THIN LAYER CHROMATOGRAPHIC TECHNIQUES FOR DETECTION OF SUDAN DYES IN PALM OIL

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ABSTRACT

Sudan dyes I, II, III & IV are synthetic, oil-soluble, red-coloured azo dyes. These dyes are suspected genotoxic carcinogens and have been used in the adulteration of palm oil products. Many experimental studies on animal specimen have confirmed the formation of tumour due to the presence of different Sudan dyes in food products. In this study, a Simple Thin Layer Chromatographic method is described for the qualitative detection of Sudan-I, II, III & IV in palm oil gotten from different markets. The study includes extraction of sample, preparation of Thin Layer Chromatography (TLC) plates, Separation, detection and visualization of dyes on TLC plates. The dyes were extracted with solvent Acetonitrile. Sample extracts were decanted and analysed by simple thin layer chromatography in hexane, chloroform and acetic acid solvent system. The chromatograms of samples and standards were stained with Iron (ii) chloride stain and further evaluated in term of R_f values. Out of the five samples analysed the R_f values of the colouring spots from the three samples L2, L3 and L4, matched the standards (S2). It is therefore, concluded that samples L2, L3 and L4 contain Sudan IV dye. Without using any derivation, the limit of detection (18mg/L for S2 (Sudan IV dye) and 10mg/L for S_I (Sudan III dye) was determined by visually determining if a spot was visible or not at a certain concentration of standard dye.

Keywords: Thin Layer Chromatography; Palm oil; Sudan I, II, III and IV, Iron (ii) chloride stain

INTRODUCTION

In the food industries, colour is the most distinguished and significant characteristic of food products [1]. Many colouring agents are often added to different foods to make them visually appealing and for promotion of sales [2]. The colouring matter in food may be natural or synthetic [1]. They may also be classified as water soluble and oil soluble. Natural colours consist of chlorophyll, Caramel, Annatto, Carotenes, Curcumin, Capsainthin etc. Synthetic colours are of importance as they are widely used in different foods. Synthetic colours are further classified as acidic and basic dyes [3]. There are certain synthetic colorants that are unpermitted due to the adverse effects they have on the body. Sudan dyes are a

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family of compounds in the class of azo dyes that are used for different industrial and scientific applications [4]. They find applications in the industries and analytical research [3] and are used in paints, cosmetic products, for colouring plastics and other synthetic materials [2]. Examples of sudan dyes include Sudan I, Sudan II, Sudan III and Sudan IV, Sudan red B, Sudan red 7B, Sudan red G, Sudan orange G, Sudan black, Di-methyl yellow, Para red [3]. Sudan dyes are however, illegally used as food adulterants [5], particularly in red palm oil because of their intense red-orange colour and low price [3]. A large part of Sudan findings and reports are from palm oil. Since 2003, reports by RASFF showed that around 20% cases of Sudan dye adulteration are from palm oil. Palm oil is contaminated mostly with Sudan IV (less often with Sudan I) and originates from various African countries [6]. Sudan dyes are fat soluble azo dyes but insoluble in water. This characteristic makes it blend well in palm oil. Marketers involved in such act usually use low quality palm oil such as palm oil sludge. The dyes are added to enhance the ugly colour and improve the visual aesthetic of palm oil sludge. However Sudan I-IV dyes as food additives are not permitted worldwide due to their carcinogenicity [5]. In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) has raised concern over the use of Azo dye, popularly known as Sudan Dye, to make palm oil look reddish and attractive. The Director-General of NAFDAC, Prof. Moji Christianah Adeyeye, raised the concern at a sensitization campaign in Anambra State [7]. Sudan dyes are indirect carcinogens (classified as category 3 carcinogens by IARC) and are therefore banned from the use in foods in Nigeria [3]. In certain clinical experimentations, these azo dyes were metabolized to possible carcinogenic colourless amines, in the liver of mammalians [8]. Also Sudan dyes are used in cosmetic products and animal testing has found that isomers of Sudan III cause allergic reaction [8].

