ISOLATION AND IDENTIFICATION OF COLOUR ADDITIVES IN SOME FOOD PRODUCTS

By

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DEDICATION

To the soul of my brother

Abdeen

To my parents

Family members, and

To my dear friends

and colleagues

With love and respect

Elsayda

Acknowledgement

I am grateful to Allah, my special praise and thanks for giving me the health, strength and patience to conducted this research.

Iam greatly indebted to my supervisor, Dr. Khogali Elnour Ahmed Ishag, for helpful, valuable supervision and guidance throughout this work. I wish to express my great gratitude to my friend Huyam Mahmoud for great assistance and help.

My gratitude also extended to the staff of Food Science and Technology, Faculty of Agriculture, University of Khartoum for their help and encouragement.

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My thanks and appreciation are extended to my all friends and to every body who helped me during this study.

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ABSTRACT

Fourteen different types of food commodities were analyzed for colouring materials added which were then identified against reference colouring substance. The food materials studied were samples of jams, powdered juices, sweets and soft drinks, randomly collected from local markets.

A wool dying technique was used for isolation of synthetic food colours. The colouring materials were retained by the wool and stripped with dilute ammonia solution (1 part of strong ammonia to 50 part of water).

The mixed colours were separated by paper and thin layer chromatography (TLC). In both chromatographic techniques, the separations were done using different solvents. The separation of colours on TLC-plates were better and sharper than those with paper chromatography. Furthermore, using the solvent system of trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) system gave better separation on paper than on the solvent ammonia solution: water (1: 99) where more spots were resolved. Similarly, solvent n-butanol: absolute ethanol: 2 N ammonium hydroxide system (60: 20: 20) was better resolving on TLC than on iso amyl alcohol: glacial acetic acid:

water system (40: 20: 20). All samples of food material studied contained only synthetic colours except the soft drink (maaza) which was found to contain natural yellow colouring material in addition to the synthetic sunset yellow colour. The Rf-values measured for the reference food colours were found to be similar to the corresponding colouring matters in the food samples tested.

The soft drinks yellow miranda, red fanta toot and red vita were found to contain food colours of carmosine, sunset yellow, amaranth and tartrazine, and certain unidentified onces. A side of the soft drinks, all other food samples gave single spot colour component.

بسم الله الرحمن الرحيم

خلاصة الأطروحة

تحت دراسة أربعة عشر نوعاً من السلع الغذائية المختلفة لمحتواها من المواد الملونة فيها مقارنة بمواد مرجعية ملونة. شملت المواد الغذائية تحت الدراسة عينات مربات، بودرة عصائر، حلوى ومشروبات غازية تم جمعها عشوائياً من السوق.

تم إستخدام طريقة الصبغ بالصوف لعزل الألوان الغذائية الصناعية حيث يقوم الصوف بالإحتفاظ بالمواد الملونة الصناعية ثم تنزع بواسطة محلول أمونيا مخفف (1part of strong ammonia to 50 part of water).

الألوان المختلطة تم فصلها بواسطة الكروماتوغرافيا الورقية و الكروماتوغرافيا الطبقة الرقيقة. في هاتين الطريقتين تم الفصل بإستخدام أنواع مختلفة من المذيبات.

لقد كان فصل الألوان بكروماتوغرافيا الطبقة الرقيقة أفضل وأوضح مقارنة بكروماتوغرافيا الورقية. المذيب trisodium citrate: ammonia solution : water (2: 5: 95, w/v/v) أعطى فصل أفضل على الورقة مقارنة بالمذيب ammonia solution : water (1: 99) حيث تم فصل مكونات أكثر. المذيب n-butanol: absolute ethanol: 2N ammonia hydroxide (60: 20: 20) أفضل تحليلاً مقارنة بالمذيب isoamyl alcohol: glacial acetic acid: water (40: 20: 20) على الطبقة الرقيقة. كل العينات تحت الدراسة تحتوى على ألوان صناعية عدا المشروب الغازي مازا الذى يحتوى على مكون أصفر باهت طبيعي بالإضافة للمكون أصفر غروب الشمس الصناعي. قياس Rf-value للألوان المرجعية وجد مشابه للمكونات الملونة للعينات التي تم تحليلها.

وجد أن المشروبات الغازية ميرندا صفراء، فانتا حمراء وفيتا حمراء تحتوى على صبغات ضعيفة التركيز لم يتم تحديدها علماً بأن الصبغات الرئيسية تم مقارنتها بالألوان المرجعية. بجانب المشروبات الغازية وجد أن كل العينات الأخرى أعطت مكون ملون واحد.

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CHAPTER ONE

INTRODUCTION

The quality of food, apart from microbiological aspects, is generally based on colour, flavour, texture and nutritive value. One of the most important sensory quality attributes of a food is colour. This is because no matter how nutritious, flavourful or well textured a food may be, it is un likely to be eaten unless it has the right colour (Ashkenazi *et al*; 1991).

Colours and flavours are used in manufactured food to make it more appealing. Colour is probably one of the first characteristic perceived by the senses and indispensable to the modern day consumer a means for the rapid identification an ultimate acceptance of food (Weast, 1972).

Synthetic organic food colours are substances that can be added to food in solution or dispersion. They are superior to the natural dye extract in tinctorial power, consistency of strength, range and brilliance of shade, stability and ease of application (Bell, 1990; Walford, 1980b).

The use of colourants as additives for food and drinks is a significant factor to food manufacturer and consumer alike in determining the acceptability of processed food. For manufacturer, added colourants assist in ensuring batch- to- batch uniformity and reinforce colourants that are already present but are les intensive that the consumer would expect.

For the consumer, added colourant help to restore the original appearance of foods whose natural colourant content has been reduced

by processing treatment, also to provide appealing and readily identifiable products. Food must not to be coloured to simulate a higher level of nutritionally important components or worse, to mask poor quality or spoilage (Blenford, 1985).

A short term of food additives, and particularly food colours, was hyperactivity in some children, believed to form an allergic response. In long term some of these food colours are believed to be carcinogenic and some colours, mostly reds, have been banned from food use (Katz, 2000).

In recent years, food additives in general, and colours in particular, have increasingly come under investigation for evaluation of their safety in use. At present, in many developed countries only about ten dyes are permitted for use as food colouring agents, and many others have been banned in the last two decades due to their toxicity and carcinogecity (Francis, 1985; Gilhooley *et al.*, 1962). Tartrazine, sunset yellow, carmosine, amaranth, ponceau 4R and brilliant blue are synthetic organic azo dyes that may be present in common foods (sweets, drinks, jams, etc).

1.2. The objectives of this study were to:

1. Extract, isolate and identify colouring materials added to some permitted food products.
2. Compare these colours to reference permitted ones.
3. Assess methods used for extraction and identification.

CHAPTER TWO

LITERATURE REVIEW

2.1. General:

Colour refers to human perception of coloured materials – red, green, blue, etc. A colourant is any chemical, either natural or synthetic, that imparts colour. Foods have colour because of their ability to reflect or emit different quantities of energy at wavelengths able to stimulate the retina in the eye. The energy to which the eye is sensitive is referred to as visible light, depending on individuals sensitivity, encompasses wavelength of approximately 380–770 nm. This range makes up a very small portion of the electromagnetic spectrum. In addition to obvious colours (hues), black, white and intermediate grays are also regarded as colours (Vonelbe and Schwartz, 1996).

2.2. Food colour history:

Meggos (1995) stated that, the addition of colourants to food is thought to have occurred in Egyptian cities, where candy makers around 1500 BC added natural extracts and wine to improve the appearance of the products. Up to the middle of the 19^{th} century ingredients, such as the spice saffron, were added for decorative effect to certain foodstuff.

Following the industrial revolution both the food industry and processed food developed rapidly. The addition of colour, via mineral and metal based compounds, was used to disguise low quality and adulterated foods, some more lurid examples according to Downham and Collins (2000) were:

- Red lead (Pb_3O_4) and vermillion (HgS) which were routinely used to colour cheese and confectionery.
- Copper arsenate which was used to recolour used tea leaves for resale. It also caused two deaths when used to colour a dessert in 1860.

Toxic chemicals were used to tint certain candies and pickles. Historical records show that injuries, even deaths, resulted from tainted colourants.

Walford (1980a) pointed out that the first synthetic colour (Maurine) was developed by Sir William Henry Perkin and, by the turn of the century, unmonitored colour additives had spread through Europe in all sorts of popular foods, including Ketchup, mustard, jellies and wine. Sellers at that time offered more that 80 artificial colouring agents; some were intended for dyeing textiles, not foods. Many colour additives had never been tested for toxicity or other adverse effects.

In the year 1900 the bulk of chemically synthesized colour were derived from aniline. When was known to be a constituent of coal tar dyes. Such dyes were loosely called “aniline dyes” irrespective of being actually derived from aniline or “coal tar dyes”. The term “synthetic dyes” is now preferred, since primaries are increasingly obtained from petroleum sources rather than from coal tar (Allen, 1971; Downham and Collins, 2000).

2.3. Classification of food colourants (colour additives):

According to the Food and Drug Administration (FDA), food colourants are classified as either certified or exempt from certification (un- certified). The certified colour are synthetic organic compounds.

Manufacturers must submit a sample from each production batch to the FDA for certification (Hallagan, 1991; Boyd, 1998). The exempt certification includes natural colours and nature identical (Meggos, 1984; Vonelbe and Schwartz, 1996).

Meggos (1984) reported that there are two types of synthetic organic compounds for certified colours namely dyes and lakes. Dyes are water – soluble compounds which manifest their colouring power by being dissolved. The dyes, both primary and blends, are produced in a number of market forms. The most important are powders,

granules, liquids, non flashing blends, pastes and dispersions. Each form suits a specific need, and each possesses it's own advantages. The dyes are used to colour a variety of food products, including beverages, dry mixes, baked goods, dairy products, confections, sausage casings and pet foods. Each application requires a different form of dye; e.g., powders or granules are used for beverages, pastes or dispersions for baked goods and confectionary and liquid colours for dairy products.

There are no FDA limits as to the amount of dye one can use, but good manufacturing practice (GMP) suggests that the dyes must be used at less than 300 ppm. This is more than sufficient to colour the vast majority of the products, with the exception of dark-coloured products, such as chocolate, which may require more.

According to U.S. Food and Drug Administration (1993), lakes are the water insoluble form of the dye. Lakes are more stable than dyes and are ideal for colouring products containing fats and oil or items lacking sufficient moisture to dissolve dyes. Typical uses include coated tablets, and hard candies and chewing gums.

Meggos (1984) showed that lakes generally have better light, chemical stability, and thermal stability than the dyes but are more expensive. The synthetic colours, both dyes and lakes, have good

functional and application proprieties. But like any other food ingredients, they should be handled with care to avoid unnecessary problems. For example, when solutions of the dyes are prepared, the solubility limits should not be exceeded, to prevent precipitation and speckling in the finished product. Compared to uncertified colours, the certified colours (synthetic colours) have many advantages including high tinctorial strength, cost efficiency, good thermal, light and chemical stability, consistent quality and ample supply.

2.4. Synthetic colours and uses:

The first artificial colours were mineral pigments, later supplanted by coal-tar dyes. Some dyes have been proved to be carcinogenic so their uses were prohibited by food regulations, 1957 (Martin, 1965).

According to U.S. Food and Drug Administration, table 1 shows the colour additives which were certifiable for food use:

Table (1) Certified food colours and their common uses in foods

Name(common name)	Colour	Common food uses
FD & C Blue No. 1 (Brilliant Blue) FCF	Bright blue	Beverages, dairy products powders Jellies, confections, condiments, icings syrups, extract.

FD & C Blue No. 2 (Indigotine)	Royal blue	Baked goods, cereals, snake foods, ice cream, confection, cherries.
FD & C Green No. 3 (Fast Green FCF)	Sea green	Beverage, puddings, ice cream, sherbert cherries confections, baked goods, dairy products.
FD & C Red No. 40 (Allura Red AC).	Orange-red	Gelatins, puddings, dairy products confections, beverage condiment.
FD & C Red No. 3 (Erythrosine)	Cherry-red	Cherries in fruit cocktail in canned fruits for salads, confections, backed goods, dairy products, snacks foods.
FD & C Yellow No. 5 (Tartrazine)	Lemon yellow	Custards, beverages, ice cream, confections, preserves, cereals.
FD & C Yellow No. 6 (Sunset yellow)	Orange	Cereals, baked goods, snack foods, ice cream, beverages, dessert powders, confections.

