003): Federal Institute for Risk Assessment, Dyes Sudan I to IV in food
7. Chandrasekharan, N. and Kalyana, S. (1995): Minor Components in Edible Oils and Fats; Their Nutritional Implications. Palm Oil Developments..
8. Charnley Berris, (2008): Arguing over adulteration: the success of the Analytical Sanitary Commission Division of History and Philosophy of Science, University of Leeds, Leeds, LS2 9JT
9. Choo, Y.M. (1995) Carotenoids from palm oil, Palm Oil Developments 22,1-6
10. Ciba Foundation Symposium on Vitamin E. (1983): Biology of Vitamin E, Pitman, London.
11. DG SANCO, (2005): Final report of a mission carried out in Ghana in order to assess the control systems in place for Sudan dyes adulteration in palm oil. European Commission Health & Consumer Protection Directorate-General

file:///C:/Users/FDAFSMD_WS05/Downloads/Report%207672%20of%20year%202005%20unsecured%20(1).pdf

12. EC (2015b) European Commission, Food Safety [Online] Food Additives Available from: https://webgate.ec.europa.eu/sanco_foods/main/?event=substances.search&substances.pagination=1 [Accessed: October 7 2015]
13. EFSA (2005): "Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission to review the toxicology of a number of dyes illegally present in food in the EU" The EFSA Journal (2005) 263, 1–71, online content: http://www.efsa.europa.eu/etc/
14. Food and Drug Administration (2009): Economically Motivated Adulteration; Public Meeting; Request for Comment. [Electronic Version]. Fed Register 74(64):15497–9. http:// edocket.access.gpo.gov/2009/E9–7843.htm. Accessed January 23, 2012.
15. Ghana Statistical Service, (2012): 2010 Population and Housing Census, Final Results
16. Grocery Manufacturers Association. (2010): Consumer product fraud: deterrence and detection.
17. Gurr, M.I. (1984): Role of fats in nutrition, Elsevier Applied Science, London. pp.95-146
18. Hartley, C.W.S. (1984): The Oil Palm, Longman, London. pp.697-780,
19. Hayenga Ingrid (2011), Sudan Red Dye Standards, New standards and deuterated standards for the reliable analysis of these carcinogenic compounds in foodstuffs, AnalytiX Volume 8 Article 4
20. Hornstra, G. (1987): Dietary Lipid and Cardiovascular Disease: Effects of Palm Oil. International Oil Palm/Palm Oil conference. 29th June - 1 July, Kuala Lumpur.

21. Hornstra, G. (1987): Dietary Lipid and Cardiovascular Disease: Effects ofPalm Oil. International Oil Palm/Palm Oil conference. 29th June - 1 July, Kuala Lumpur.
22. Howson, C.P.; Eileen T. Kennedy, and Abraham Horrwitz (1998): Prevention of micronutrient deficiency, tools for policy makers and public health workers, committee on micronutrient deficiencies Board on International Health, Food and Nutrition, p. 14. National Academy Press.
23. IARC (1987). Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Supplement 7. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer, Lyon, France.
24. Jalani, B.S. and Cheah, S.C. (1997): The role of biotechnology in making oil palm the major supplier of edible and renewable industrial feedstock, Palm Oil Technical Bulletin 3, (4) 2-4.
25. Johnson Renée (2014): Food Fraud and "Economically Motivated Adulteration" of Food and Food Ingredients. Congressional Research Service
26. Mensah N.K., (1999): Effect of Processing methods on the quality of Palm oil in Ghana. A thesis presented to the Department of Nutrition and Food Science, University of Ghana, in partial fulfilment for the degree of master of philosophy in food science
27. Moore JC, John Spink and Markus Lipp, (2012): Development and Application of a Database of Food Ingredient Fraud and Economically Motivated Adulteration from1980 to 2010
28. Moore JC, Lipp M, Griffiths JC. (2011): Preventing the adulteration of food protein. FoodTechnol. US Pharmacupia, Journal of Food Science. Vol.77, Nr.4, 2012

29. Public Health Act, 2012 (2012): Act 851

30. Püntener, A. and Page, C (2004), European Ban on Certain Azo Dyes, Quality and Environment.

31. Rapid Alert System for Food and Feed (RASFF), (2005): Annual Report of the Functioning of the RASFF 2004. Version 2 of 06-04-2005. Available on: http://europa.eu.int.comm/food/food/rapidalert/report2004en.pdf

32. Schier JG, Rubin C, Miller D, Barr D, McGeehin M. (2009) Medication-associated diethylene glycol mass poisoning: a review and discussion on the origin of contamination. Journal of Public Health Policy, Vol. 30, No. 2 (Jul., 2009), pp. 127-143

33. Spink J, Moyer DC. (2011b): Defining the public health threat of food fraud. Journal of Food Science and other resources from MSU's Food Fraud Initiative (http://foodfraud.msu.edu/).

34. Spink J. (2010): A food manufacturer's perspective on the prevention of economically motivated adulteration. Paper presented the Inst. of Food Technologists Annual Meeting and Food Expo, July 18–20, 2010 in Chicago, Ill. Symposium: Compendial quality standards for food ingredients—an efficient first-line defense against economically motivated adulteration.

35. Spink J. (2011a). The challenge of intellectual property enforcement for agriculture technology transfers, additives, raw materials, and finished goods against product fraud and counterfeiters. Journal Intellect Property Rights 16(2):183–93.

