Detection of Synthetic Colours in Selected Foods & Beverages Available in Colombo District, Sri Lanka

G. Kumudu M. Rajapaksha¹, M.A. Jagath Wansapala², A. Buddhika G. Silva³

^{1, 2} Department of Food Science and Technology, University of Sri Jayewardenepura, Sri Lanka

³ Department of Nutrition, Medical Research Institute, Borella, Sri Lanka

Abstract: Colour is used in foods and beverages to make them more attractive to increase consumer's acceptability. Synthetic food colours are often preferred over natural colours today because they are less expensive, more stable, blend more easily, add no flavor and can be used in tiny amounts because they are more intense. Various food products available in the market may contain harmful nonpermitted colors. The consumption of eatables mixed with non-permitted colors, may lead to potential health hazards, such as increased mortality, retardation of growth, , decreased food intake and fertility rate. It also causes damage to organs like kidney, liver, heart, lungs, eyes etc. All these colors also have mutagenic properties and most of them have identified as potential carcinogens. Even, permitted food colors can prove to be toxic if used or consumed indiscriminately. In this study, different types of food stuffs available at Colombo district were analyzed for the detection of the added synthetic colours. A total of 120 samples of different food stuffs including Boondi, Bubble gum, Seeni murukku, Coconut toffee, Burfi, Muscut, Ice packet, Sherbet drink, Jelly, Bottled fruit drinks (each n=12) were collected from small and medium scale vendors and supermarkets in Colombo district from August to September in 2016. The samples were tested using Thin Layer Chromatography (TLC) method and UV-Spectrophotometric method by using synthetic colour standards which are permitted according to the food act regulations of Sri Lanka. The results of this study revealed the frequency of occurrence of permitted colours as well as indiscriminate use of non-permitted colours in some foods and beverages available in Colombo district.A majority of samples (85%) contained only permitted colours. Tartrazine (55.83%) was the frequently used synthetic colour in analyzed food stuffs. Subsequently, 30.83%, 25%, 20.83%, 14.17%, 3.33%, and 3.33% of total samples contained Carmosine, Sunset yellow, Brilliant blue FCF, Ponceau 4R, Erythrosine and Allura red respectively. Indigo carmine and Fast green FCF did not contained any tested samples. Only 9.2% of samples did not contain any synthetic colour. But 5.8% of samples contained a non-permitted colour namely Alizarin. Thus, frequency of occurrences of synthetic food colours is still high and incidence of the use of non-permitted colour in foods and beverages is at unsatisfactory level in Colombo district.

Keywords: Food, Synthetic colours, Thin layer Chromatography, UV-Visible Spectrophotometer, Food safety.

1. Introduction

According the data published in many countries it is showed that food intoxications are increasing. They are observed that coloured food products are the major source for food intoxication. Adding attractive colours can enhance the appetizing value of foods and beverages. Therefore most food manufacturers use many colours in their products including confectioneries, fruit drinks, soft drinks, jams and jellies, etc. (Asfaq and Masud, 2002)

In recent years, many synthetic colours have been widely used as color additives and substitutes for natural colours (Alves et al., 2008), as synthetic colours are often preferred over natural colors because they are more stable, add no flavor, are less expensive, blend more easily and can be used in smaller amounts because they are more intense. Many of synthetic colours are originally derived from coal tar and are commonly called coal-tar dyes, they contain the azo group. Colour chemicals are active chemicals; therefore it requires much care (Asfaq and Masud, 2002). Some of these colours and their metabolites can pose potential health risk to human beings and may even be carcinogenic, particularly if consumed in large amounts (Robens et al., 1980). In the past, artificial colours had been linked with hyperactivity in children (Redlinger and Nelson, 1993). According to the food act regulations of 2011 only nine synthetic colours are permitted to be used as colouring substances in foods in Sri Lanka. They are (E numbers in parentheses): Carmosine (Azorubine) (E 122), Ponceau 4R (E 124), Erythrosine (E 127), Allura Red (E 129), Sunset Yellow FCF (E110), Tartrazine (E 102), Indigotine (Indigo carmine) (E132), Brilliant Blue FCF (E 133), Fast Green FCF (E 143). Also the propotion of the synthetic colouring substance present shall not exceed as a single component or in combination, of 100 mg per 1 kg, of food or beverage ready for consumption. (The Food (Colouring Substances) Regulations - 2011, Sri Lanka)