Martin (1965) pointed that the artificial colourings used in food were mainly prepared for colouring textiles and prints and, in their process of manufacture, they may contain small amounts of arsenic, copper, and lead so may be inherently toxic. Others have antispectic properties, for which they are used in medicine. Dyes may mask defects in food products and so deceive the purchaser.

2.5. Classification of synthetic food colours:

Synthetic colours are chemically synthesized pigments that do not occur in nature. The Leatherhead Food Research Agency (LFRA) suggests in its ingredients Handbook (1997) that synthetic colours can be divided into various categories depending on their chemical structures:

- Azo dyes – E102 (Tartrazine), E110 (Sunset yellow FCF), E124 (Ponceau 4R, Cochineal Red A).
- TPM (triphenyl methane/triaryl mehane)-E133 (Brilliant Blue FCF), E142 (Green S).
- Quinoline – E104 (Quinoline yellow).
- Indigoid – E132 (Indigotine, Indigo carmine).
- Xanthene – E127 (Erythrosine).

2.6. Description of synthetic food colours :

Walford (1980b) stated that the descriptions of synthetic colours for food are:

2.6.1. Azo food colours:

In this group of colours the chromophoric system consists essentially of the azo group (Fig.2.1.) in association with one or more aromatic systems:

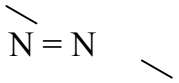

Fig. 2.1. Azo group

There may be one or more azo groups present in the colour molecule, these are the Monoazo, Diazo, Triazo, Tetrakisazo and polyazo dyes according to whether there are 1, 2, 3, 4 or more azo groups present. The orange of shades covered by the azo group of food colours is very wide and includes red, orange, yellow, blue, violet, brown and black. Azo dyes having a green shade are very limited and none is used as food colours.

2.6.2. Triarylmethane food colours:

The chromophoric system consists of a central carbon atom joined to three aromatic rings generally with hydroxyl, amino and substituted amino substituents in the para position acting as auxochromes (Fig.2.2). The triarylmethane food colours are generally bright green and blue in shade but red and violet hues are also available

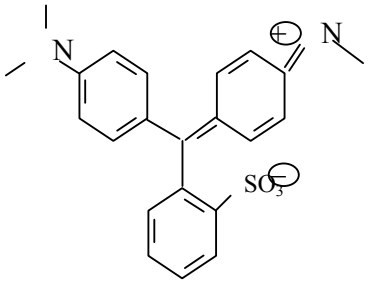

Fig. 2.2. Triarylmethane chromophoric system

2.6.3. Xanthene food colours:

The chromophoric system is the xanthene or dibenzo-1, 4-pyran heterocyclic ring system with amino or hydroxyl groups in the meta position with respect to the oxygen bridge. Generally a further aromatic ring is attached to the xanthene system (e.g. Fig.2.3), analogous to the triarylmethane dyes. The shade of the dye stuff depends on the other substituents and auxochromes present in the molecule. The group gives rise to brilliant red and greenish yellow dyes with fluorescence present in some of the colours. Erythrosine is the only xanthene dye permitted in the EEC or the USA for use in food colouring.

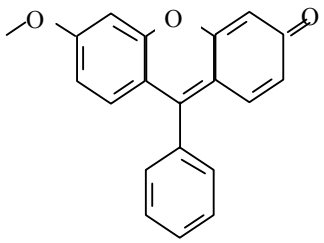

Fig. 2.3. Xanthene chromophoric system with phenyl substituents

2.6.4. Quinoline food colours:

The chromophoric system is the quinophthalone or 2-(2-quinolyl)-1, 3 indandione heterocyclic system (Fig.2.4). In addition the quinoline dyes invariably contain a small amount of the isomeric

phthalyl derivatives (Fig. 2.5). Bright greenish yellow shades with poor light fastness are characteristic of the group. Quinoline yellow is the only dye in this group of importance for use in food colouration

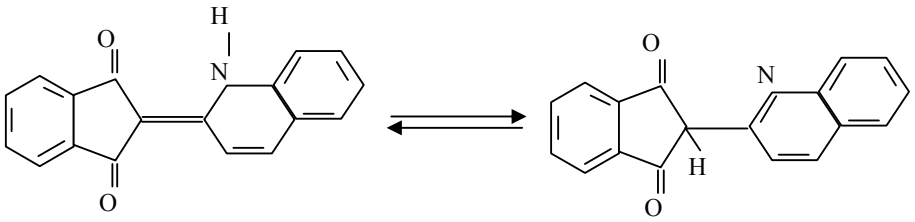

Fig. 2.4. Structure of Quinoline colours

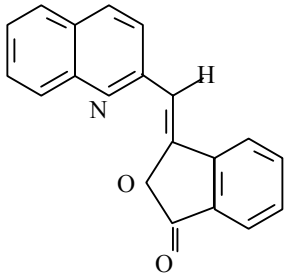

Fig. 2.5. Phthalyl derivative occurring in quinoline colours

2.6.5. Indigoid food colours:

The indigoid group of food is based on synthetic equivalents of naturally occurring indigo. Colour is due to a resonance hybrid of structures (Fig.2.6) or a tetrapole structure (Fig.2.7). The only food colour of importance in this group is indigo carmine.

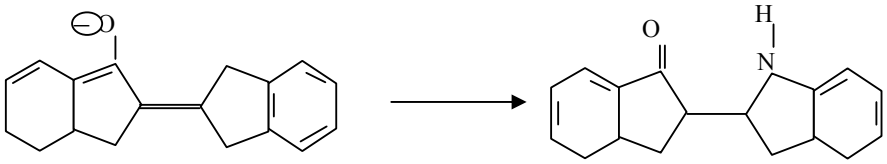

Fig. 2.6. Resonance hybrid structure of Indigo

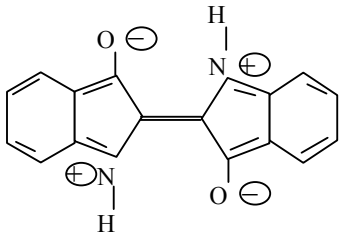

Fig. 2.7. Tetrapole structure of Indigo

2.7. Natural colours:

Natural food colours are extracted and isolated from different plants and animals. As they have no harmful effect, they can be used in food in any amount. These colours are less stable, less bright and not uniform but they are very expensive, moreover it is also difficult to find the exact shade required for different food products (Davis *et al.,* 1964; Maurer *et al.,* 1980).

Ihekoronye and Nogoddy (1985) stated that foods are made up of chemical compounds and among the more interesting groups are the pigments. These natural colours are highly susceptible to chemical change as in fruit ripening and meat ageing. They are also sensitive to chemical and physical effect during food processing. Excessive heat alters virtually all natural pigments. Chopping and grinding also generally change food colours. This is because many of the plant and animal pigments are organized in tissue cells and pigment bodies,

such as the chloroplasts which contain green chlorophyll. When these cells are broken by grinding and chopping the pigments leach and are partially destroyed on contact with air (Potter, 1978).

2.8. Source of natural colours:

Not all food colours come from true plant and animal pigments. Another source of colour comes from the action of heat on sugar which is termed carmalisation. Dark colours may also result from certain chemical interaction between sugars and proteins known as the browning reaction or the Millarad reaction. Natural colours are classified into four major groups which include the chlorophylls, cartenoids, anthocyanines and anthoxanthins. Colours belonging to the latter two groups also are referred to as flavonoids and include the tannins (Potter, 1978).

The principal natural organic colouring matters reported as Martin (1965) are shown in the following table (2):

Table (2) Common natural food colours and their sources

Colour material	Source	Colour
Alkanet	Roots of plant	Red
Annatto	Fruit of shrub	Yellow
Caramel	Burnt sugar	Reddish
Carotene	Carrot and other plants	Yellow
Chlorophyll	Leaves of plants	Green
Cochineal	Dried female insect	Red
Cocoa red	Cocoa beans	Red
Indigo	Easter fern	Blue
Litmus	Lichens	Red
Orchil	Lichens	Red
Flavine	Bark of tree	Yellow
Osage orange	Dried fruit	Yellow
Persian berries	Fruit of thorn bush	Yellow
Safflower	Flower of species saffron	Red
Saffron	Dried stigmas and petals of crocus plant	Yellow
Sandalwood	Red sandal wood	Red
Tumeric	Root of Indian plant	Yellow

2.9. Nature – identical colours:

Basf (1997) stated that nature identical colours have been developed to match their counterparts in nature. The most common pigments that are synthesized are cartenoids.

According to the leatherhead food Research Agency (LFRA) in its Ingredient Handbook (1997) beta-carotene, which is found naturally in carrots, for example, was the first carotenoid to be synthesized and was marketed in 1954. Since then, a number of other carotenoids have been synthesized, including E161g (canthaxanthin), E160e (beta-apo-8-carotenal (C30)) and E160 f (ethyl ester of beta-apo-8-carotenic acid (C30). Some of these colours are only commercially available in the nature-identical form. Although they are produced by chemical means nature-identical colours have similar attributes to natural colours, and their nature-identical status enables food manufactures to claim that their products contain "no artificial colours".

2.10. Colour additives regulation:

U.S. Food and Drug Administration (1993) stated that in 1900, there were about 80 man-made colour additives available for use in foods. At that time there were no regulations regarding the purity and uses of these dyes. The food and Drug Act of 1906 permitted or listed seven man-made colour additives for use in foods. The Act also

established a voluntary certification program, which was administered by U.S. Department of Agriculture (USDA), hence man-made colour additives became known as certifiable colour additives. To avoid confusing colour additives used in food with those manufactured for other uses, three categories of certifiable colour additives were created:

- Food, Drug&Cosmetic (FD&C)-colour additives with application in foods, drugs or cosmetics.
- Drug & cosmetic (D&C)-colour additives with the applications in drugs and / or cosmetics.
- External Drug & Cosmetic (External D& C)-colour additives with applications in externally applied drugs and in externally applied cosmetics.

In 1960, the colour additive Amendments to the FD& C Act placed colour additives on a provisional list and required further testing using up-to – date procedures. One section of the amendment known as the Delaney Clause, prohibits adding colourant to any food substance that has been shown to cause cancer in animals or man regardless of the dose. Under the amendments, colour additives exempted from certification also are required to meet rigorous safety standards prior to being permitted for use in foods. According to the

Nutrition Labeling and Education Act of 1990, a certifiable colour additive used in food must be listed in the ingredient statement by its common or usual name. All label printed after July 1, 1991 must comply with this requirement.

2.11. Benefits of added colouring in foods:

According to the U.S Food and Drug Administration (1993) the primary reasons of adding colours to foods include:

- To offset colour loss due to exposure to light, air, extremes of temperature, moisture and storage conditions.
- To correct natural variation in colour. Off coloured foods are often incorrectly associated with inferior quality. For example some tree ripened oranges are often sprayed with Citrus Red No. 2 to correct the natural orangy – brown or mottled green colour of their peels (Masking inferior quality, however, is an unacceptable use of colours).
- To enhance colours that occur naturally but at level weaker than those usually associated with a given food.
- To provide a colourful identify to foods that would otherwise be virtually colourless. Red colours provide a pleasant identity to strawberry ice while lime sherbet is known by its bright green colour.

- To provide a colourful appearance to certain foods. Many candies and holiday treats are coloured to create a festive appearance.
- To protect flavours and vitamins that may be affected by sunlight during storage.
- To provide an appealing variety of wholesome and nutritious foods that meet consumer's demands.

2.12. Food colours and health hazard:

Daniel (1962) reported that food colours were one of the earliest groups to be suspected as possible carcinogenic substance because of their supposedly close relationship to coal – tar colours. A very large proportion of the food colours currently in use have been tested for carcinogenic activity by the oral route. Two colours, ponceau 3R and Ponceau MX produced hepatic tumours in rats and mice when given by the oral route and were promptly withdrawn because they were suspected of having carcinogenic activity. Exhaustive tests on other food colours by the oral route have failed to reveal any carcinogenic potential in any of the colouring tested (Grasso and Golberg, 1966; Grice et al., 1961)

Different studies elsewhere revealed that analysis of various food products with respects to the added colours showed the

concentration of food colours in different food products ranges from 15 – 20 mg/kg, which was within the minimum permissible limit. It was found that a few foods manufactured by unorganized private sector and small vendors did contain colours in higher concentration than permitted range (Biswas and Chatterjee,1994).