36. Spink J. (2011b): Defining food fraud and the chemistry of the crime for imported food products. In: Ellefson W, Zach L, editors. Import food safety. Washington, DC: Inst. of Food Technologists.
37. Spink J. and D.C. Moyer, (2011): "Defining the Public Health Threat of Food Fraud," Journal of Food Science, Journal, Food Science. 76(9):R157–163.
38. Sundram K. (n.d.) Palm Oil: Chemistry and Nutrition Updates [Online] Available from: http://www.americanpalmoil.com/pdf/DR%20Sundram.pdf [Accessed: October 6, 2015]
39. Vasireddi, S.P. (2013): Workshop on food defense awareness for food business operators and exporters. Ppt presentation Hyderabad, India.
40. Wolf, G. (1982): Is dietary Beta-carotene an anti-cancer agent? Nutr. Revs 40, 257

APPENDIX

Appendix 1: Results of HPLC analysis of palm oil samples for Sudan dyes from ten markets in Accra

NAME OF MARKET	SAMPLES	SUDAN DYES				PERCENTAGE ADULTERATION
		Sudan I	Sudan II	Sudan III	Sudan IV	
Madina Market	1	×	×	×	×	80
	2	×	√	×	√	
	3	×	√	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Dome Market	1	×	√	×	√	100%
	2	×	√	×	√	
	3	×	×	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Dansoman Market	1	×	√	×	√	100%
	2	×	√	×	√	
	3	×	√	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Agbogbloshie Market	1	×	×	×	√	100%
	2	×	×	×	√	
	3	×	×	×	√	
	4	×	×	×	√	
	5	×	×	×	√	
Makola No 2 Market	1	×	√	×	√	100%
	2	√	√	×	√	
	3	×	√	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Mallam Market	1	×	√	×	√	100%
	2	×	√	×	√	
	3	×	√	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Mallam Atta	1	×	×	√	√	100%

Market	2	×	√	×	√	
	3	×	√	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Tema Community 1 Market	1	×	×	×	√	100%
	2	×	√	×	√	
	3	×	√	×	√	
	4	×	√	×	√	
	5	×	√	×	√	
Ashaiman Market	1	×	×	×	√	100%
	2	×	×	×	√	
	3	×	×	×	√	
	4	×	×	×	√	
	5	×	×	×	√	
Kaneshie Market	1	×	×	×	√	80%
	2	×	√	×	√	
	3	×	√	×	√	
	4	×	×	×	√	
	5	×	×	×	×	

Appendix 2: Result of branded prepackaged products sampled from supermarkets

NAME OF MARKET	**SAMPLES**	**SUDAN DYES**				**Percentage contamination**
		Sudan I	**Sudan II**	**Sudan III**	**Sudan IV**	
Branded Prepackaged Products from Supermarkets	1	×	×	×	√	60
	2	×	×	×	√	
	3	×	×	×	√	
	4	×	×	×	×	
	5	×	×	×	×	

Appendix 3: Percentage adulteration of palm oil samples with Sudan dyes from the open market

NAME OF MARKET	PERCENTAGE SUDAN DYE ADULTERATION			
	Sudan I	**Sudan II**	**Sudan III**	**Sudan IV**
Madina Market	0	80	0	80
Dome Market	0	80	0	100
Dansoman Market	0	100	0	100
Agbogbloshie Market	0	0	0	100
Makola No 2 Market	20	100	0	100
Mallam Market	0	100	0	100
Mallam Atta Market	0	80	20	100
Tema Community 1 Market	0	80	0	100
Ashaiman Market	0	0	0	100
Kaneshie Market	0	40	0	80

Appendix 4: Questionnaire to determine the source of Palm oil and Sudan IV Dye

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

QUESTIONNAIRE TO ASCERTAIN THE SOURCE OF PALM OIL AND SUDAN IV DYE

Name of Market ……..

Name of Palm oil Vendor ……..

Telephone ……..