If synthetic colours are consumed indiscriminately, it is not safe even they are permitted. Human studies indicated that food colours, (even natural or synthetic) can induce wide range of allergic reactions only in sensitive or atoptic individuals. Most of the foodborne diseases reported are due to the consumption of non-permitted textile colours or abuse of colours (**Babu and Shenolikar**, 1995).Considering the importance of the subject, this research is aimed to detect the presence of non-permitted food colours in different food products and to identify the frequency of each permitted synthetic colours in foods and beverages obtained from different areas of Colombo district in Sri Lanka.

2. Literature Review

Food colours

Food colour is any dye, pigment or any other substance that imparts colour when it is added to a food or beverage. It is a food additive primarily use for adding or restoring colour to a food or beverage. They can be identified in many forms as liquids, gels, pastes and powders. Food colours are used both in commercial food production and in domestic cooking. Colours are divided into four categories as natural, natureidentical, synthetic and inorganic colours. (Mortensen, 2006)

Food colours are also divided into two categories as permitted food colours and non-permitted food colours. (**Kapoor, 2006**) The United States Food and Drug Administration has been classified the permitted colours as 'Exempt from certification' or 'Subject to certification', both of them are subject to safety standards prior to their approval for use in foods. Certified colours are produced synthetically and mostly used as they impart an intense and uniform colour, are less expensive, and blend more easily to create a variety of hues. Colours those are exempt from certification include pigments came from natural sources. Nature derived colour additives are typically more expensive than certified colours and may added unintended flavours to foods. (International Food Information Counsil and U.S. Food and Drug Administration, 2010)

Synthetic food colours

Synthetic food colors are substances of chemicals which do not synthesis in nature and have been made by human. These colors are water-soluble and can be used in foods without any further processing (Bachalla, 2016).

Synthetic food colour is one of the main food additive groups. Most of them are acidic substances. They are used widely to optimize and compensate for food color, because freshness, ripeness, and flavor are all associated with food color (Wilson and Bahna 2005). Many of synthetic colours are originally derived from coal tar and are commonly called coal-tar dyes, they contain the azo group. Colour chemicals are active chemicals, therefore it requires much care when use them (Asfaq and Masud, 2002).

Usage of food colours

Human are attracted to foods and beverages which having pleasant colors, therefore addition of attractive colors can enhance the appetizing value and the palatability of food and beverage for the consumers. Therefore, most food manufacturers use variety of colors in their food products. (NIN Hyderabad, 1994). Most of food manufacturers use different colours in their bakery products such as cakes biscuits and pastries. Similarly colours are often used in the manufacture of confectioneries, soft drinks, fruit drinks, icecreams, toffees, jams and jellies, Snack foods, coloured candies, chewing gums, coloured ice- balls etc, by both street vendors and large manufacturers. Even in house level, colours are used to colour rice and other dishes, mainly to give them a more appetizing look (Saleem1 et al., 2013, Ashfaq and Masud, 2002).

Reasons for using synthetic colours in food articles.

Colorings have neither nutritional value, nor flavor, but they are very important in terms of marketability and acceptability (**Redlinger and Nelson, 1993**). Synthetic colors are used instead of natural colors in many foods as natural colours are unstable and easily degraded during food processing. High consistency to light, pH and oxygen, color monotony, low pollution are advantages of synthetic colours compared to natural colours. (**Alves et al., 2008**). Also synthetic colourings are often preferred over natural colorings because they are more stable, add no flavor, are less expensive, blend more easily and can be used in smaller amounts because they are more intense.

In recent years, many synthetic colours, chiefly azo dyes have been widely used as color additives and substitutes for natural colours. These colorus are used to make food more attractive, hide defects, improve superficial features of the products, and also to replace natural color additives that cannot stand the preparation process (Schuster and Gratzfeld, 1995).