The health hazard due to consumption of food colours has also been reported by FAO/WHO in 1994.

A naphylactic reactions after consuming natural colours like annatto were exhibited by certain individuals (Nish *et al.*, 1991). Food anaphylaxis following ingestion of carmine, a natural dye extracted from the cochnineal insects was reported in women at a dose of 1mg/kg body wt. although the ADI is 0 – 5 mg/kg body weight (Beardowin and Kanny, 1995).

Tartrazine has been reported to be associated with irritability, restlessness and sleep disturbance in a topic or hypertensive children aged between two and fourteen years (Rowe and Rowe, 1994). Other permitted food colour such as sunset yellow and ponceau4R have also been implicated in adverse reactions in patients with chronic urticaria (Lockey, 1977).

Babu and Shenolikar (1995) reported that toxicity experiments showed toxic effects of food colours at relatively high levels of 1 –

5%, however, such levels are not normal. The main symptoms found were allergies. Some food brone diseases were reported due to consumption of non-permitted textile colouring materials in the diet.

Murdoch *et al.* (1987) showed that azo dyes are still used extensively throughout the world in spite of the reported health hazards. Some of the more common azo colours include amaranth (red), azorubine (red), brilliant black (blue), sunset yellow (yellow), carmosine (red) and tartrazine (yellow), most of which are allergens as demonstrated in their potential to induce histamine release by leucocytes of normal and urticaria patients

2.13. Qualitative analysis of synthetic colours:

2.13.1. Extraction:

Dyes can be extracted from foods by adsorption and desorption with polyamide, which retains all acid coal-tar dyes. Unlike the wool dyeing technique which is now rarely used, this procedure does not bring about changes in the dyes, so there should be no effect on the subsequent chromatographic and spectrophotometric examination (Lehmann *et al.,* 1970).

It was reported in Pearson (1976) that preliminary treatments can be done for different food categories prior colour identification. The different food categories involve:

a. Non-alcoholic liquids, e.g. soft drinks.

b. Soluble solids foods, e.g. jams, sweets, icings.

c. Starch based foods, e.g. cakes, custard powder, golden raising powder.

d. Candied fruits.

e. Product with a high fat content, e.g. sausages, meat and fish pastes.

2.13.2. Separation and identification:

Many analytical methods have been developed for the qualitative and quantitative analysis of food colour. These include: thin layer chromatography (Francis, 1985; Harada *et al.,* 1991), UV/VIS spectrometry (Love, 1984; Furia, *et al.,* 1980), DCSPE (dynamic column-solid phase extraction) system (Gilhooley *et al.,* 1972). Caplliary electrophoresis, C_{18} cartridage (Talsky *et al.,* 1978). Or various of these techniques likes.

Tripathi *et al.* (2004) described a simple method for the extraction, separation and determination of synthetic colours in ice cream samples. They used a wool dyeing technique and eluted the colours with 5% ammonia solution. The mixed colours were separated by paper chromatography using trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v). The recovery with neutral detergent for spiked colours was about 90%, namely for sunset yellow FCF, tartrazine,

carmosine, ponceau 4R, brilliant blue FCF and fast green FCF, however, for erythrosine it was much lower whereas indigo carmine could not be covered. The detergents used were triton X-100 and Tween 20. The sensitivity of the method was about 1 ppm.

Ishikawa *et al.* (2004) used a technique of capillary electrophoresis (CE) with photodiode array detection for the analysis of 12 synthetic food colours. The dyes were cleaned up by solid phase sep-Pak C18 and eluted with methanol, then separated by CE and the results were found to be similar to those with paper chromatography.

Suzuki *et al.* (1994) used the technique of capillary electrophoresis for the separation of synthetic food dyes; namely: Erythrosine (R-3), Phloxin (R-104), Rose Bengal (R-105), Acid Red (R-106),Amaranth (R-2), New coccine (R-102) and Allura Red Ac (R-40). The identification was by absorbance spectra of each peak. They used sodium phosphate buffer solution pH 8.0 with sodium dodecyl sulfats SDS, however, substitution of beta-cyclodextrin for SDS improved the separation for R-2 and R-102.

Synthetic food colourants in drinks and in instant drink powder were determined by high performance ion chromatography (Chen *et al.,* 1998). They used an anion–exchange analytical column with very low hydrophobicity and visible absorbance detection. The separation was done by gradient elution with HCl – acetonitrile which included clean up of the column as well.

According to Gonzalez *et al.* (2003), natural and synthetic colourants in dairy food samples could be determined at low concentration of micro g/ml using liquid chromatography–diode array detection. They described a simple novel sensitive screening method for food colourants where synthetic and natural colourants could selectively adsorbed on cotton or RP-C18 sorbent columns respectively.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Materials:

Samples of different food products containing colouring matters were collected randomly from different markets, namely: jams, powdered concentrates of artificial flavoured juices, sweets and soft drinks.

3.1.1. Jams:

Two samples of jams of abocefain (A_1) and Saeed (A_2) products as imported and local products respectively.

3.1.2. Juices:

Two different samples of coloured concentrated powdered drinks of arwa pineapple flavoured powder (B_1) and orange flavoured powder (B_2). Which are commercially known as Tang powders.

3.1.3. Sweets:

Two samples of sweets of yellow colour soft sweet (C_1) locally called lackoom and hard sweet (C2), which are usually displayed in the market during the celebration of the birth–day of prophet Mohamed (Mawlid)

3.1.4. Soft drinks:

The samples of the soft drinks included the following commercial refreshing products:

Yellow Maaza D_1

Red Miranda D_2

Yellow Miranda D_3

3.1.4.4. Red Fanta toot D_4

Red Pasiganos D_5

Yellow Mango vita D_6

Yellow Fanta D_7

Red Vita D_8

3.2. General methods for identification:

According to the Association of Puplic Analysts (APA, 1960), Bfmira (1963), Stanley and Kirk (1963), Lehmann *et al.* (1970) and Gilhooley *et al.* (1962) and reviewed by Minor (1972) the general scheme for identifying coal – tar dyes present in foods involves:

1. Preliminary treatment of the food.
2. Extraction and purification of the dye from the prepared solution of the food.
3. Separation of the mixed colours if more than one is present.
4. Identification of the separated colours.

3.2.1. Preliminary treatment of the food:

The preliminary treatment was done according to Pearson method (1976) which involves removing interfering substances and obtaining dye in acid solution prior boiling with wool.

a. Non-alcoholic liquids, e.g. soft drinks: As most of the foods in this group are acidic they can usually be treated directly with wool. Otherwise, acidify slightly with acetic acid or potassium hydrogen sulfate.

b. Soluble solid foods, e.g. jams, sweets, icings: were dissolved in water and diluted, then treated directly with wool.

3.2.2. Method for colours extraction:

White knitting wool may contain fluorescing materials which may appear as a fluorescent spot on the chromatogram. To overcome this interference, white knitting wool was boiled in dilute solution of ammonia (1+4), washed with water and then with acetic acid (1+4), and finally washed with water again (Ranganna, 1986).

Procedure:

Fifty ml of the sample (soft drinks) were taken in a 250 ml beaker, if liquid (soft drinks). For soluble solid material, like jams, sweets, coloured concentrated powdered juices, 250 g were dissolved by mixing with water and made up to approximately 100 ml with water. The solution was acidified, if not already acidic, with acetic acid or dilute HCl (1+9). Four to five pieces of white clean woollen yarn 5cm in length, were washed as described by Ranganna (1986) and immersed in the beaker, the content of the beaker were boiled for 10–20min. After the wool has taken up all synthetic colours, it was removed from the beaker, washed under running tap water and finally with distilled water. The wool was transferred to a small beaker and the colour was stripped from the dyed wool by boiling in a dilute solution of ammonia (1 part of strong ammonia to 50 parts of water). The used wool was discarded and the coloured solution was concentrated by evaporation on water bath. After the removal of ammonia the coloured solution was filtered through filter paper and evaporated to a small volume for spotting. The coloured concentrate was stored in stopper glass bottle and kept in refrigerator for further analysis.

3.2.3. Chromatographic identification of colours:

Separation and identification was accomplished by paper chromatography (PC) and thin layer chromatography (TLC) as an adsorption form. Separation is accomplished by differential adsorption of components in the sample of different stationary phase (Dixon and Renyk, 1982). The position of compounds on a TLC and PC are often described by the retention factor (RF) which is according to Ranganna (1986).

$$Rf = \frac{Distance\ travelled\ by\ the\ spot\ of\ the\ food\ colour}{Distance\ travelled\ by\ the\ solvent\ front}$$

Identification of separated colours of the samples was carried out using ascending paper chromatography (PC) and thin layer chromatography (TLC).

3.2.3.1. Ascending paper chromatography:

The extracted colours concentrates were separated using ascending paper chromatography. (Association of Public Analysts, UK, 1960 and Maureen, 1966).

Whatman No. 1 filter paper (20 × 20cm) was used. Extracts of colours as well as reference colour compounds were spotted on the same paper using 10µ capillary tubes. The spots were dried with blowing hot air using hair drier to avoid diffused spots. The paper was

placed in a two litre tank containing about 50 ml solvent. Two solvents were used separately namely:

Solvent I (S-I) ammonia solution: water (1:99) and

Solvent II (S-II) trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v)

The development was allowed until the solvent front had reached a height of 12cm from the base line. Then the paper was removed and dried. The colour and number of the separated spots were recorded and the Rf-value for each spot was calculated.

3.2.3.2. Thin layer chromatography (TLC):

3.2.3.2.1. Preparation of the thin layer:

According to Krishnaprasad *et al.* (1970) the preparation of silica gel thin layer plates were made using two impregnating solutions separately. First fifty grams silica gel were shaken for 2 min with 80 ml of 1.25% solution of disodium salt of ethylene diaminetetraacetic acid (EDTA) in 250 ml stoppered conical flask. The slurry was spreaded using spreader making 0.5 mm thick layer on 5 glass 20 x 20cm. The coated plates were allowed to dry at room temperature, then activated at 105º C for two hours. The hot plates were stored and allowed to cool down in a desicator over blue silica. In the second type: fifty grams of silica gel were slurried with fifty ml

of 1% Tris solution (Tris (hydroxyl methyl) amino methane) and spreaded to a thickness of 0.5 mm on 5 glass plates 20 × 20cm.

The coated plates were allowed to dry at room temperature, then activated at 105º C for two hours. The hot plates were stored and allowed to cool down in a desiccator over blue silica.

Stationary phase: The stationary phase used was silica gel.

Type: silica gel for TLC

13% gypsum was added as a binding agent which contains inorganic fluorescent indicator for near wave UV

Method of development: One dimensional ascending development to tank saturation was used.

Mobile phase:

Solvent I (S-III): Isoamylalcohol: glacial acetic acid: water (40: 20: 20)

Solvent II (S-IV): n-butanol: absolute ethanol : 2Na ammonium hydroxide (60: 20: 20)

Application of samples:

Extracts of colours as well as reference colour compounds were spotted on the same plate by means of 10µ capillary tubes. They were applied 1 to 2 cm from the bottom of the plate. Mobile phase No. I (SIII) was used for plate containing EDTA and mobile phase No. 2(S-IV) for plates containing Tris solution, as solvents. The TLC plates

were placed in the chromatographic chamber containing the appropriate solvent and the solvents was allowed to run for a certain distance from the base line. Time required was about 90 min. The plates were allowed for air drying and the Rf-values were calculated.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1. Extraction of food colours:

The wool technique was found to be effective in retaining synthetic food colours which were then obtained by stripping with ammonia solution from all samples. Pre washing with tap water which usually remove natural colours showed that all samples analysed contained only synthetic colours except sample D_1 (maaza) which contained natural colours (D_1I) and light synthetic yellow colours (D_1II). These results are comparable with Tripathi *et al.* (2004) who worked in synthetic colours in ice-cream samples as well as with Ashfaq and Masud (2002) who worked on some ready to eat foods and used the wool dying technique for extraction of colours with 5% ammonia solution and the recovery was about 90%, namely for sunset yellow FCF, tartrazine, carmosine, ponceau 4R, brilliant blue FCF and fast green FCF as reported by Tripathi *et al.* (2004).