Gender Male ◯ Female ◯

Age 18 –30 ◯ 31 – 40 ◯ 41 – 50 ◯ 51 and above ◯

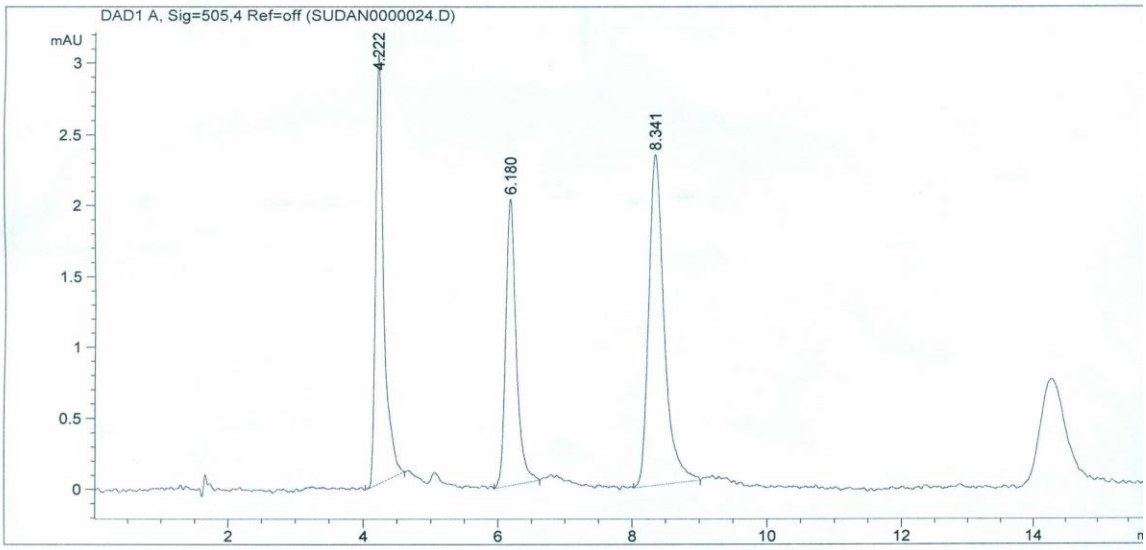
Distributor ◯ Retailer ◯

QUESTION	**RESPONSE**
How long have you been selling palm oil?	
Which production areas do you get your palm oil from?	
Do you know Sudan IV dye?	
Have you used Sudan IV in your palm oil before or do you know people who use it?	
Why is the Sudan IV dye added to palm oil?	
At what point is the Sudan IV dye added?	
Do you know the source of the Sudan IV dye?	

Appendix 5 Some Chromatograms representing the results from spike palm oil with Sudan I, II, III, IV

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-16 09-18-15\SUDAN0000024.D
Sample Name: standard chk

Acq. Operator	: Leslie Owusu-Ansah	Seq. Line	: 13
Acq. Instrument	: HPLC 1260	Location	: Vial 2
Injection Date	: 9/16/2015 4:02:57 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Sequence File	: C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-16 09-18-15\SUDAN.S		
Method	: C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-16 09-18-15\SUDAN DYE.M (Sequence Method)		
Last changed	: 9/16/2015 9:53:43 AM by Leslie Owusu-Ansah		
Sample Info	: SUDAN I,II,III,IV 1ppm		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

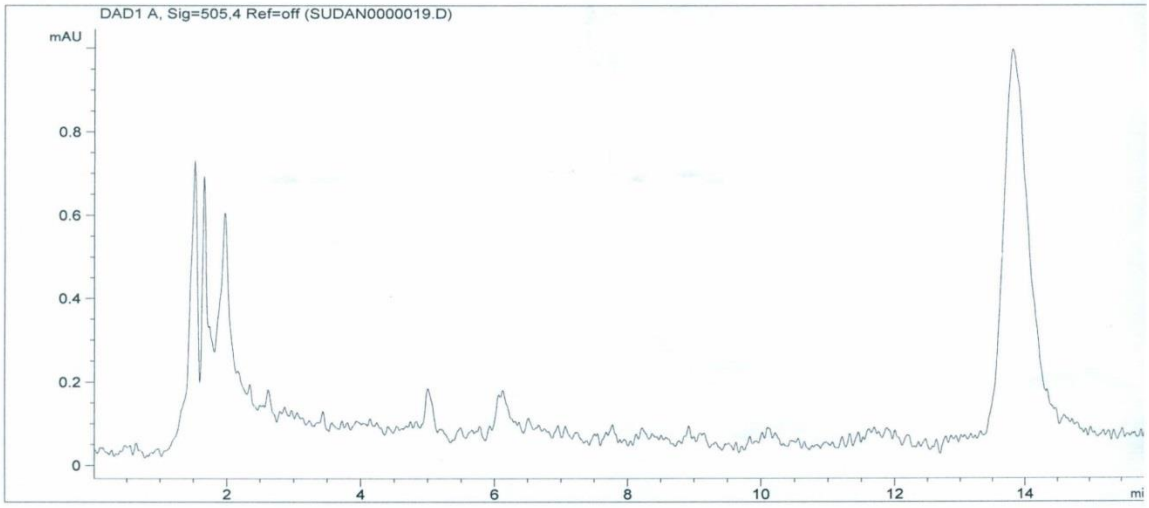
Signal 1: DAD1 A, Sig=505,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.222	BB	0.1215	25.28402	3.02560	29.7901
2	6.180	BB	0.1653	22.04939	2.02062	25.9790
3	8.341	BB	0.2458	37.54037	2.33248	44.2308
Totals :				84.87379	7.37870	

HPLC 1260 9/16/2015 4:19:02 PM Leslie Owusu-Ansah Page 1 of 1

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 20-41-46\SUDAN0000019.D
Sample Name: Madina 3

Acq. Operator	: Leslie Owusu-Ansah	Seq. Line	: 11
Acq. Instrument	: HPLC 1260	Location	: Vial 15
Injection Date	: 9/18/2015 2:00:30 AM	Inj	: 1
		Inj Volume	: 20.000 µl
Sequence File	: C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-17 20-41-46\SUDAN.S		
Method	: C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 20-41-46\SUDAN DYE.M (Sequence Method)		
Last changed	: 9/17/2015 8:41:46 PM by Leslie Owusu-Ansah		
Sample Info	: Madina 3		