Other reasons to use synthetic food colours are; Consumers demand color and variety in foods, The traders make their products look superior, attractive and thereby increase of sales and profit, Consumer carelessness, ignorance, indifference and lack of organized action of check the menace, Inadequate enforcement of foods and absence of deterrent punishment for offenders (**Bachalla**, 2016). Also to offset colour loss due to exposure to extremes of temperature, light, air, moisture and storage conditions, to enhance colour of foods and to provide colour to colourless foods, to compensate for natural or seasonal variations in food raw materials, to meet consumer expectations (International Food Information Council and U.S. Food and Drug Administration, 2010).

Global purview for food colours

In near past, food colors have been subjected to rigid toxicological examination and with the results most of the countries now have a very short list of permitted colors or additives. Different countries permit different synthetic food colors. Sri lanka permits only nine synthetic food colours (The Food (Colouring Substances) Regulations - 2011, Sri Lanka). The USA permits seven food colors, including Fast Red (which is prohibited in India & Sri Lanka), Iran and Australia permit thirteen each and in the European Union (EU) sixteen synthetic food colors are permitted. European countries harmonized the regulations, and controls on synthetic colours in food items from EU directives. Each country is attempting to review these controls by surveillance work (Padmaja, 2004). According to Pakistan pure food rules (1965) only eighteen synthetic colors are permitted in specified food items. Usage of black and brown colours is completely banned in developed countries as they contain ingredients(1956-1996. FAO/IPCS/WHO. harmful Geneva).

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Harmful effects & Health risk of synthetic food colours

Synthetic food colours in foods is the main source of food intoxication and surveys have been conducted to determine the presence of non-permitted colors in different food products (**Koutsogeorgopoulou et al., 1998**).Food colors have toxic effect on human body (EB, 2012). Synthetic colors in foods and beverages may lead to various complications such as weakening of the immune system, decreased lymphocyte and WBC count, vitamin B6 deficiency, abortion, hyperactivity of children, decreased IQ of children, carcinogenicity, anaphylactic reactions, idiosyncrasy, urticarial and asthma (Hinton, 2000; Geoffrey and Felix, 1991).

Exposure of animals to semi toxic doses of Sunset blue leads to make changes in total lipid storage of the animal body. This would influence their metabolism and may leads to hazardous hepatic injuries such as necrosis (**Mathur et al.**, **2005**). The studies have illustrated that even topical administration of colors may end in harmful effects for human health. For instance, a study carried out by the Food and Drug Administration (FDA) in 1990 revealed the color Fast green #3 which is used in cosmetic products with external use and drugs leads to thyroid cancer in male rats. Therefore FDA disapproved the color as using the color is associated with danger (**Hinton**, **2000**).

According to the Delany Clause, "No additive shall be seemed safe if it is found to induce cancer when ingested by man or animal." This law is applied for additive substances, colors and animal drugs. If colors taken by human induce cancer, which is repeatable and the disease is not secondary tohormonal and nutritional factors or physiological imbalance, then it is match with the clause (IFIC, 2011). Public introduction of natural colors and emphasizing their advantages would meet the diversity of customers' preference and also maintain community health. Further it would encourage producers to use natural colors in their products. For instance, substitution of synthetic red colors with anthocyanin in foods and beverages would be helpful, since anthocyanin have different properties such as decreasing vascular permeability, anti-inflammatory effects and prevention of capillary fragility (Farzianpour et al., 2012; Egan et al., 2007). Also saffron can be used as a substitute instead of synthetic colours which is a natural color with several medicinal properties, including the anticancer effects (Shamsa et al., 2009).

Other toxic effects of synthetic food color on human health are; they are carcinogenic and damage to kidneys and adrenals, they lower the red cell count and hemoglobin concentration and allergic reactions, they inhibit dopamine uptake by nerve ending, these colors are associated with irritability, sleep disturbance and restlessness in hypertensive, cause exhibited symptoms glossitis (Inflation of tongue), effects on liver and intestine. Allergic responses vary from uticaria to dermatitis, angioedema, etc, and they lead to Ear infections, Asthma,Autism, Dyslexia, Eczema,etc when consumption of high level of synthetic food colours (Bachalla, 2016).