4.2. Identification of extracted dyes by paper chromatography:

Fourteen samples were separated by paper chromatography using two different solvent systems: S-i ammonia solution: water (1: 99)and

S-ii: trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

Table 1 and Fig. 4.1 shows that extracts from three products namely: soft sweet (C_1), red Miranda (D_2) and red pasgianos (D_5) as well as reference compounds gave separated spots using solvent system (S-I). Samples C_1 and D_2 which were isolated from soft sweet and red miranda each gave one coloured component with yellow and red colour respectively. While as sample D_5 extracted from red pasgianos was separated into three coloured components of red, yellow and blue colours. They were identified in relation to amaranth (magenta red), ponceau 4R (strawberry red), carmosine (red), tartrazine (lemon yellow), sunset yellow (orange) and brilliant blue (turquoise blue) as reference compounds. It can be inferred from table 1 (Fig. 4.1) that sample C_1 which gave a yellow coloured component with Rf-value of 0.80 comparatively similar to tartrazine with Rf-value 0.81 while sample D_2 showed one red coloured component with Rf- value 0.46 and it can be similar to amaranth with Rf 0.48. Sample D_5 was found to contain a mixture group of coloured components which were red Rf, 0.56, yellow Rf, 0.89 and blue Rf, 0.94. They were similar to amaranth Rf, 0.48, tartrazine Rf, 0.81 and brilliant blue Rf, 0.96 respectively.

Fig. 4.2 demonstrates the separation of the same samples of C_1, D_2 and D_5 except that the solvent system used was S-II instead of solvent system S-I.

Table 1: Rf- valuesof food colours from soft sweet (C_1), red miranda (D_2) and red pasgianos (D_5) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-I	[2*]S-II	[1*]S-I	[2*]S-II
C_1	Yellow	Yellow	0.80	0.58
D_2	Red	Red	0.46	0.12
D_5	1\ Red	1\ Red	0.56	0.13
	2\ -	2\ Red	-	0.25
	3\ Yellow	3\ Yellow	0.89	0.67
	4\ Blue	4\ Blue	0.94	0.89
* Amaranth	3* Magenta red		0.48	0.18
*Ponceau 4R	3* Strawberry red		0.86	0.46
* Carmosine	3* Red		0.42	0.13
* Tartrazine	3* Lemon yellow		0.81	0.48
* Sunset yellow	3* Orange		0.60	0.13
*Brilliant blue	3* Turquoise blue		0.96	0.88

*Reference compounds.

1* Ammonia solution : water (1: 99).

2* Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

3* Downham and Collins (1999).

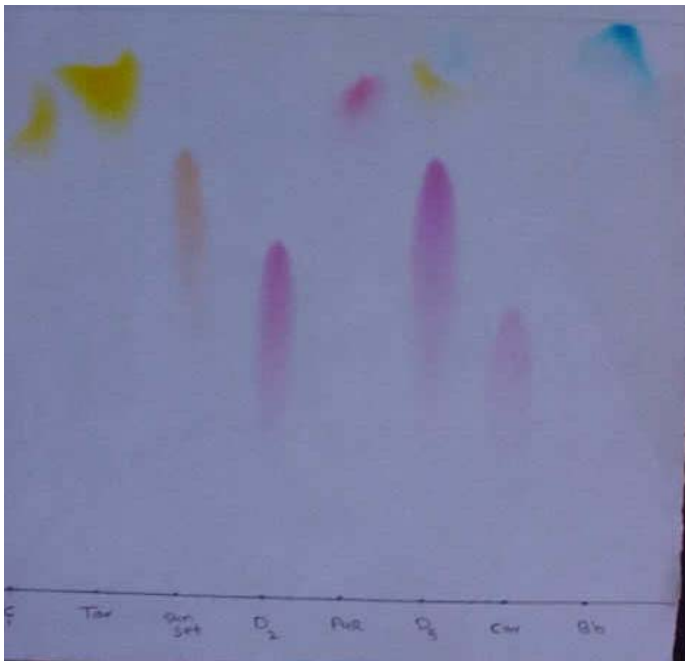

Fig. (4.1). Paper chromatograms of coloured food extracts* in solvent system ammonia solution: water (1: 99) in comparison to reference coloured compounds**.

**P4R: ponceau4R
*D_5: red pasgianos
**Car: carmosine
**Bb: brilliant blue
**A: Amaranth
*C_1: soft sweet
**Tar: Tartrazine
**Sunset: sunset yellow
*D_2: red miranda

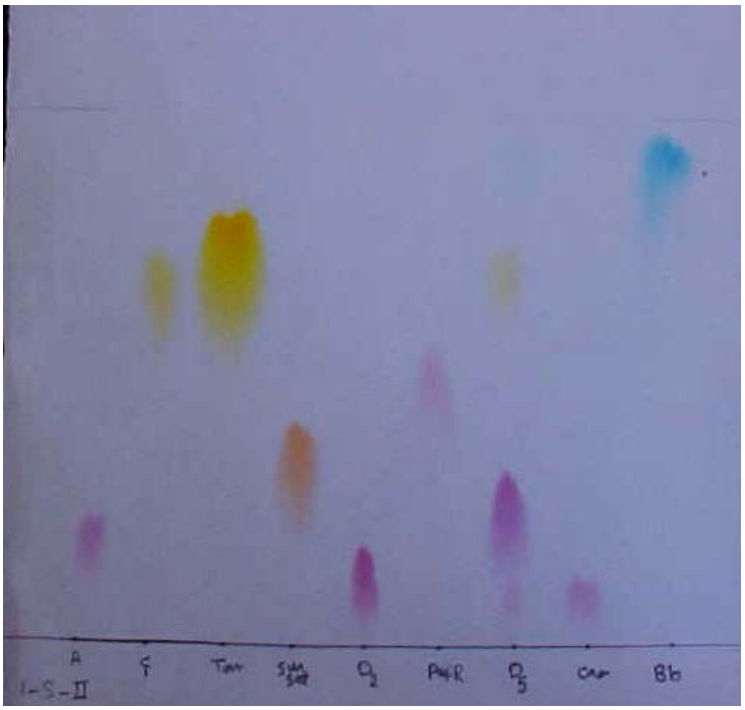

Fig. (4.2). Paper chromatogram of coloured food extracts* in solvent system trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) in comparison to reference coloured compounds**.

*D_2: Red miranda
**P4R: ponceau4R
*D_5: Red pasiginos
**Car: Carmosine
**Bb: Brilliant blue
**A: Amaranth
*C_1 : Soft sweet
**Tar: Tartrazine
**Sunset: Sunset yellow

Table 2: Rf- values of food colours from abocefain jam (A_1), hard sweet (C_2), yellow miranda (D_3) and red fanta toot (D_4) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-I	[2*]S-II	[1*]S-I	[2*]S-II
A_1	Red	Red	0.36	0.08
C_2	Yellow	Yellow	0.84	0.73
D_3	1\ Orange	1\ Orange	0.59	0.58
	2\ Pale orange	2\ Pale orange	0.95	0.47
D_4	1\ Red	1\ Red	0.31	0.09
	2\ Pale brown	2\ Pale brown	0.97	0.64
* Amaranth	3* Magenta red		0.44	0.41
*Ponceau 4R	3* Strawberry red		0.77	0.79
* Carmosine	3* Red		0.31	0.09
* Tartrazine	3* Lemon yellow		0.80	0.74
* Sunset yellow	3* Orange		0.57	0.63
*Brilliant blue	3* Turquoise blue		0.90	0.88

*Reference compounds.

1* Ammonia solution: water (1: 99).

2* Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

3* Downham and Collins (1999).

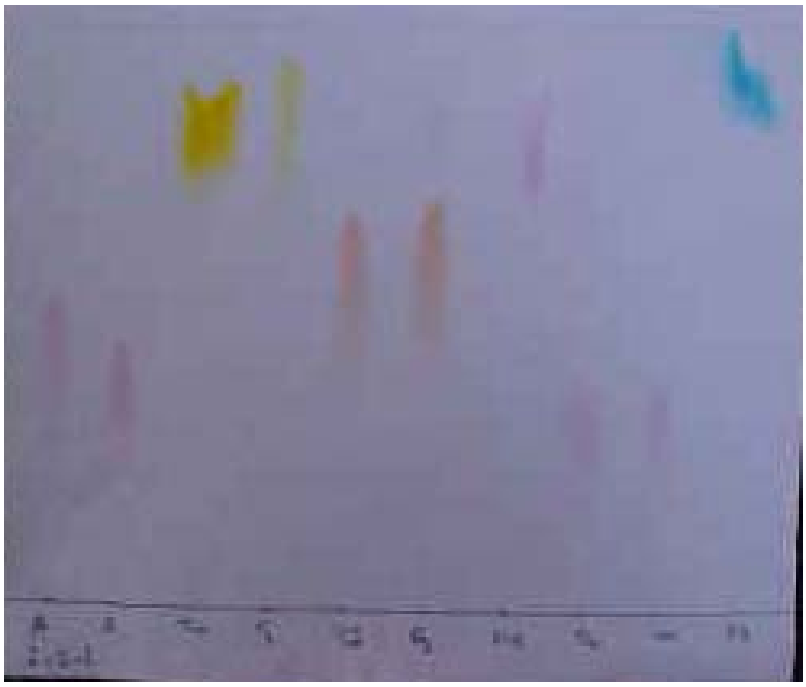

Fig. (4.3). Paper chromatogram of coloured food extracts* in solvent system trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) in comparison to reference coloured compounds**.

*D_3: yellow miranda
**P4R: ponceau4R
*D_4: Red fanta toot
**Car: Carmosine
**Bb:Brilliant blue
**A: Amaranth
*A_1 Abocefain jam
**Tar: Tartrazine
*C_2: Hard sweet
**Sunset: Sunset yellow

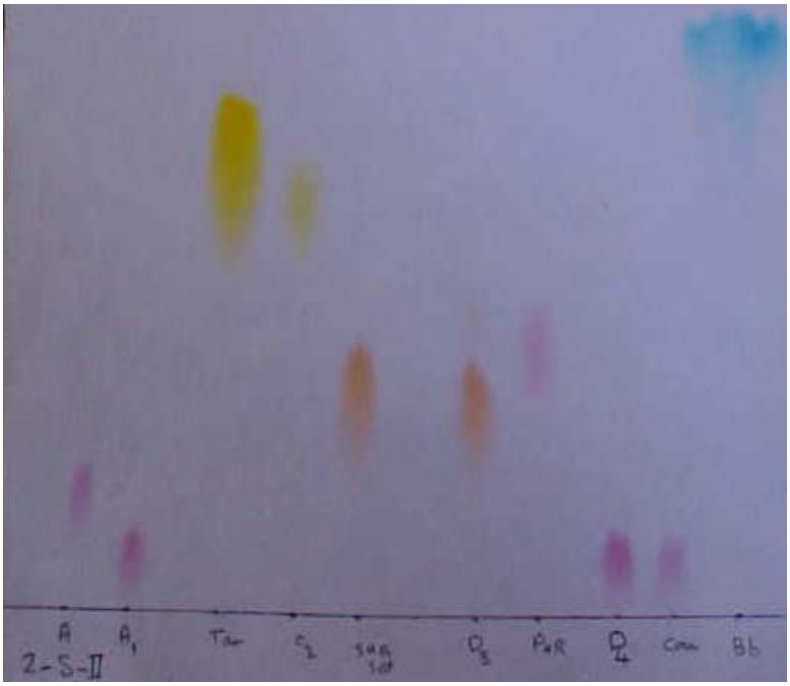

Fig. (4.4). Paper chromatogram of coloured food extracts[*] in solvent system trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) in comparison to reference coloured compounds[**].

[*]D_3: yellow miranda
[**]A: Amaranth
[**]P4R: ponceau4R
[*]A_1 Abocefain jam
[*]D_4: Red fanta toot
[**]Tar: Tartrazine
[**]Car: Carmosine
[*]C_2: Hard sweet
[**]Bb: Brilliant blue
[**]Sunset: Sunset yellow

Table 1 (Fig. 4.2) shows that sample C_1 which gave a yellow coloured component with Rf-value 0.58 similar to tartrazine with Rf-value 0.48 while sample D_2 gave one red coloured component with Rf- value 0.12 and was similar to amaranth with Rf- value 0.18. Sample D_5 was found to contain four components which were red with Rf 0.13, red with Rf 0.25, yellow with Rf 0.67 and blue with Rf 0.89. They were identified in relation to reference compounds: carmosine Rf 0.13, amaranth Rf 0.18, tartrazine Rf 0.48 and brilliant blue Rf, 0.88 respectively.

It can be concluded from Fig. 4.2 that solvent system S-II was more powerful than system S-I also sample D_5 gave additional red coloured component with Rf- value 0.25 which was shifted when solvent system S-I was used.