Area Percent Report

Sorted By	:	Signal
Multiplier	:	1.0000
Dilution	:	1.0000

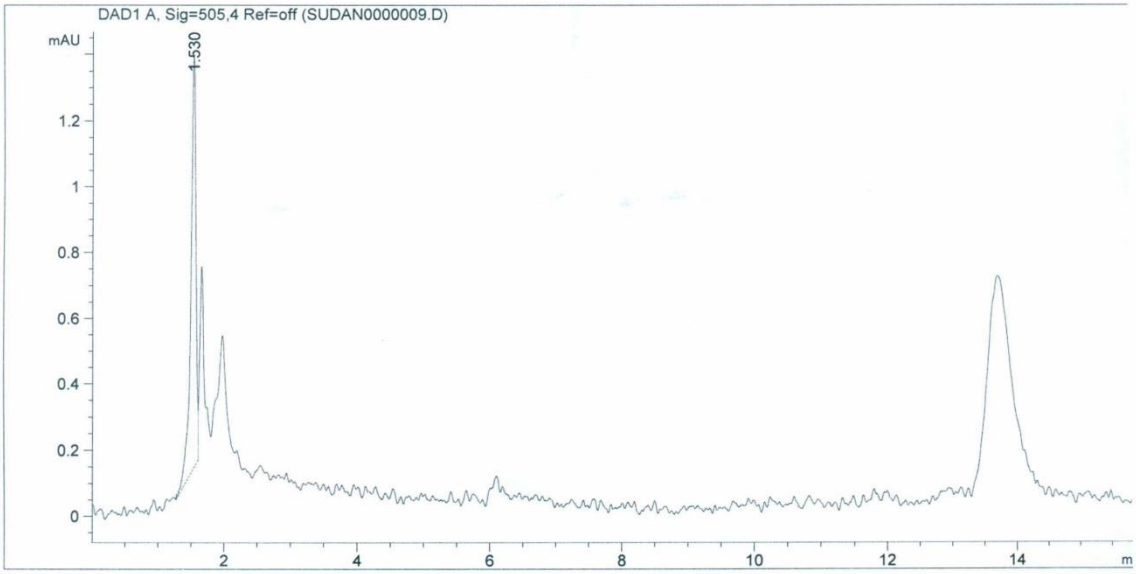
Use Multiplier & Dilution Factor with ISTDs

No peaks found

*** End of Report ***

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 20-41-46\SUDAN0000009.D
Sample Name: Domi 3

Acq. Operator	: Leslie Owusu-Ansah	Seq. Line	: 6
Acq. Instrument	: HPLC 1260	Location	: Vial 10
Injection Date	: 9/17/2015 11:04:18 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Sequence File	: C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-17 20-41-46\SUDAN.S		
Method	: C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 20-41-46\SUDAN DYE.M (Sequence Method)		
Last changed	: 9/17/2015 8:41:46 PM by Leslie Owusu-Ansah		
Sample Info	: Domi 3		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

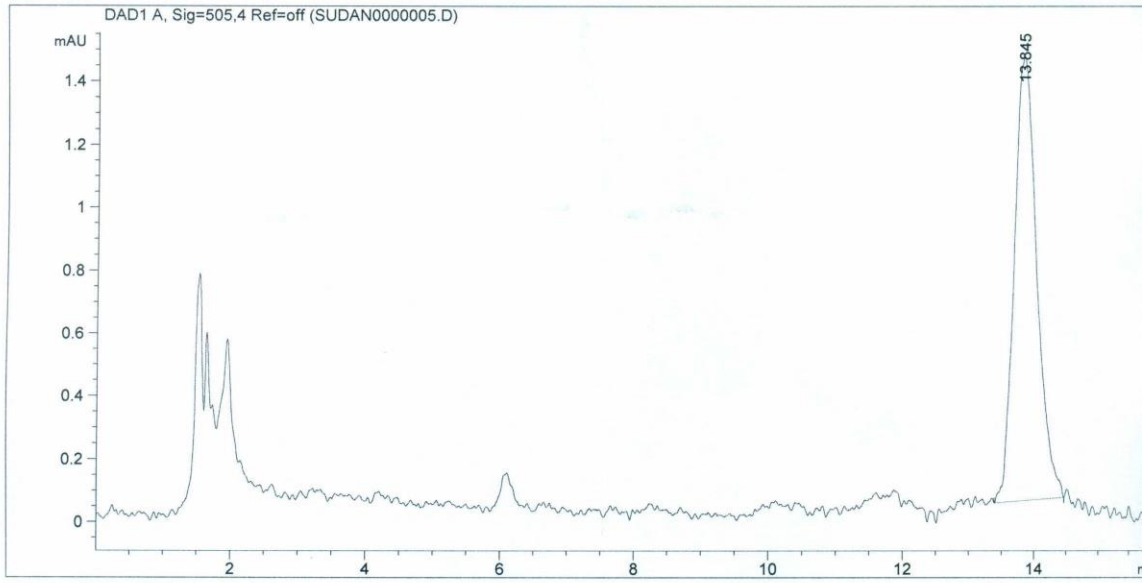
Signal 1: DAD1 A, Sig=505,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.530	BV	0.0812	6.58121	1.25373	100.0000
Totals :				6.58121	1.25373	

*** End of Report ***

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-16 09-18-15\SUDAN0000005.D
Sample Name: Dansoman 1