MATERIALS AND METHODS

Reagents and Materials

Analytical grade Sudan III and Sudan IV were purchased. Analytical grade Acetonitrile, hexane, chloroform, and acetic acid were used. The solvent system was made up of hexane, chloroform, and acetic acid in the ratio of (60:40:2). Silica gel TLC grade was used on plates of 10×7 cm.

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For the staining system, Iron (ii) chloride tetra-hydrate and ethanol were used. Palm oil for the determination of adulteration were bought from market in Benin-City.

Lab equipment

TLC Chamber, Glass Capillary Tubes, Lab tape, pencil**,** Gloves and nose masks.

Preparation of Oil Samples

Twelve palm oil samples were collected from local markets in Benin-City including one pure control sample which was gotten from the New Mill in NIFOR. Pure samples were also adulterated with Sudan III and Sudan IV dyes to obtain standards. 5ml of sample was dissolved in 5 ml of the solvent acetonitrile. The mixture was agitated vigorously for 1 minutes and allowed to stand for 15 minutes. The top layer of acetonitrile containing the extracted dye was decanted and ready for spotting.

Preparation of Iron (ii) chloride stain

The ferric chloride ($FeCl_2$) stain is highly specific, and is used mainly to visualize phenols (ArOH). Fe^{2+} in $FeCl_2$ is oxidized to Fe^{3+} which forms coloured complexes with phenols. This reaction can aid the visualization of the Sudan dyes on the chromatogram developed after TLC. This is because Sudan dyes have phenol groups that can be attacked by Fe^{3+} ions.

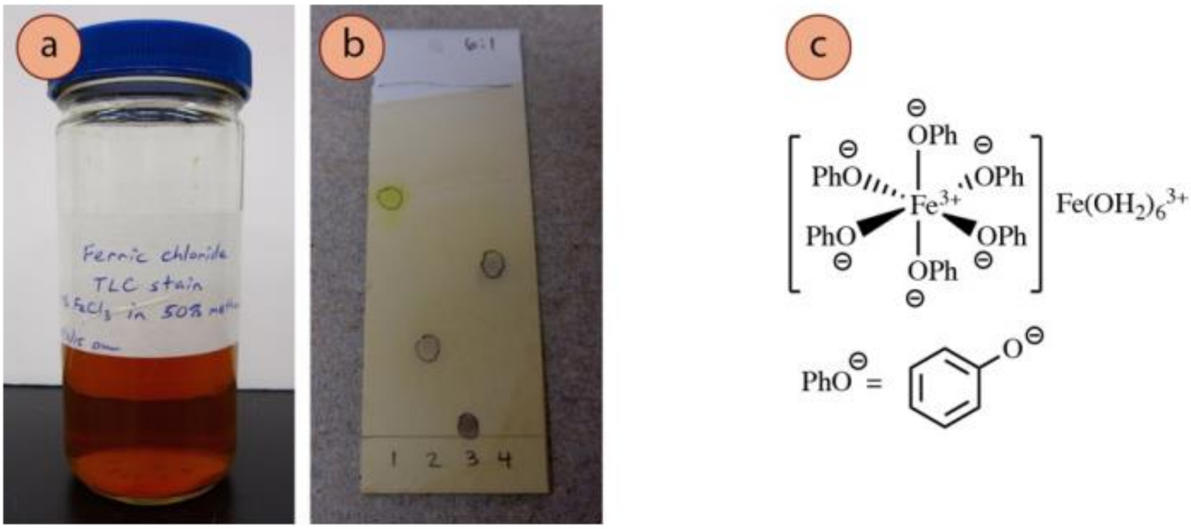

Fig 1: a. $FeCl_2$ stain reagent jar b. TLC plate with various dye spots stained with $FeCl_2$ c. generic phenol-fe^{3+} coloured complex [9].

The Preparation of Iron (ii) chloride stain is done by mixing 1% of $FeCl_2$ in water and ethanol in a 1:1 ratio.

Experiment

Samples and standards were applied as spots in triplicate using glass capillary tubes on a prepared TLC plate (10×7mm). Distance from the left and right edge of the plate was 10 mm and from the lower edge was 8 mm. The distance between the spots was kept at mm. 6 spots were applied on a single plate.