Table 2 (fig. 4.3) showed that the extracts from four samples namely, abocefain jam (A_1), hard sweet (C_2), yellow miranda (D_3) and red fanta toot (D_4) gave separated coloured spots using solvent system S-I. Sample A_1 which gave a red coloured component with Rf -value 0.36 was similar to carmosine with Rf-value of 0.31 while sample C_2 which gave a yellow coloured component with Rf-value 0.84 was similar to tartrazine with Rf -value 0.80. Sample D_3 was found to be resolved into two coloured component of orange and pale orange with

Rf -values 0.59 and 0.95 respectively. The orange component of sample D_3 was found to be similar to sunset yellow of Rf 0.57 while the pale orange component was not related to any of the reference compounds used and might be a derivative of sunset yellow. Sample D_4 had red and pale brown components with Rf- value of 0.31 and 0.97 respectively. The red coloured component was similar to carmosine with Rf- value 0.31 while the pale brown component with Rf 0.97 remained unidentified.

Fig. 4.4 shows the separation of the same sample of A_1, C_2, D_3 and D_4 except that the solvent system S-II was used instead of system S-I. Samples A_1 and C_2 which were isolated from abocefain and hard sweet each gave one coloured component, red with Rf 0.08 and yellow with Rf 0.73 respectively. They were identified against reference compounds carmosine Rf 0.09 and tartrazine Rf 0.74 respectively. Sample D_3 which gave two coloured component; orange with Rf-value 0.58 and was similar to sunset yellow with Rf-value 0.63 and pale orange component with Rf-value 0.47 which could not be identified against any of the reference compounds used. Sample D_4 which was isolated from red fanta toot gave two coloured component with red component Rf 0.09 which was similar to carmosine Rf 0.09 and pale brown component Rf 0.64 which was not identified. These

results indicate that all isolated samples of A_1, C_2, D_3 and D_4 were separated by paper chromatography using two different solvent systems S-I and S-II and they gave similar coloured components which were different in Rf -values.

Table 3 (Fig. 4.5) shows that the chromatographic separation of three coloured food commodities from : orange flavoured powder (B_2), yellow fanta (D_7) and red vita (D_8) gave clear spots using solvent system S-I. Sample B_2 had one colour component which appeared as yellow spot with Rf 0.67. It was comparatively similar to tartrazine with Rf -value 0.69. Also sample D_7 gave an orange spot which had an Rf- value 0.48. It was found to be identical to sunset yellow with Rf-value 0.49. Sample D_8 was found to be resolved into three components: blue Rf of 0.05, red Rf of 0.32 and yellow Rf of 0.73. The blue component could not be related to any of the reference compounds used and might be a derivative of brilliant blue. The red Rf of 0.32 and yellow Rf of, 0.73 coloured components of sample D_8 were identified against reference compounds amaranth Rf of, 0.31 and tartrazine Rf of, 0.69 respectively.

Table 3: Rf- values of food colours from orange flavoured powder (B_2), yellow fanta (D_7) and red vita (D_8) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-I	[2*]S-II	[1*]S-I	[2*]S-II
B_2	Yellow	Yellow	0.67	0.59
D_7	1\ -	1\ Red	-	0.05
	2\ Orange	2\ Orange	0.48	0.25
D_8	1\ deep blue	1\ deep blue	0.05	0.03
	2\ Red	2\ Red	0.32	0.11
	3\ Yellow	3\ Yellow	0.73	0.56
* Amaranth	3* Magenta red		0.31	0.13
*Ponceau 4R	3* Strawberry red		0.79	0.38
* Carmosine	3* Red		0.25	0.06
* Tartrazine	3* Lemon yellow		0.69	0.61
* Sunset yellow	3* Orange		0.49	0.25
*Brilliant blue	3* Turquoise blue		0.92	0.83

*Reference compounds.

1* Ammonia solution: water (1: 99).

2* Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

3* Downham and Collins (1999).

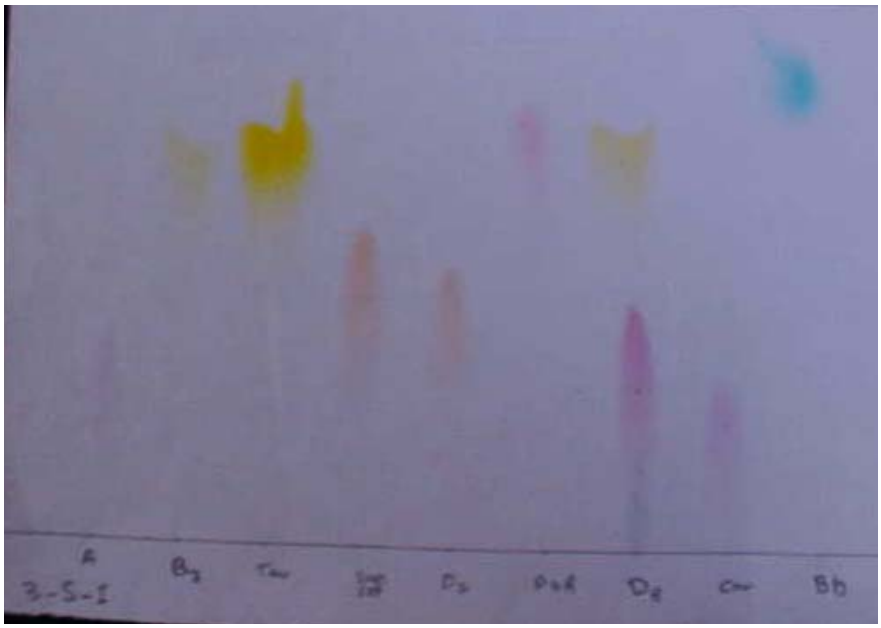

Fig. (4.5). Paper chromatogram of coloured food extracts[*] in solvent system ammonia solution: water (1: 99) in comparison to reference coloured compounds[**].

[**]A: Amaranth
[**]P4R: ponceau4R
[*]B_2: Orange flavour powder
[*]D_8: Red vita
[**]Tar: Tartrazine
[**]Car: Carmosine
[**]Sunset: Sunset yellow
[**]Bb: Brilliant blue
[*]D_7: Yellow fanta

Fig. (4.6). Paper chromatogram of coloured food extracts[*] in solvent system Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) in comparison to reference coloured compounds[**].

[*]D_7: Yellow fanta
[**]P4R: ponceau4R
[*]D_8: Red vita
[**]Car: Carmosine
[**]A: Amaranth
[*]B_2: Orange flavour powder
[**]Tar: Tartrazine
[**]Sunset: Sunset yellow
[**]Bb: Brilliant blue

Fig. 4.6 demonstrates the separation of the same samples of B_2, D_7 and D_8 except that the solvent system used was S-II instead of solvent system S-I. It can be inferred from table 3 and fig. 4.6 that sample B_2 which gave a yellow spot with Rf-value 0.59 was similar to tartrazine Rf, 0.61. While sample D_7 was found to contain two coloured components of red Rf, 0.05 and orange Rf, 0.25. They comparatively similar to carmosine with Rf value 0.06 and sunset yellow with Rf -value 0.25. Sample D_8 was resolved into three coloured components which were blue Rf, 0.03, red Rf, 0.11 and yellow Rf, 0.56 respectively. The blue component was not identified while red and yellow components were similar to amaranth with Rf-value 0.13 and tartrazine with Rf- value 0.61 respectively. These results show that the solvent system S-II was more powerful for the separation of sample D_7 which gave an additional component red Rf, 0.05 and it was not separated when system S-I was used.

Table 4 (Fig. 4.7) shows the extracts of the separated samples of arwa pinapple flavoured powder (B_1), saeed jam (A_2) and yellow mango vita (D_6) using solvent system S-I. Sample B_1 which gave a yellow spot with Rf-value 0.74 was comparatively similar to reference compound tartrazine with Rf-value 0.75 while sample A_2 which had a red coloured spot Rf 0.70 which was similar to ponceau 4R Rf of ,

0.81.Sample D_6 was separated into two components of oranges Rf of, 0.50 and yellow Rf of, 0.79 which were similar to sunset yellow with Rf of, 0.63 and tartrazine with Rf -value 0.75 respectively.

Table 4 (Fig. 4.8) demonstrates the separation of samples B_1, A_2 and D_6 using solvent system S-II instead of S-I. Samples B_1 and A_2 which were isolated from arwa pinapple flavoured powder and saeed jam respectively; each had one spot with yellow Rf of, 0.51 and red Rf of, 0.29 and were identified as tartrazine Rf of, 0.51 and ponceau 4R Rf of, 0.28 respectively. Sample D_6 contained three components: orange, red and yellow. The Rf-values of these components were 0.17, 0.29 and 0.46 and they were comparatively similar to sunset with Rf-value 0.19, ponceau 4R Rf of, 0.28 and tartrazine Rf of, 0.51 respectively.

The separation of samples B_1, A_2 and D_6 on using two different solvent systems S-I and S-II show that solvent system S-II was more polarity than solvent system S-I. Also solvent S-II gave an additional component with Rf-value 0.29 from sample D_6 which was not obtained when solvent S-I was used.

Table 4: Rf- valuesof food colours from arwa pinapple flavoured powder (B_1), saeed jam (A_2) and yellow mango vita (D_6) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-I	[2*]S-II	[1*]S-I	[2*]S-II
B_1	Yellow	Yellow	0.74	0.51
A_2	Red	Red	0.70	0.29
D_6	1\ Orange	1\ Orange	0.50	0.17
	2\ -	2\ Red	-	0.29
	3\ Yellow	3\ Yellow	0.79	0.46
* Amaranth	3* Magenta red		0.51	0.09
*Ponceau 4R	3* Strawberry red		0.81	0.28
* Carmosine	3* Red		0.39	0.08
* Tartrazine	3* Lemon yellow		0.75	0.51
* Sunset yellow	3* Orange		0.63	0.19
*Brilliant blue	3* Turquoise blue		0.92	0.80

*Reference compounds.

1* Ammonia solution: water (1: 99).

2* Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

3* Downham and Collins (1999).

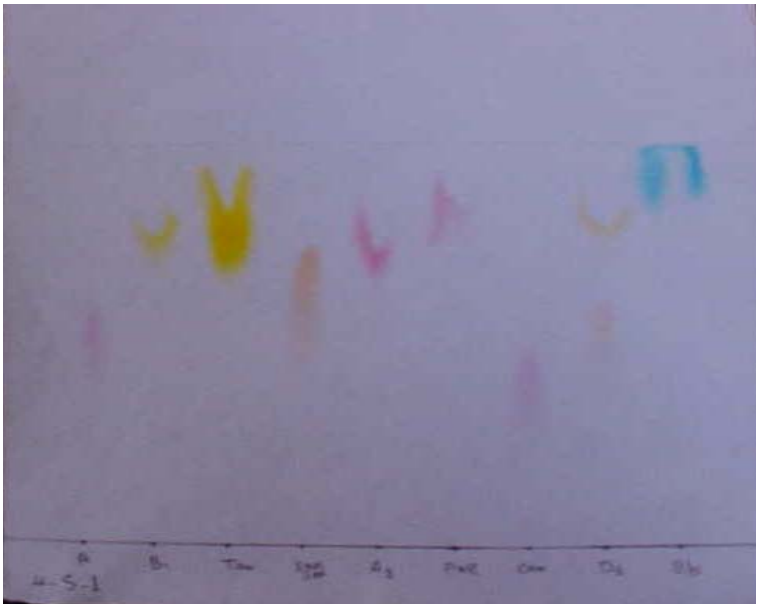

Fig. (4.7). Paper chromatogram of coloured food extracts[*] in solvent system ammonia solution: water (1: 99) in comparison to reference coloured compounds[**].