3. Materials and Methods

3.1 Sample Collection

A total of 120 samples of ten food categories including boondi, bubble gum, seeni murukku, coconut toffee, barfi, muscut sherbet drinks, jelly, ice packets and fruit drinks (each n=12) were collected randomly from different small and medium scale vendors and super markets in Colombo district from August to September in 2016.

3.2 Chemicals and Reagents

Colour standards: nine synthetic food colourants; Carmoisine /Azorubine (E 122), Ponceau 4R (E 124), Erythrosine (E 127), Allura Red (E 129), Sunset Yellow FCF (E 110), Tartrazine (E 102), Indigotine/ Indigo carmine (E 132), Brilliant Blue FCF (E 133), Fast Green FCF (E 143).

Chromatography jar and lid, Glass capillary tubes

Stationary phase; Thin Layer Chromatography plate (silica coated aluminum foils, $20 \text{ cm} \times 20 \text{ cm}$).

Mobile phase; n-Butanol, Distilled water and Glacial Acetic Acid.

3.3 Sample pre-preparation and Colour Extraction

Synthetic colours were isolated according to method described by **Farzianpour** *et al.*, **2013** with modifications. In this method, before colour isolation, semi- solid and solid foods were dissolved in water.

Sample pre-preparation

Solid & Semi-solid samples

About 10g of ground food samples were taken in to a beaker and sufficient amount of petroleum ether was added to it. It was mixed very well and upper layer was discarded after allowing settle for about 1 minute. This step was repeated until get a colourless pet-ether solution. Then 2% ammonia in 70% ethanol solution was added to the sample and was mixed well. Mixture was warmed and then it was allowed cooling and settling down the starch. Upper layer was filtered into a centrifuge tube and all remained starch and solid materials were settled down by centrifugation at 2.5×10^3 rpm for 15 minutes. Upper layer was filtered and evaporated the solution by using a water bath.

Liquid samples

About 60 ml of each sample was centrifuged at 2.5×10^3 rpm for 15 minutes and then 50 ml from upper layer was measured into small conical flasks.

Colour extraction

Each prepared sample was acidified using 2M acetic acid and pure sheep wool thread of around 3 cm which was conditioned boiling in hot water for 3-5 minutes, was dipped in each acidified sample and it was kept in a water bath for around 30 minutes until colour components absorbed to the wool. Then the coloured sheep wool was washed under running tap water for 1-2 minutes and was dipped in 2M ammonia solution. It was kept again in a water bath and the wool thread was taken out when it was decolourized and the coloured solution was concentrated by evaporation. The dry color was then collected and dissolved in few drops of water and stored in a stoppered glass bottle for further analysis.

3.4 Thin Layer Chromatography Analysis

Colour standards and extracted colour samples were dissolved separately in few drops of distilled water. Then samples were spotted with standards in similar distances from each spot on a pencil line driven on TLC plate parallel to the plate bottom line and 2 cm away from it. For that separate cleaned capillary tubes were used. Each spots was allowed to dry about for 5 minutes and spotting step was repeated.

Then the chromatogram was run in a saturated chromatogram jar with n-Butanol: Distilled water: Glacial Acetic Acid (20: 12:10 v/v) system. When the solvent front was arrived about 3 cm from the top line of the TLC plate, it was taken out from the jar after solvent front was marked on the plate by using a pencil and it was allowed to dry.

3.5 Calculations

The identities of each separated spots were recorded. Then the retention factor (R_f values) for each separated colour spot was calculated using;

 $R_{f} \, value = \, \frac{Distance \, of \, the \, center \, of \, the \, sample \, spot \, from \, the \, origin}{Distance \, of \, the \, solvent \, from \, the \, origin}$

The position of each colour spot was taken from its center.

3.6 UV-Spectrophotometer Analysis

A tiny amount of standard colours were dissolved in 9 ml of distilled water with 1 ml of ethanol. Here all permitted synthetic food colours in SL were used as colour standards. According to the results of 3.4, colours which are identified as non- permitted were removed off with the silica layer from the thin layer chromatogram and dissolved in 4.5 ml of distilled water with 0.5 ml of ethanol in a test tube. Upper liquid layer was filtered off in to another test tube and were used as samples. These samples were prepared and used within 5 days from 3.4 step.