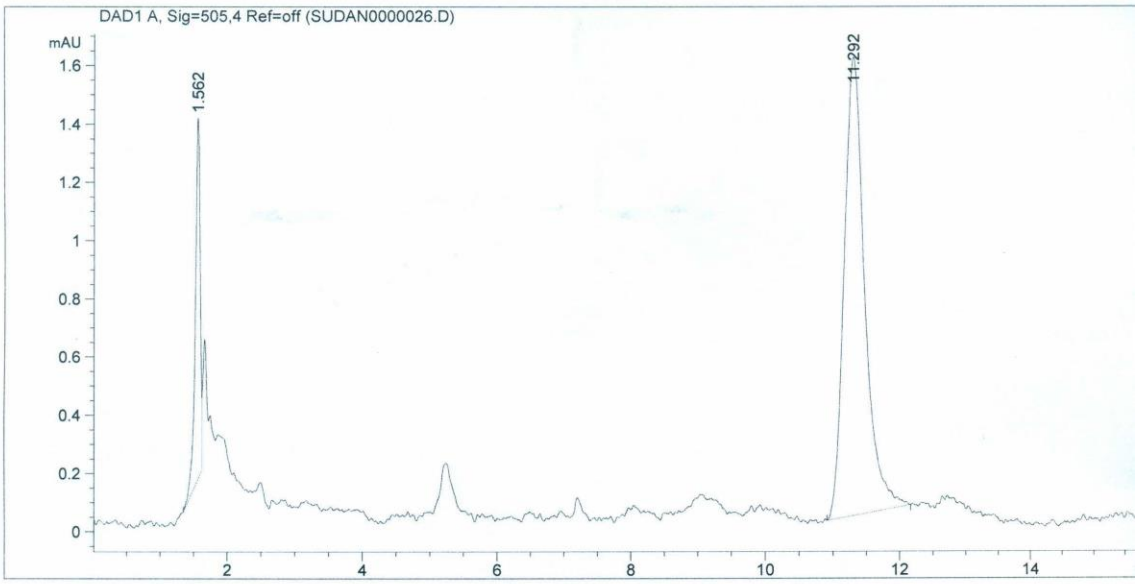
```
==========================================================================
Acq. Operator    : Leslie Owusu-Ansah              Seq. Line :   3
Acq. Instrument  : HPLC 1260                        Location : Vial 3
Injection Date   : 9/16/2015 10:28:05 AM                 Inj :   2
                                                  Inj Volume : 20.000 µl
Sequence File    : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-16 09-18-15\SUDAN.S
Method           : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-16 09-18-15\SUDAN DYE.M (Sequence
                   Method)
Last changed     : 9/16/2015 9:53:43 AM by Leslie Owusu-Ansah
Sample Info      : Dansoman 1
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=505,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1  13.845 BB    0.3108   34.20190    1.41110 100.0000

Totals :                    34.20190    1.41110


==========================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-25 08-08-59\SUDAN0000026.D
Sample Name: Agbogbloshie 2

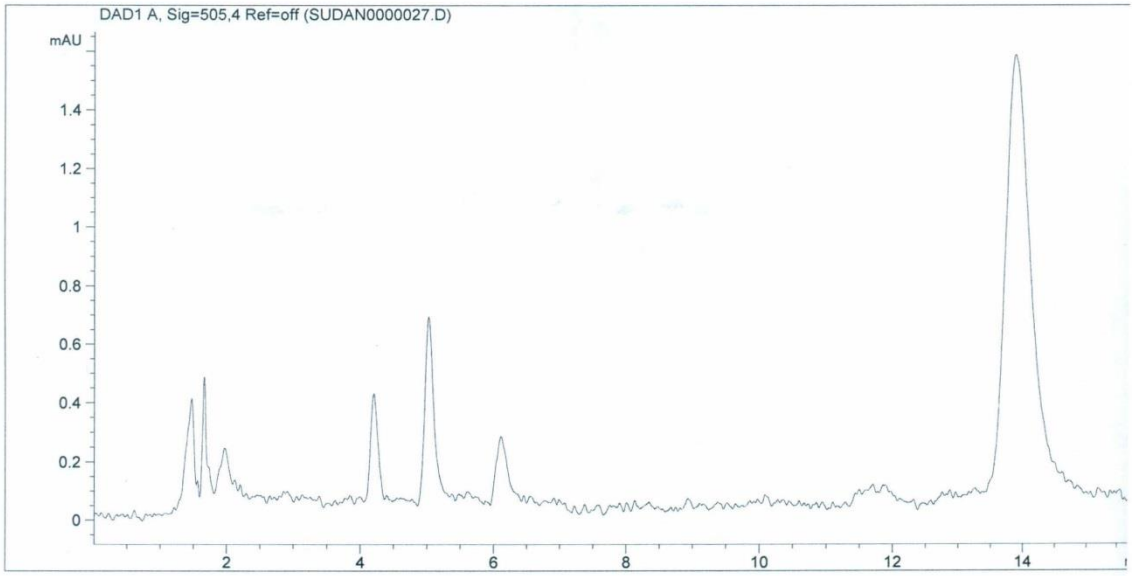
```
==========================================================================
Acq. Operator    : Leslie Owusu-Ansah              Seq. Line :  14
Acq. Instrument  : HPLC 1260                       Location : Vial 4
Injection Date   : 9/25/2015 3:30:55 PM                Inj :   1
                                                  Inj Volume : 20.000 µl
Sequence File    : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-25 08-08-59\SUDAN.S
Method           : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-25 08-08-59\SUDAN DYE.M (Sequence
                   Method)
Last changed     : 9/25/2015 8:08:59 AM by Leslie Owusu-Ansah
Sample Info      : Agbogbloshie 2
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=505,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]    [mAU*s]     [mAU]      %
----|-------|----|-------|----------|----------|--------|
   1   1.562 BV    0.0772    6.31498    1.24438  15.8566
   2  11.292 BB    0.3123   33.51048    1.57477  84.1434