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Development of the plate was performed in saturated TLC jar with different solvent systems and the development time was 20 minutes. For visual evaluation, the chromatograms were visualised by placing the TLC plates in the jar containing the prepared Iron (ii) chloride stain. After 10 minutes, the separated dyes were stained and evaluated under normal light. The distances moved by solvent and the spots were measured and compared between standards and samples.

RESULTS AND DISCUSSIONS

Sudan IV and III were selected for the study because of their colour, health risk and relevance in reported cases of palm oil adulteration. Sudan IV and III were used to prepare standard oil mixtures of concentration 100ppm (0.01%) and labelled S1 and S2 respectively. Out of twelve samples purchased from the local market in Benin-City only five samples were suspected to be adulterated. Five oil samples (L1, L2, L3, L4, L5) were taken randomly that include a control sample L5, which was gotten from the New Mill in NIFOR.

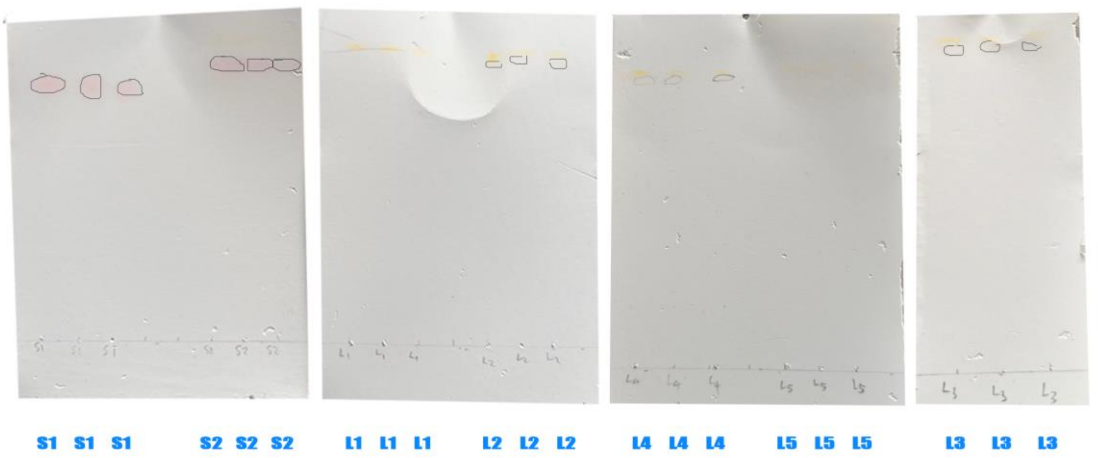

Figure 2: Chromatogram of Samples and Standards after staining

The chromatograms of sample and standards before staining are shown in figure 1. The chromatograms of sample and standards after staining are shown in figure 2. The results were expressed in terms of R_f (retardation Factor) (Table 1). Mean of the R_f values were calculated from three triplicate spots of the given sample/standard.

The standard S1 and S2 showed the grand mean R_f values 0.91 and 0.99 respectively. The relative R_f values of five samples analyzed are shown below in Table 1. There was no dye spot observed in L1 and L5 while L2, L3 and L4 have R_f values of 1. 0.99, 0.98 respectively confirming the presence of the suspected dyes S2 in all three samples.

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The limit of detection was based on visual evaluation of colouring spot.