[**]P4R: ponceau4R
[**]Car: Carmosine
[*]D_6: Yellow mango vita
[**]Bb: Brilliant blue
[**]A: Amaranth
[*]B_1: Arwa pinapple flavoured powder
[**]Tar: Tartrazine
[**]Sunset: Sunset yellow
[*]A_2: Saeed jam

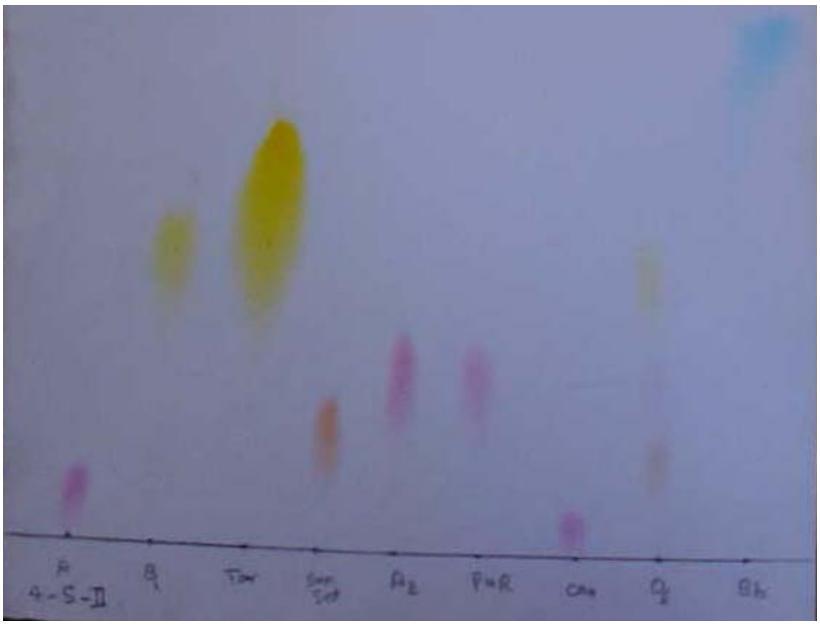

Fig. (4.8). Paper chromatogram of coloured food extracts[*] in solvent system Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) in comparison to reference coloured compounds[**].

[**]P4R: ponceau4R
[**]Car: Carmosine
[*]D_6: Yellow mango vita
[**]Bb:Brilliant blue

[**]A: Amaranth
[*]B_1: Arwa pinapple flavoured powder
[**]Tar: Tartrazine
[**]Sunset: Sunset yellow
[*]A_2: Saeed jam

Figures. 4.9 and 4.10 show the chromatograms of yellow maaza (D_1). The coloured material of remained sample D_1 using wool and then gave a pale yellow component when washed under tap water to give D_1I. The remained colour on wool was stripped from wool by dilute ammonia solution and gave D_1II. Samples D_1I and D_1II were separated using two solvent systems S-I and S-II against reference compounds tartrazine and sunset yellow.

It can be inferred from table 5, and Fig. 4.9 that samples D_1I and D_1II in comparison with reference compounds tartrazine and sunset yellow by using solvent system S-I. Sample D_1I was not separated while sample D_1II which gave an orange spot with Rf-value 0.58 was similar to sunset yellow with Rf-value 0.57.

Figures. 4.9 and 4.10 revealed that sample D_1I was not separated in both solvent S-I and S-II, while sample D_1II appeared as an orange spot with Rf-value of 0.38 which was similar to sunset yellow with Rf-value 0.38 in solvent system S-II.

Similarly Tripathi *et al.* (2004) working on ice cream and Ashfaq and Masud (2002) working in different ready to eat foods were able to separate synthetic food colours with paper chromatography using solvent trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

Table 5: Rf- values of food colours in samples D_1I, D_1II against reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-I	[2*]S-II	[1*]S-I	[2*]S-II
D_1 I	-	-	-	-
D_1 II	Orange	Orange	0.58	0.38
* Tartrazine	3* Lemon yellow		0.44	0.24
* Sunset yellow	3* Orange		0.57	0.38

*Reference compounds.

1* Ammonia solution: water (1: 99).

2* Trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v).

3* Downham and Collins (1999).

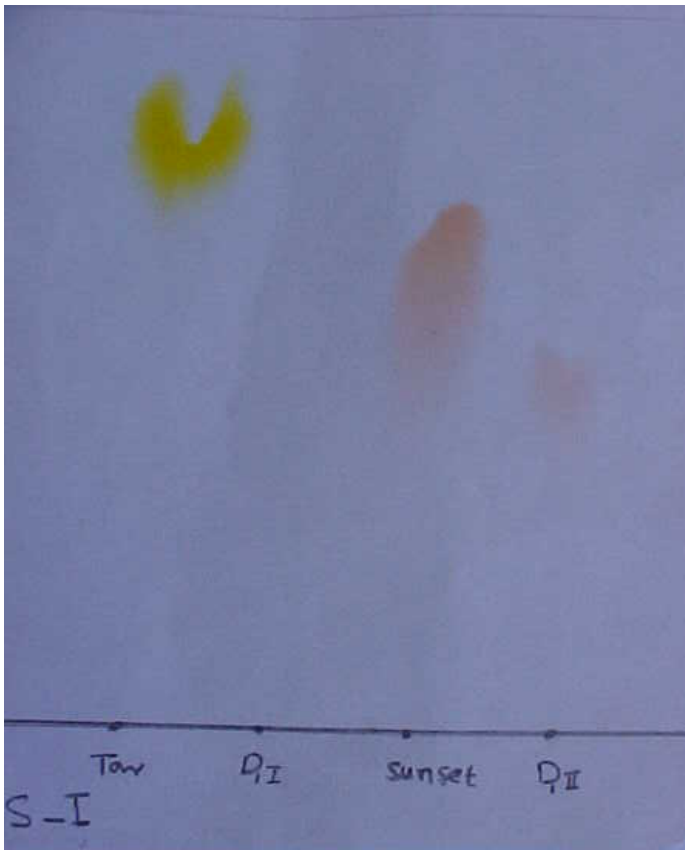

Fig. (4.9). Paper chromatogram of coloured food extracts* of sample yellow maaza (D_1) in solvent system Ammonia solution: water (1: 99) in comparison to reference coloured compounds**.

*D_1I: Natural colour
*D_1II: Synthetic colour
**Tar: Tartrazine
**Sunset: Sunset yellow

Fig. (4.10). Paper chromatogram of coloured food extracts[*] of sample yellow maaza (D_1) in solvent system Trisodium citrate: Ammonia solution: water (2: 5: 95, w/v/v), in comparison to reference coloured compounds[**].

[*]D_1I: Natural colour
[*]D_1II: Synthetic colour
[**]Tar: Tartrazine
[**]Sunset: Sunset yellow

4.3. Identification of extracted dyes by thin layer chromatography:

Thin layer chromatography**(TLC)** method was used for determination of fourteen different food commodities. Two solvent system were applied separately for separation:

(S-III): Iso-amyl alcohol : glacial acetic acid : water (40: 20: 20).

(S-IV): n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20).

Plate 4.1 shows that the chromatographic separation of extracts from three coloured food products namely: soft sweet (C_1), red miranda (D_2) and red pasgianos (D_5) gave clear spots using solvent system S-III.

Table 6 and plate 4.1 show that sample C_1 had one yellow coloured component with Rf of, 0.17 which was comparable to tartrazine Rf of, 0.17. While sample D_2 gave a red component with Rf of, 0.40. It was similar to carmosine Rf of, 0.39. Sample D_5 was found to be resolved into three components: red Rf of, 0.15, blue Rf of, 0.28 and red Rf of, 0.39. These components were similar to amaranth Rf of, 0.2, brilliant blue Rf of , 0.28 and carmosine Rf of , 0.39.

Table 6 and plate 4.2 revealed that the samples C_1 and D_2 which were isolated from soft sweet and red miranda using solvent system S-

IV each gave one coloured components: yellow Rf of, 0.53 and red Rf of, 0.83. They were comparatively similar to tartrazine Rf of, 0.53 and carmosine Rf of, 0.80. Sample D_5 was resolved into four components: yellow Rf of, 0.53, red Rf of, 0.59, blue Rf of, 0.61 and red Rf of, 0.80. These components were comparable to tartrazine Rf of, 0.53, amaranth, Rf of, 0.67 brilliant blue Rf of, 0.63 and carmosine Rf of, 0.80 respectively.

From plate 4.1 and 4.2 the chromatographic separation of samples C_1, D_2 and D_5 indicated that the solvent system S-IV was more polar and powerful than solvent system S-III as it gave additional yellow coloured component from sample D_5 and the spots were removed up the plates that component was not separated when solvent system S-III was used.

Table 6: Rf- values of food colours from soft sweet (C_1), red miranda (D_2) and red pasgianos (D_5) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-III	[2*]S-IV	[1*]S-III	[2*]S-IV
C_1	Yellow	Yellow	0.17	0.53
D_2	Red	Red	0.40	0.83
D_5	1\ -	1\ Yellow	-	0.53
	2\ Red	2\ Red	0.15	0.59
	3\ Blue	3\ Blue	0.28	0.61
	4\ Red	4\ Red	0.39	0.80
* Amaranth	3* Magenta red		0.20	0.67
*Ponceau 4R	3* Strawberry red		0.17	0.50
* Carmosine	3* Red		0.39	0.80
* Tartrazine	3* Lemon yellow		0.17	0.53
* Sunset yellow	3* Orange		0.33	0.73
*Brilliant blue	3* Turquoise blue		0.28	0.63

1* Iso-amylalcohol: glacial acetic acid : water (40: 20: 20).

2* n-butanol: absolute ethanol : 2N ammonium hydroxide (60: 20: 20).

*3 Downham and Collins (1999).

*Reference compounds.

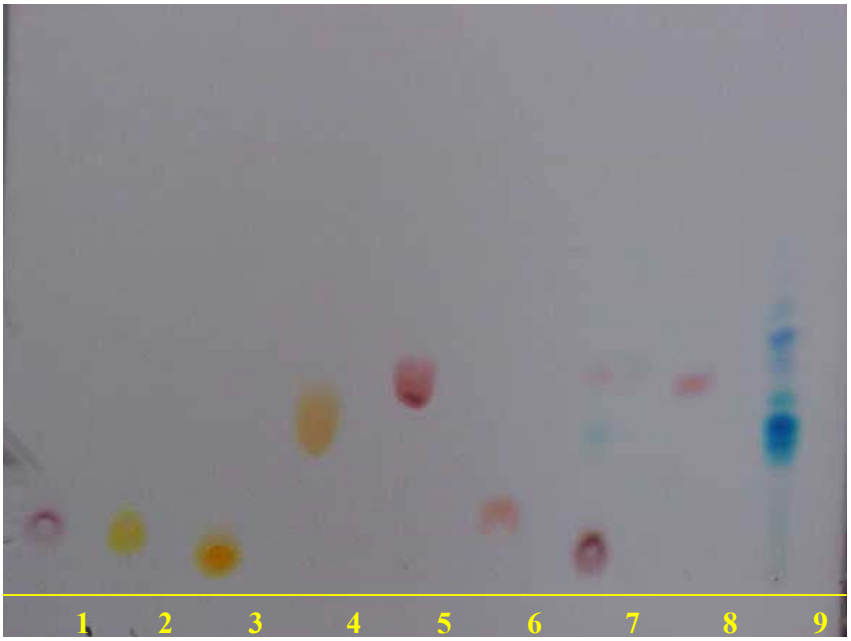

Plate (4.1). Thin layer chromatogram of coloured food extracts* in solvent system iso-amyl alcohol: glacial acetic acid: water (40: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *C_1: Soft sweet
3. **Tartrazine
4. **Sunset yellow
D_2 Red miranda
6. **Ponceau4R
7. *D_5Red pasigianos
8. **Carmosine
9. **Brilliant blue

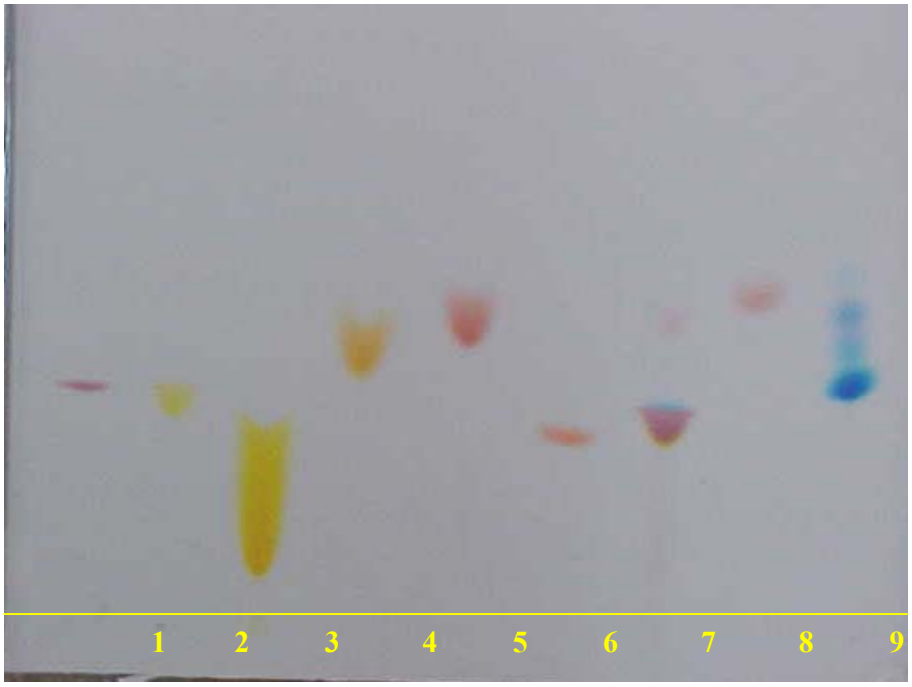

Plate (4.2). Thin layer chromatogram of coloured food extracts* in solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *C_1: Soft sweet
3. **Tartrazine
4. **Sunset yellow
5. *D_2: Red miranda
6. **Ponceau4R
7. *D_5: Red pasigianos
8. **Carmosine
9. **Brilliant blue