The maximum wave length, λ_{max} was measured for each colour standards and samples with wave length range of 380nm – 800 nm using UV-visible spectrophotometer. λ_{max} value of samples was compared with λ_{max} value of each standards to confirm whether the sample colours were permitted or non-permitted.

4. Results and Discussion

The study was carried out to find out the presence of nonpermitted colours in food samples and to identify the frequency of each permitted synthetic colours in foods and beverages obtained from different areas of Colombo district in Sri Lanka. In this study, TLC method was used basically to determine the synthetic colours which were added to sampled food types and then UV-spectrophotometric method was used for confirmation of non-permitted colours.

Figure 1 shows percentage of colour additives identified in the foods and beverages available in small and medium scale vendors and super markets in Colombo district.





As figure 1 indicates, Among the 120 of food samples, 5.8% of samples contained synthetic colours which are nonpermitted to use in foods by the Sri Lankan food act regulations. **Saleeml et al., 2013** also identified that nonpermitted colours are contained in different low-grade and cheap food products. Figure 1 shows about 85% of samples contained synthetic colours which are permitted. Only 9.2% of samples did not contain any synthetic colour additives. Therefore, as a total 90.8% of samples contained synthetic colours. Therefore, it is essential to create awareness at different levels about the toxicity of food colors either the use of non-permitted colors or excessive use of permitted food colors.

Table 1 shows only three of ten food types as Boondi, Muscut and Sherbet drinks contained Non-permitted colours. Also it shows all samples of Boondi, Bubblegum, Burfi, Muscut and Jelly food types contained synthetic colours and more samples of other food types also contained synthetic colours. Only few samples did not contain any synthetic colour. Therefore as a total, Synthetic colours were presence in higher percentage of food samples in each category.

Table 1: Distribution of synthetic colourswith food typ	e^{b}
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	With synthetic	With synthetic	Without
Type of sample	colours-	colours- Non-	synthetic
	permitted	permitted	colours
Boondi	91.7	8.3	0.0
Bubble gum	100.0	0.0	0.0
Seeni murukku	83.3	0.0	16.7
Coconut toffee	91.7	0.0	8.3
Burfi	75.0	25.0	0.0

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Muscut	100.0	0.0	0.0
Ice packet	91.7	0.0	8.3
Sherbet drink	66.7	25.0	8.3
Jelly	100.0	0.0	0.0
Fruit drink	50.0	0.0	50.0
Total	85.0	5.8	9.2

^bData are precented as percentage.



Figure 2: Types of synthetic colours observed in the samples.

Figure 2 clearly indicates that all permitted synthetic colours except Indigo carmine and Fast green FCF were used in food and beverages as a food additive. From analyzed food samples, 85% of samples contained permitted synthetic colours; they are Tartrazine 55.83%, Carmosine 30.83%, Sunset yellow 25%, Brilliant blue FCF 20.83%, Ponceau 4R 14.17%, Erythrosine 3.33% and Allura red 3.33%.

The mostly used permitted synthetic colourants in analyzed food products were Tartrazinefollowed by Carmosine, Sunset yellow, Brilliant blue FCF, Ponceau 4R, Erythrosine and Allura red. Indigo carmine and Fast green FCF were not used in any analyzed food samples in Colombo district.

Nadia A. and Tariq M., 2002 observed that Tartrazine is the frequently used synthetic colour in almost all kinds of food products especially in sugar confectioneries. It is matched with the experimental results of this study.

Although tartrazine is a permitted yellow colour, it 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**). A typical case of anaphylactoid purpura associated with tartrazine has been reported (**Wuthrich, 1993**). According to **Lockey, 1977**, other food colours such as sunsets yellow and Ponceau 4R have also been implicated in adverse reactions in patients with chronic urticarial.

Tartrazine is an approved azo dye which presents in many food products. However its sensitivity has been reported. Its sensitivity is mainly manifested by asthma andurticaria, the mechanism of sensitivity is not clear and is called pseudo allergy (**Dipalma**, **1990**); the management of pseudo allergy consists of avoidance of foods that contain tartrazine. It has been reviewed that azo dyes (Allura Red, Sunset yellow, and Tartrazine) can be reduced to aromatic amines by the intestinal micro flora and hence causes intestinal cancer (**Chung and Stevens.**, **1978**).