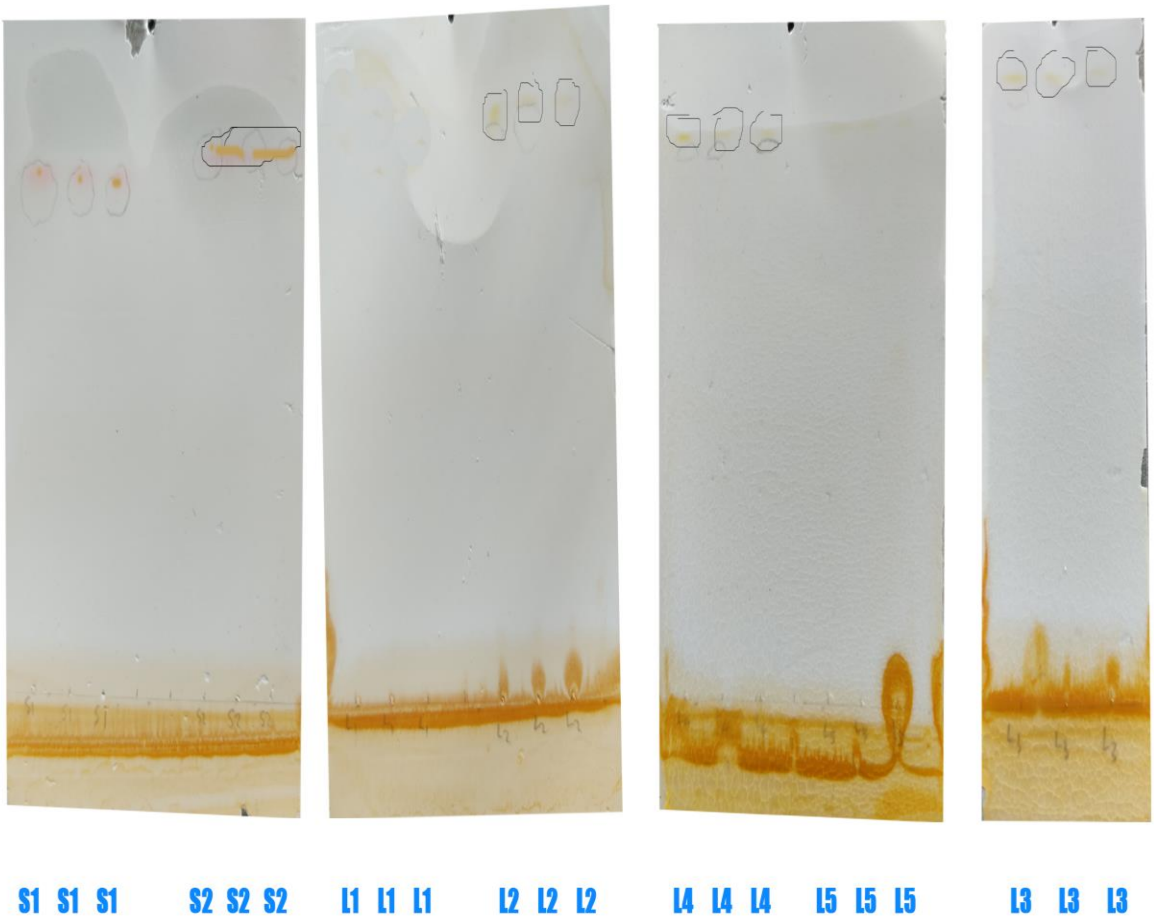

Figure 2: Chromatogram of Samples and Standards after staining

Table 1: Table showing R_f value

Colouring spot	Distance travelled by solvent (cm)	Distance travelled by spot (cm)	R_f value
S_1	7	6.4	0.91
S_2	7	6.9	0.99
L_1	7	None	None
L_2	7	7	1
L_3	7	6.9	0.99
L_4	7	6.85	0.98
L_5	7	None	None

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In Table 1, the performance of sample extraction and TLC separation was cross checked by internal standard procedure, in which pure sample (L5) was spiked using dye concentrations ranging from 20mg/L to 2mg/L of standard dye (S1 and S2). The limit of detection was based on visual evaluation of colouring spot. When standard dyes were spiked in control (crude palm oil), the spots were visible up to 18mg/L for S_2 (Sudan IV dye) and 10mg/L for S1 (Sudan 111 dye).

CONCLUSION

The R_f values of colouring spots from the three samples L2, L3 and L4, matched the standards (S2). It therefore, shows that samples L2, L3 and L4 contain Sudan IV dye. Therefore, more market survey and sampling of palm oil needs to be carried out in Benin-City and other major towns in Edo state and other cities in Nigeria to determine level of adulteration of palm oil.

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