Totals :                    39.82546    2.81915


==========================================================================
                          *** End of Report ***

HPLC 1260 9/25/2015 3:47:00 PM Leslie Owusu-Ansah                Page   1 of 1
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 20-41-46\SUDAN0000027.D
Sample Name: Makola 2

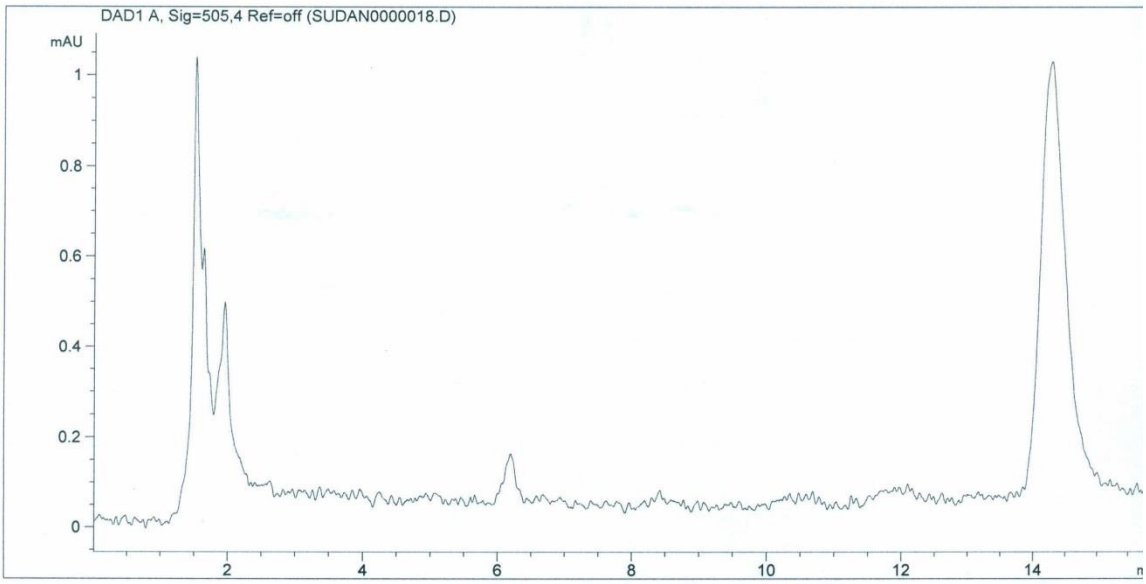
```
==========================================================================
Acq. Operator    : Leslie Owusu-Ansah            Seq. Line :  15
Acq. Instrument  : HPLC 1260                      Location : Vial 19
Injection Date   : 9/18/2015 4:21:21 AM              Inj :   1
                                                 Inj Volume : 20.000 µl
Sequence File    : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-17 20-41-46\SUDAN.S
Method           : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 20-41-46\SUDAN DYE.M (Sequence
                   Method)
Last changed     : 9/17/2015 8:41:46 PM by Leslie Owusu-Ansah
Sample Info      : Makola 2
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

==========================================================================
                           *** End of Report ***
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-16 09-18-15\SUDAN0000018.D
Sample Name: Mallam Market 3

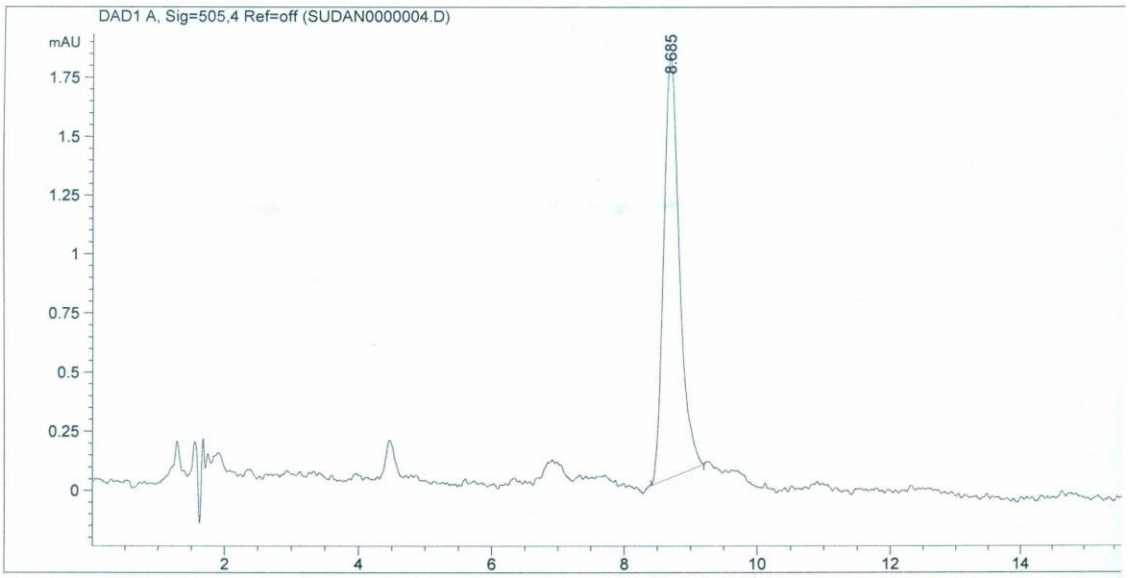
```
==========================================================================
Acq. Operator    : Leslie Owusu-Ansah              Seq. Line :  10
Acq. Instrument  : HPLC 1260                        Location : Vial 10
Injection Date   : 9/16/2015 2:17:19 PM                Inj :   1
                                                  Inj Volume : 20.000 µl
Sequence File    : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-16 09-18-15\SUDAN.S
Method           : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-16 09-18-15\SUDAN DYE.M (Sequence
                   Method)
Last changed     : 9/16/2015 9:53:43 AM by Leslie Owusu-Ansah
Sample Info      : Mallam Market 3
```