Therefore it is necessary to limit usage of synthetic colours, especially tartrazine in foods. And international system of regulations must be followed while using the permitted colors in foods to assure the implementation of safety strategies, as required.

 Table 2: Synthetic colour distribution with the colour of the food/drink^c

Synthetic colour	Red	Orange	yellow	Green	Brown	Blue
Carmosine	60.5	18.2	0.0	33.3	50.0	0.0
Allura red	10.5	0.0	0.0	0.0	0.0	0.0
Ponceau 4R	34.2	9.1	0.0	0.0	33.3	0.0
Erythrosine	10.5	0.0	0.0	0.0	0.0	0.0
Tartrazine	18.4	40.9	84.4	100.0	50.0	0.0
Sunset yellow	5.3	86.4	15.6	4.8	50.0	0.0
Indigo carmine	0.0	0.0	0.0	0.0	0.0	0.0
Brilliant blue FCF	0.0	0.0	0.0	95.2	66.7	100.0
Fast green FCF	0.0	0.0	0.0	0.0	0.0	0.0
Unknown	18.4	0.0	0.0	0.0	0.0	0.0
Total	100	100	100	100	100	100

^cData presented as percentage.

Table 2 clearly indicates that Tartrazine is the frequently used colour in Yellow (84.4%) and Green (100%) food products. Brilliant blue is the mostly used colour in Brown (66.7%) and Blue (100%) food products. Carmosine and Sunset yellow was the mostly used colour in Red (60.5%) and Orange (86.4%) food products respectively.

The overall colour distribution of the permitted food colors revealed that tartrazine in blend with brilliant blue FCF is the usually used color indicated in a variety of colored food items, followed by sunset yellow in blend with ponceau 4R, erythrosine and carmosine, carmosine, ponceau 4R, brilliant blue FCF in blend with erythrosine and tartrazine (**Saleeml** et al., 2013)

	Table 5. The results of ron-permitted colour contained samples.							
Sample No.	Food type	Food colour	Distance of the center of the unknown sample spot from the origin	Distance of the solvent front from the origin	R _f Value	Other colours identified in same sample on the same TLC		
1	Boondi	Red	4.0	14.3	0.28	Allura red, Ponceau 4R, Sunset yellow		
49	Barfi	Red	4.0	14.3	0.28	Carmosine, Ponceau 4R		
50	Barfi	Red	4.1	14.3	0.29	Carmosine, Ponceau 4R		
52	Barfi	Red	4.1	14.3	0.29	Carmosine, Ponceau 4R		

 Table 3: TLC results of Non-permitted colour contained samples.

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86	Sherbet drinks	Red	4.0	14.3	0.28	Carmosine, Ponceau 4R
88	Sherbet drinks	Red	3.8	14.3	0.27	Carmosine, Ponceau 4R
89	Sherbet drinks	Red	4.1	14.3	0.29	Carmosine, Ponceau 4R, Tartrazine

Table 3 indicates the $R_{\rm f}$ value of the colour identified as nonpermitted is nearly 0.28 on silica layered aluminum foil chromatography by running with the mobile phase of nbutanol : distilled water : glacial acetic acid (20 : 12 : 10 V/V). All the non-permitted colours were found in the samples are the same one as they were appeared as yellow spots in TLC and $R_{\rm f}$ value were nearly similar in all the samples (table 3). Furthermore the non-permitted colour was found in red food samples. The non-permitted colour was yellow in acidic medium as the used mobile phase was acidic. But subsequently it turned to red within 5-7 days when the plate gets more dried.

According to confirmation of non-permitted colour by UV-Spectrophotometric method, λ_{max} values of permitted synthetic colours are shown in table 3 and the, λ_{max} values of the colour identified as non-permitted in samples are shown in table 4.

Table 4: λ max values of permitted synthetic colour standards

Colour standard	λ_{max} value / nm				
Carmosine	515				
Ponceau 4R	507				
Erythrosine	526.3				
Allura red	502.5				
Sunset yellow	483				
Tartrazine	427				
Indigo carmine	609				
Brilliant blue FCF	629.5				
Fast green FCF	623.5				

Table 5: λ max values of non-permitted colour samples prepared within 5 days from TLC step.