Plate 4.3. shows the separation on thin layer chromatogram of the extracted samples: abocefain jam (A_1), hard sweet (C_2), red miranda (D_3) and red fanta toot (D_4) using solvent system S-III. Table 7 indicates that sample A_1 had one red component with Rf of, 0.51.It was similar to carmosine with Rf of, 0.41 while sample C_2 had one yellow coloured component Rf of, 0.17 and was similar to tartrazine Rf of, 0.17. Sample D_3 contains two coloured components: Pale orange Rf of, 0.11 and orange Rf of, 0.36. The pale orange component was not related to any of the reference compounds used, while the orange component was comparable to sunset yellow Rf of, 0.36. Sample D_5 gave a red component with Rf-value 0.53.It was similar to carmosine with Rf, 0.41.

Plate 4.4 demonstrates the chromatographic separation of the same samples: A_1, C_2, D_3 and D_4 except that the solvent system S-IV was used instead of solvent system S-III samples A_1, C_2 and D_4 each gave one coloured component red Rf of, 0.77, yellow Rf of, 0.47 and red Rf of, 0.65 which were comparatively similar to carmosine Rf of, 0.76, tartrazine Rf of, 0.47 and carmosine Rf of, 0.76 respectively. Sample D_3 gave a pale orange Rf of, 0.43, which was not related to any reference compounds used, and orange Rf of, 0.6 was similar to sunset yellow Rf of, 0.6.

Table 7: Rf- values of food colours from abocefain jam (A_1), hard sweet (C_2) yellow miranda (D_3) and red fanta toot (D_4) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-III	[2*]S-IV	[1*]S-III	[2*]S-IV
A_1	Red	Red	0.51	0.77
C_2	Yellow	Yellow	0.17	0.47
D_3	1\ Pale orange	1\ Pale orange	0.11	0.43
	2\ Orange	2\ Orange	0.36	0.6
D_4	Red	Red	0.53	0.65
* Amaranth	3* Magenta red		0.20	0.57
*Ponceau 4R	3* Strawberry red		0.19	0.4
* Carmosine	3* Red		0.41	0.76
* Tartrazine	3* Lemon yellow		0.17	0.47
* Sunset yellow	3* Orange		0.36	0.60
*Brilliant blue	3* Turquoise blue		0.53	0.57

1* Iso-amylalcohol: glacial acetic acid : water (40: 20: 20).

2* n-butanol: absolute ethanol : 2N ammonium hydroxide (60: 20: 20).

*3 Downham and Collins (1999).

*Reference compounds.

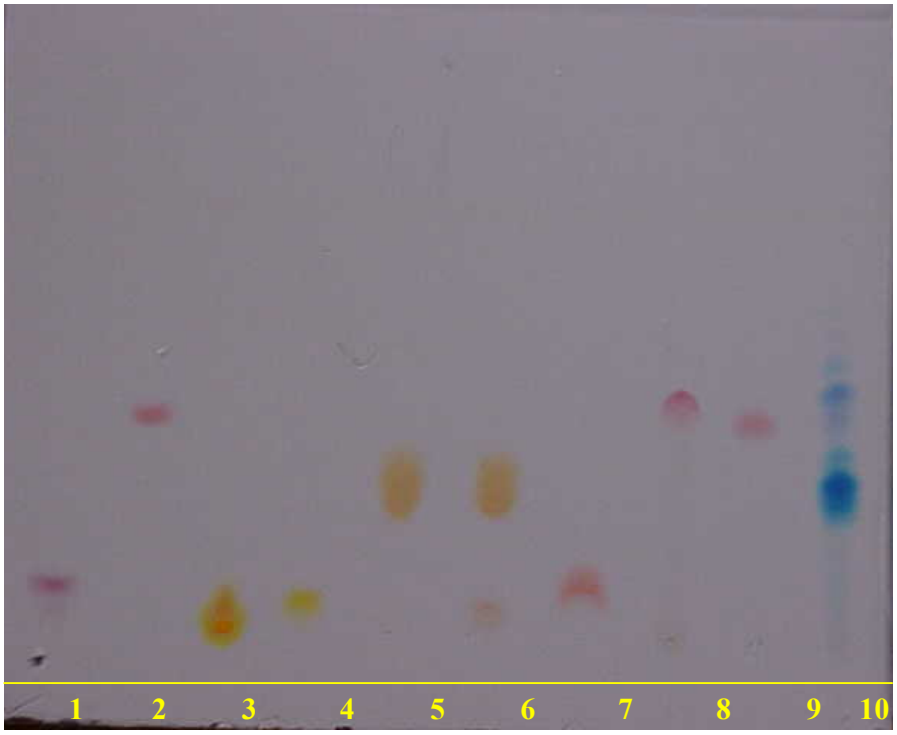

Plate (4.3). Thin layer chromatogram of coloured food extracts[*] in solvent system iso-amyl alcohol: glacial acetic acid: water (40: 20: 20) in comparison to reference coloured compounds[**].

1. [**]Amaranth
2. [*]A_1: Abocefain jam
3. [**]Tartrazine
4. [*]C_2: Hard sweet
5. [**]Sunset yellow
6. [*]D_3: Yellow miranda
7. [**]Ponceau4R
8. *D_4: Red fanta toot
9. [**]Carmosine
10. [**]Brilliant blue

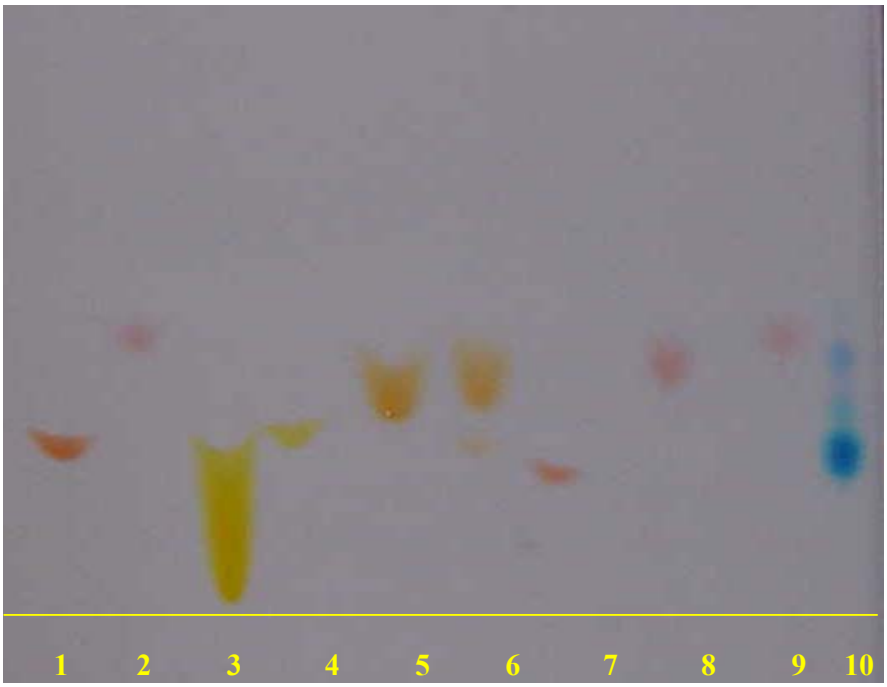

Plate (4.4). Thin layer chromatogram of coloured food extracts* in solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *A_1: Abocefain jam
3. **Tartrazine
4. *C_2: Hard sweet
5. **Sunset yellow
6. *D_3: Yellow miranda
7. **Ponceau4R
8. *D_4: Red fanta toot
9. **Carmosine
10. **Brilliant blue

Table 7shows the chromatographic separation of samples: A_1, C_2, D_3 and D_4 using solvent system S-III and S-IV. Both solvents gave similar separation but with different Rf-values.

Plate 4.5 shows that the three sampls isolated from orange flavoured powder (B_2), yellow fanta (D_7) and red vita (D_8) gave clear spots on TLC plates using solvent system S-III. Sample B_2 had one coloured component which appeared yellow with Rf of, 0.23.It was similar to tartrazine Rf of, 0.23 also sample D_7 gave one orange coloured component which had an Rf-value 0.42. It was found to be identical to sunset yellow with Rf-value 0.42. Sample D_8 was found to be resolved into two components: yellow Rf of, 0.23 and red Rf of, 0.47. They were identical to tartrazine Rf of, 0.23 and carmosine Rf of, 0.50 respectively.

Plate 4.6 shows the separation of the same samples of B_2, D_7 and D_8 on thin layer chromatographic using solvent system S-IV. It can be inferred from table 8 that samples B_2 and D_7 which were isolated from orange flavoured powder and yellow fanta each gave one coloured component. They were yellow with Rf of, 0.57 and orange Rf of, 0.75 and they were identical to tartrazine Rf of, 0.5 and sunset yellow Rf of, 0.75. Sample D_8 contains a mixture group of coloured components: deep blue Rf, 0.21, yellow Rf of, 0.57 and red

Rf of, 0.80. The blue coloured component could not be related to any of the reference compounds used and might be derivative of brilliant blue. The yellow and red coloured components of sample D_8 were identified against reference compounds tartrazine Rf of, 0.57 and carmosine Rf of, 0.80 respectively.

Plate 4.6 show that sample D_8 had additional deep blue coloured components in solvent system S-IV.It was more polarity than S-III. The deep blue component could not be separated when solvent system S-III was used.

Table 8: Rf- values of food colours from orange flavoured powder (B_2), yellow fanta (D_7) and red vita (D_8) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-III	[2*]S-IV	[1*]S-III	[2*]S-IV
B_2	Yellow	Yellow	0.23	0.57
D_7	Orange	Orange	0.42	0.75
D_8	1\ -	1\ Deep blue	-	0.21
	2\ Yellow	2\ Yellow	0.23	0.57
	3\ Red	3\ Red	0.47	0.80
* Amaranth	3* Magenta red		0.27	0.67
*Ponceau 4R	3* Strawberry red		0.23	0.43
* Carmosine	3* Red		0.50	0.80
* Tartrazine	3* Lemon yellow		0.23	0.50
* Sunset yellow	3* Orange		0.42	0.75
*Brilliant blue	3* Turquoise blue		0.60	0.90

1* Iso-amylalcohol: glacial acetic acid : water (40: 20: 20).

2* n-butanol: absolute ethanol : 2N ammonium hydroxide (60: 20: 20).

*3 Downham and Collins (1999).

*Reference compounds.