```
==========================================================================
                          Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

==========================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-29 07-10-18\SUDAN0000004.D
Sample Name: Mallam Atta 2

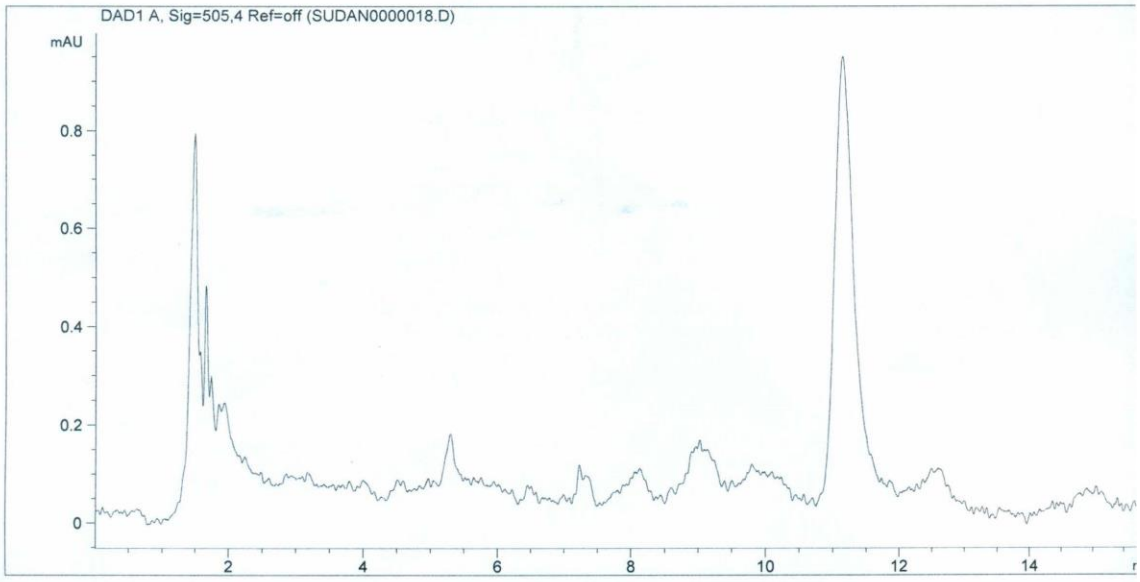
```
Acq. Operator   : Beatrice Aberdey Mensah        Seq. Line :   3
Acq. Instrument : HPLC 1260                      Location : Vial 3
Injection Date  : 9/29/2015 8:04:33 AM               Inj :   1
                                                 Inj Volume : 20.000 µl
Sequence File   : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-29 07-10-18\SUDAN.S
Method          : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-29 07-10-18\SUDAN DYE.M (Sequence
                  Method)
Last changed    : 9/29/2015 7:10:18 AM by Beatrice Aberdey Mensah
Sample Info     : Mallam Atta 2
```




```
=====================================================================
                        Area Percent Report
=====================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs


Signal 1: DAD1 A, Sig=505,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   8.685 BB    0.2420   29.35238    1.78333 100.0000

Totals :                    29.35238    1.78333


=====================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-25 08-08-59\SUDAN0000018.D
Sample Name: Tema 3

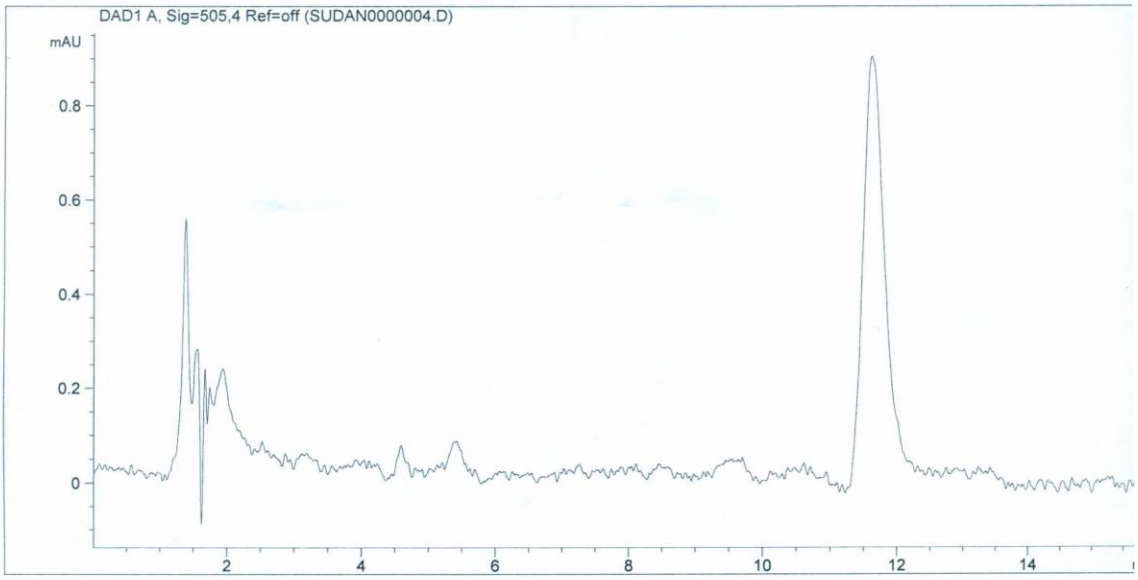
```
==========================================================================
Acq. Operator    : Leslie Owusu-Ansah            Seq. Line :  10
Acq. Instrument  : HPLC 1260                      Location : Vial 10
Injection Date   : 9/25/2015 1:09:53 PM              Inj :   1
                                                 Inj Volume : 20.000 µl
Sequence File    : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-25 08-08-59\SUDAN.S
Method           : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-25 08-08-59\SUDAN DYE.M (Sequence
                   Method)
Last changed     : 9/25/2015 8:08:59 AM by Leslie Owusu-Ansah
Sample Info      : Domi  3
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