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Sample no.	λ_{max} Value / nm
1	433
49	433
50	432
52	433.5
86	433
88	433
89	418.8

Table 4 and table 5 confirm that , the λ_{max} value of the non-permitted colour is 433 nm $\,$ and also it is not matched with the λ_{max} value of any permitted colour in Sri Lanka in the same medium.

UV-spectrum and λ max value of sample no.86 (sherbet drink sample) is shown in Figure 3 and in figure 4.



Figure 3: UV-Spectrum of sample no. 86 (sherbet drink sample)



Figure 4: λmax value of sample no. 86 (sherbet drink sample)

As Table 4 shows, the λ_{max} value of the non-permitted colour is 433 nm in distilled water with ethanol medium. According to **Anouar et al., 2014,** they observed the λ_{max} value at 433 nm for 1,2-dihydroxyanthraquinone in ethanol. According to **Alizarin Vankar cited in Asha et al.**, chemical compound namely 1, 2-dihydroxyanthraquinone is known as Alizarin or Mordant red and Turkey Red. It is an organic compound with molecular formula C14H8O4 that has been historically used as a prominent dye mainly for dyeing textile fabrics. Alizarin is the first dye which has been synthesized in laboratory and is now finding usage in painting industry. Figure 5 shows structure of Alizarin.



Figure 5: Structure of Alizarin

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According to **Rogers**, "Alizarin is used as an acid-base (pH) indicator in chemical analysis. It is yellow below a pH of 5.6 red above a pH of 7.2 changing to purple above 11.0. **The 2010 Handbook of Biological Dyes and Stains** says that alizarin is yellow up to pH 5.5, red at pH 6.8 to 10.1, and purple from pH 12.1. That indicates Alizarin is yellow in acidic medium and red in neutral medium.

According to the TLC results, the non-permitted colour was visible as yellow colour up to first 4-5 days and subsequently it was gradually turned into red when the solvent system fully evaporated from the plate. The used solvent system was n-butanol : distilled water : glacial acetic acid (20 : 12 : 10 v/v). Solvent system is an acidic medium. Therefore, the non-permitted colour is yellow in acidic medium and is red in neutral medium. According to the results of this study with the literature survey, the non-permitted colour was in food samples is identified as Alizarin.

5. Conclusion

Synthetic colour usage in foods and beverages is more frequent today. The consumption of edibles mixed with nonpermitted colors, may lead to potential health hazards. Even, permitted food colors can prove to be toxic if used or consumed indiscriminately. The regulations related to synthetic colours in food consider the health of human and the adulterations of foods and economic needs. The results of this study revealed the frequency of presence of permitted food colours as well as indiscriminate use of non-permitted colours in foods and beverages which are available in small and medium scale vendors and super markets in Colombo district. The used methods of detection can be used in the area of food quality control to detect whether non-permitted synthetic colours are present or not in food. Efficient analytical methods are required for evaluating toxicity and authenticity in order to determine whether synthetic colours present and the level of permitted synthetic colours in foods, to confirm the absence of added colours in food and to check the stability of colours during processing and storage. This study revealed that frequency of occurance of synthetic colours and non-permitted colours is still high. Therefore a systematic approach is needed to evaluate the level of permitted colours in foods and to determine the frequency of occurance of non-permitted colours in other foods within the country. Together it is needed to enforce and implement certain rules or regulations on the manufacture of such products to prevent ill effects of using non-permitted colours as well as permitted colours above permicible level to ensure the good food quality and food safety for human beings.

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Author Profile

G.Kumudu M.Rajapaksha is a final year (2016) undergraduate student in Department of Food Science and Technology at University of Sri Jayewardenepura who is following the degree of B.Sc. (Special) in Food Science and Technology. She has successfully completed this research project under the expert guidance of M.A. Jagath Wansapala, Senior lecturer in Food Science and Technology who holds B.Sc. in Chemistry, M.Sc. in Food Science and Technology andPhD in Food Science and A. Buddhika G. Silva, Chemist at Medical Research Institute who holds B.Sc. in Chemistry and M.Sc. in Food Science and Technology.

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