Plate (4.5). Thin layer chromatogram of coloured food extracts* in solvent system iso-amyl alcohol: glacial acetic acid: water (40: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *B_2: Orange flavoured powder
3. **Tartrazine
4. **Sunset yellow
. *D_7: yellow fanta5
6. **Ponceau4R
7. *D_8: Red vita
8. **Carmosine
9. **Brilliant blue

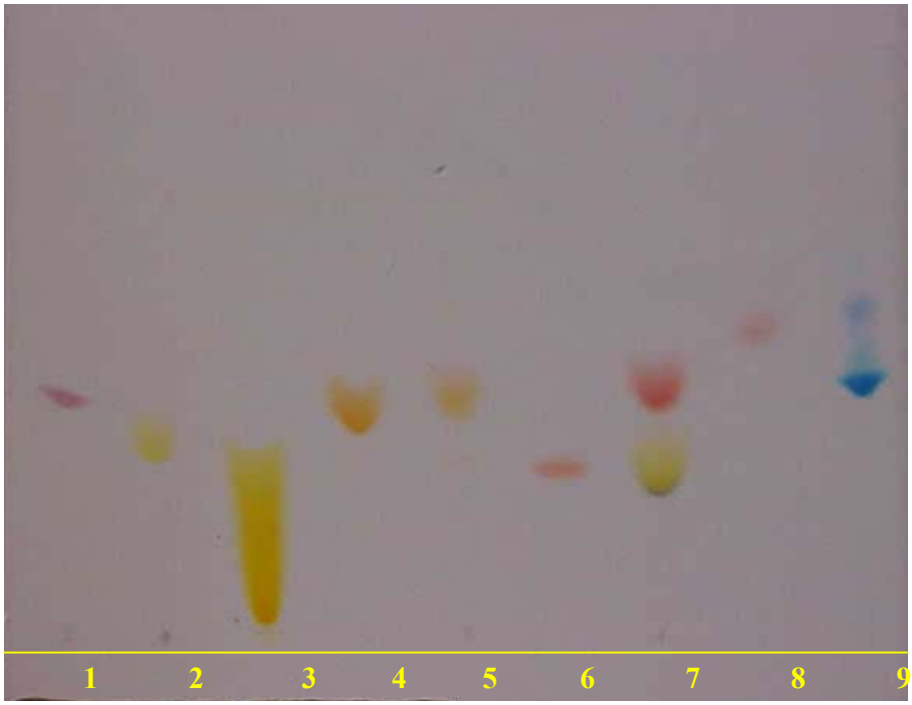

Plate (4.6). Thin layer chromatogram of coloured food extracts* in solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *B_2: Orange flavoured powder
3. **Tartrazine
4. **Sunset yellow
5. *D_7: yellow fanta
6. **Ponceau4R
7. *D_8: Red vita
8. **Carmosine
9. **Brilliant blue

Table 9 and plate 4.7 show the extraction of the separated samples of arwa pinapple flavoured powder (B_1), Saeed jam (A_2) and yellow mango vita D_6 using solvent system S-III. Sample B_1 which gave a yellow spot with Rf-value 0.20 was identical to tartrazine with Rf-value 0.20 while sample A_2 which had a red spot Rf of, 0.25 was similar to poncean 4R Rf of, 0.25. Sample D_6 was found to be resolved into three components: yellow Rf of, 0.17; red Rf of, 0.25 and orange Rf of, 0.43. These components were identified against reference colours of tartrazine Rf of, 0.20, amaranth Rf of, 0.27 and sunset yellow Rf of, 0.40 respectively.

Plate 4.8 demonstrates the chromatographic separation of the same samples B_1, A_2 and D_6 but using solvent system S-IV instead of S-III. Samples B_1 and A_2 each gave one coloured component: yellow (Rf, 0.47) and red (Rf, 0.45). They were similar to tartrazine (Rf, 0.47) and ponceau 4R (Rf, 0.45). Sample D_6 was found to contain a group of coloured components: red (Rf, 0.47), yellow (Rf, 0.50) and orange (Rf, 0.67). They were comparatively similar to amaranth (Rf, 0.60) tartrazine (Rf, 0.47) and sunset yellow (Rf, 0.67).

Plate 4.9 and table 10 show the separation of samples D_1I and D_1II using solvent system S-III sample D_1II was not separated while

sample D_1II gave an orange spot with(Rf, 0.65) it was similar to sunset with(Rf, 0.65).

Plate 4.10 and table 10 demonstrate the chromatographic separation of the extracts of samples D_1I and D_1II using solvent system S-III and S-IV separately. Sample D_1I was not separated in solvent S-III and S-IV. while sample D_1II had an orange coloured component(Rf, 0.65) was similar to sunset yellow(Rf, 0.65) in solvent S-III and also had an orange component(Rf, 0.43) was similar to sunset yellow (Rf, 0.43).

These results indicated that the solvent systems S-III and S-IV gave the same separation for sample D_1II but with different in Rf-values.

Generally, when comparing between the separation on paper and that of thin layer chromatography it was clear that the separation on TLC were sharper and clear spots were obtained. However, the number of the spots for separated colour components were similar for both paper and TLC. The separation on paper had the drawback of tailing and extended spots. The samples of jams, A_1, A_2 powdered juice B_1, B_2 and sweets C_1, C_2 gave one spot coloured components each on both paper and TLC which where also comparable with the reference colours used. Whereas the soft drinks D_1 – D_8 gave more

than one spot coloured components on separation on both paper and TLC showing that the main spot for each was comparable with the reference colour used.

Table 9: Rf-values of food colours from arwa pinapple flavoured powder (B_1), Saeed jam (A_2) and yellow mango vita (D_6) in comparison to reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-III	[2*]S-IV	[1*]S-III	[2*]S-IV
B_1	Yellow	Yellow	0.2	0.47
A_2	Red	Red	0.25	0.45
D_6	1\ Yellow	1\ Red	0.17	0.47
	2\ Red	2\ Yellow	0.25	0.50
	3\ Orange	3\ Orange	0.43	0.67
* Amaranth	3* Magenta red		0.27	0.60
*Ponceau 4R	3* Strawberry red		0.25	0.45
* Carmosine	3* Red		0.52	0.87
* Tartrazine	3* Lemon yellow		0.20	0.47
* Sunset yellow	3* Orange		0.40	0.67
*Brilliant blue	3* Turquoise blue		0.60	0.87

1* Iso-amylalcohol: glacial acetic acid : water (40: 20: 20).

2* n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20).

*3 Downham and Collins (1999). *Reference compounds.

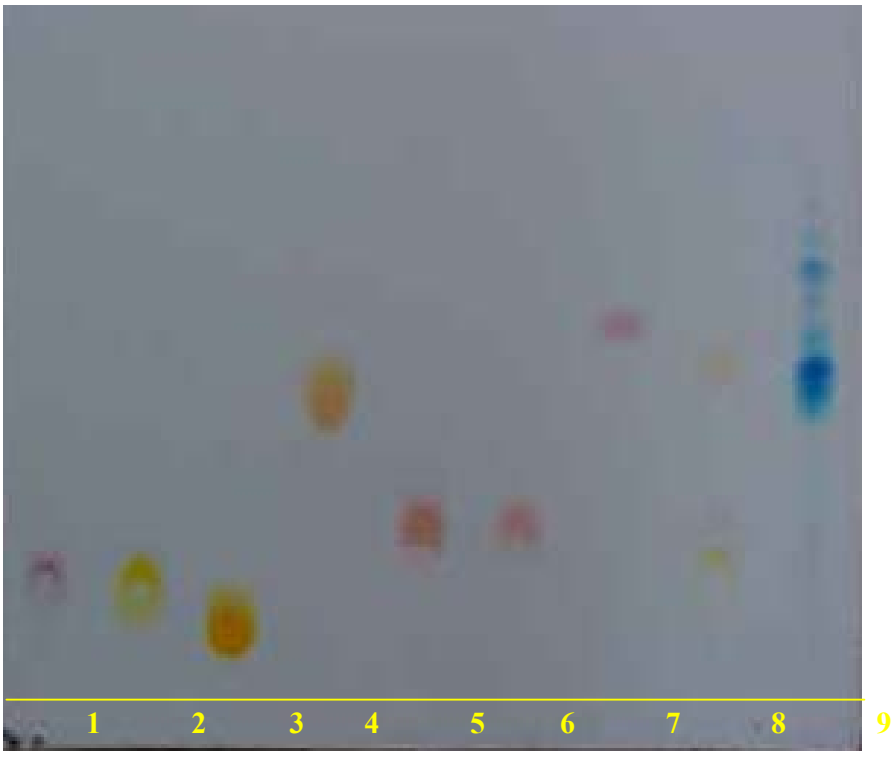

Plate (4.7). Thin layer chromatogram of coloured food extracts* in solvent system iso-amyl alcohol: glacial acetic acid: water (40: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *B_1: Arwa pinapple flavoued powder
3. **Tartrazine
4. **Sunset yellow
A_2: Saeed jam 5.
6. **Ponceau4R
7. **Carmosine
8. *D_6: Yellow mango vita
9. **Brilliant blue

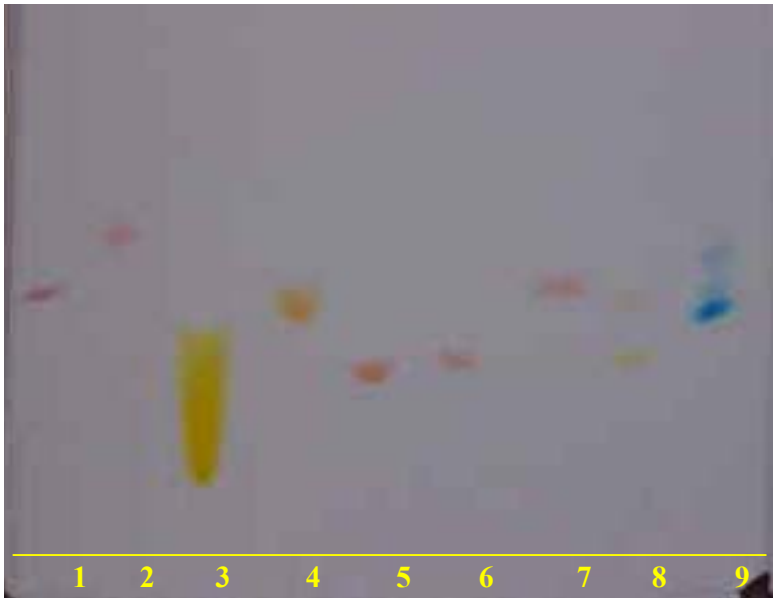

Plate (4.8). Thin layer chromatogram of coloured food extracts* in solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20) in comparison to reference coloured compounds**.

1. **Amaranth
2. *B_2: Orange flavoured powder
3. **Tartrazine
4. **Sunset yellow
5. A_2: Saeed jam
6. **Ponceau4R
7. ***Carmosine
8. *D_6: Yellow mango vita
9. **Brilliant blue

Table 10: Rf-values of food colours from samples D_1I,D_1II against reference compounds*

Sample	Colour of component in solvent		Rf-value in solvent	
	[1*]S-III	[2*]S-IV	[1*]S-III	[2*]S-IV
D_1 I	-	-	-	-
D_1 II	Orange	Orange	0.65	0.43
* Tartrazine	* Lemon yellow		0.50	0.27
* Sunset yellow	* Orange		0.65	0.43

1* Iso-amylalcohol: glacial acetic acid : water (40: 20: 20).

2* n-butanol: absolute ethanol : 2N ammonium hydroxide (60: 20: 20).

*3 Downham and Collins (1999).

*Reference compounds.

Plate (4.9). Thin layer chromatogram of coloured food extracts* in solvent iso-amyl alcohol: glacial acetic acid: water (40: 20: 20) in comparison to reference coloured compounds**.

2. D_1I: *Natural colour
4. D_1II: *Synthetic colour
1. **Tartrazine
3. **Sunset yellow

Plate (4.10). Thin layer chromatograms of coloured food extracts* in solvent n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20) in comparison to reference coloured compounds**.

2. D_1I: *Natural colour
1. **Tartrazine
4. D_1II: *Synthetic colour
3. **Sunset yellow

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions:

- The wool dying technique was found to be quite efficient for isolation of synthetic food colours.
- Paper chromatography is sufficient for separation and identification of food colouring materials.
- The solvent system trisodium citrate: ammonia solution: water (2: 5: 95, w/v/v) gave better separation with red pasgianos, yellow mango vita and red vita on paper chromatography, while the solvent system n-butanol: absolute ethanol: 2N ammonium hydroxide (60: 20: 20) gave better resolution with red pasgianos, yellow mango vita and red vita on thin layer chromatography.
- Most of the fourteen food products analysed contain single colour component except the soft drinks, red pasgianose, yellow mango vita, red vita.
- All samples contained synthetic colours only, except the soft drink maaza which contain additional natural yellow component as minor spot.

5.2. Recommendations:

- Thin layer chromatography is recommended to avoid tailing and overlapping with paper chromatography.
- More than one solvent system should be tried to optimize separation of coloured components.
- Reference colours should be included in the same chromatogram with the food samples for comparison and Rf-values measurement.
- Spectrophotometry is recommended for quantitative determination of colours as well as for confirmatory of identity of the colour at known wave length.

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