==========================================================================
                          *** End of Report ***
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-25 08-08-59\SUDAN0000004.D
Sample Name: Ashaiman 1

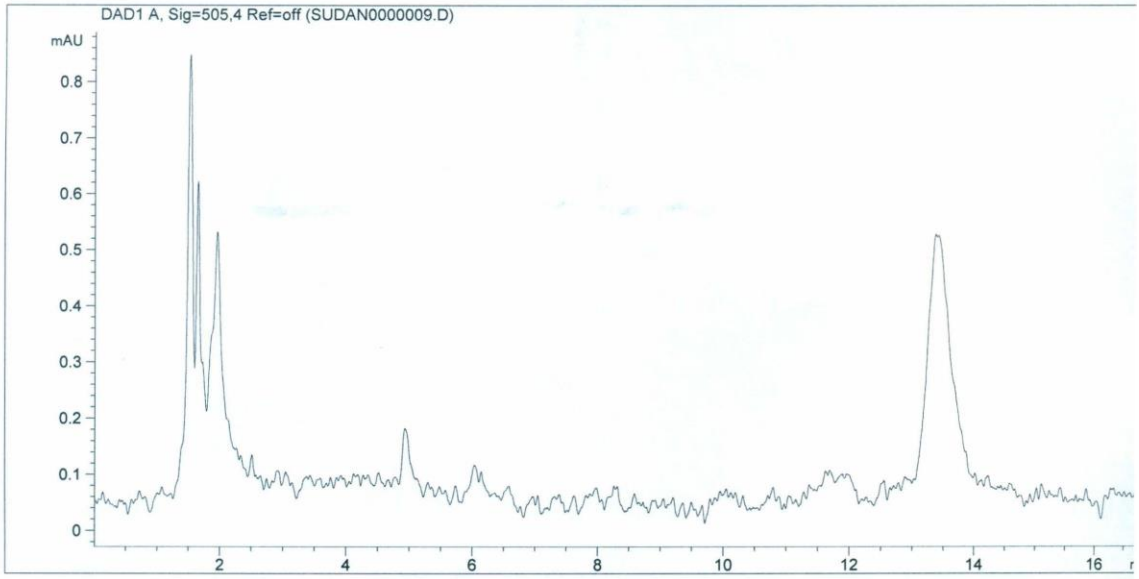
```
==========================================================================
Acq. Operator   : Leslie Owusu-Ansah          Seq. Line :   3
Acq. Instrument : HPLC 1260                   Location : Vial 13
Injection Date  : 9/25/2015 9:03:09 AM             Inj :   1
                                              Inj Volume : 20.000 µl
Sequence File   : C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-25 08-08-59\SUDAN.S
Method          : C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-25 08-08-59\SUDAN DYE.M (Sequence
                  Method)
Last changed    : 9/25/2015 8:08:59 AM by Leslie Owusu-Ansah
Sample Info     : Ashaiman 1
```




```
==========================================================================
                        Area Percent Report
==========================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

==========================================================================
                        *** End of Report ***
```

Data File C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 15-25-50\SUDAN0000009.D
Sample Name: Kaneshie 3

Acq. Operator	: Leslie Owusu-Ansah	Seq. Line	: 6
Acq. Instrument	: HPLC 1260	Location	: Vial 5
Injection Date	: 9/17/2015 5:52:02 PM	Inj	: 1
		Inj Volume	: 20.000 µl
Sequence File	: C:\Chem32\1\DATA\SudanDyes\SUDAN 2015-09-17 15-25-50\SUDAN.S		
Method	: C:\CHEM32\1\DATA\SUDANDYES\SUDAN 2015-09-17 15-25-50\SUDAN DYE.M (Sequence Method)		
Last changed	: 9/17/2015 4:41:21 PM by Leslie Owusu-Ansah		
Sample Info	: Kaneshie 3		



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

No peaks found

*** End of Report ***