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IDENTIFICATION OF SYNTHETIC FOOD COLOUR IN SELECTED CONFECTIONERIES BY UV-VISIBLE SPECTROPHOTOMETRY

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ABSTRACT
This study aimed to develop a simple and cost-effective method for identifying synthetic food colors in confectioneries using UV-Visible Spectrophotometry and Thin Layer Chromatography (TLC). This is important to ensure regulatory compliance and consumer safety. The study focused on detecting and quantifying Tartrazine (E-102), Ponceau 4R (E-124), Sunset Yellow FCF (E-110) and Brilliant Blue FCF (E-133) in confectionery samples including tutti frutti, wafers, lollipops, cakes and murukkus. Standard solutions were used to create calibration curves for UV-Visible Spectrophotometry. The calibration curves showed a linear relationship between concentration and absorbance for each colorant. The TLC method confirmed the presence of the colors in the confectionery samples. The R_f values calculated from the TLC chromatograms agreed with the standard R_f values for each colorant. The results showed that different samples of each colorant had varying concentrations. Some samples were within the permissible limits, while others exceeded the limits set by food safety authorities. The study concluded that it is important to continue monitoring the use of synthetic food colors in confectioneries because excessive consumption can have adverse health effects. The results of this study can help regulatory agencies and food manufacturers ensure compliance with food safety standards.

KEYWORDS: UV-Visible Spectrophotometry, Thin Layer Chromatography (TLC), Tartrazine (E-102), Ponceau 4R (E-124), Sunset Yellow FCF (E-110), Brilliant Blue FCF (E-133).

INTRODUCTION
Spectrophotometric methods
Spectrophotometry measures the intensity of light as a light beam travels through a sample solution to determine how much a chemical compound absorbs light. The fundamental idea is that every substance either transmits or absorbs light throughout a specific wavelength range. The amount of a known chemical compound can also be determined using this method. One of the best techniques for quantitative analysis is spectrophotometry. The number of photons or the intensity of light, absorbed after it travels through the sample solution is measured by a device called a spectrophotometer. By measuring the intensity of light observed, the spectrophotometer can also be used to determine concentrations or the amount of a known chemical substance. They can be divided into two categories based on the light source's wavelength range.

- UV-visible spectrophotometer.
- IR spectrophotometer.[1,2]

UV Spectrophotometric Methods
In pharmaceutical analysis, one of the most widely used methods is UV-visible spectrophotometry. It measures the quantity of visible or ultraviolet light that a material in a solution absorbs.[3]

Chromatographic methods
Chromatography is a crucial biophysical method that makes it possible to separate, identify and purify a mixture's constituent parts for both qualitative and quantitative study.

The foundation of chromatography is the idea that molecules in a mixture applied to a surface or into a solid and fluid stationary phase (stable phase) separate from one another as they move and are assisted by a mobile phase. Molecular properties pertaining to adsorption (liquid-solid), partition (liquid-solid), affinity, or variations in their molecular weights are the elements that have an impact on this separation process. Due to these variations, some mixture components flow quickly into the mobile phase and exit the chromatographic system more quickly, while others remain in the stationary phase longer and move more slowly.[4]

Thin Layer Chromatography

A thin glass plate coated with silica gel or aluminum oxide serves as the solid phase in thin layer chromatography. A solvent chosen according to the properties of the mixture's constituent parts serves as the mobile phase. The basic idea behind TLC is to distribute a compound between a liquid mobile phase that travels across the solid phase and a solid fixed phase that is positioned on a glass or plastic plate. A small quantity of a combination or compound is applied to the TLC plate just above the bottom. The developing chamber, which has a shallow pool of solvent just below the level where the sample was applied, is then used to develop the plate. Capillary action pushes the solvent up through the plate's particles and as the solvent passes through the mixture, each component either dissolves in the solvent and travels up the plate or stays with the solid phase. Whether the chemical moves up the plate or stays behind depends on its physical characteristics as well as its molecular structure, especially its functional groups. The solubility rule that is adhered to is "Like Dissolves Like". A compound will stay in the mobile phase for a longer period of time if its physical characteristics match those of the mobile phase. The more soluble substances will be transported up the TLC plate by the mobile phase. Chemicals with a greater affinity for the particles on the TLC plate and less solubility in the mobile phase will adhere to the plate.[5]

$$\mathbf{R_f} = \frac{\textbf{Distance travelled by solute}}{\textbf{Distance travelled by solvent front}}$$

INTRODUCTION TO SYNTHETIC COLORANTS IN THE FOOD INDUSTRY

Synthetic colorants are a vital component of the modern food industry, playing a significant role in enhancing the visual appeal of food products and contributing to consumer satisfaction.[6]

These pigments, derived from chemical synthesis, are meticulously designed to impart vibrant and consistent hues to various food items, from confectioneries to beverages.[7]

Enhancing Visual Appeal: Synthetic colorants are instrumental in creating visually appealing products that captivate consumers. They allow manufacturers to achieve precise shades and intensities, ensuring that their products stand out on store shelves and satisfy consumer expectations. This is particularly crucial for industries like confectionery, where colour plays a pivotal role in enticing customers.

Meeting Regulatory Standards: The use of synthetic colorants is subject to strict regulatory frameworks that ensure their safety for human consumption. These regulations outline permissible colorants, their maximum allowable concentrations, and labelling requirements. Adherence to these regulations is crucial for maintaining consumer trust and compliance with legal requirements.

Consistency and Cost Effectiveness: Synthetic colorants offer remarkable consistency in colour, ensuring that batches of products maintain a uniform appearance. They are also generally more cost-effective compared to natural colorants, making them a viable option for large-scale production.[8, 9]

The majority of synthetic food coloring compounds contain an azo group and are derived from coal tar. Permitted and nonpermitted are the two categories into which these colors can be separated. Many foods, including baked goods, confections, jellies and market-available beverages, employ artificial food coloring. The market's assortment of foods and drinks may include both excessive usage of approved synthetic colors and some prohibited ones. Many studies have demonstrated that artificial food coloring is a significant cause of food intoxication and can result in serious health issues like low hemoglobin levels, allergic reactions, mutations, cancers, irritability, restlessness, sleep disturbances, effects on the liver, kidney and intestine, hyperactive effects in children, ear infections, asthma and eczemas. It is also unsafe to use approved synthetic colors carelessly.[10, 11]

Different Systems for Classification of Food Colours[12]

Table 1: Different systems for classification of food colours.

CLASSIFICATION SYSTEM	GROUPS	EXAMPLES
ORGIN OF COLORANTS	Natural	Carotenoids, anthocyanins
	Synthetic	FD&C[a] colorants
	Inorganic	TiO2
STRUCTURAL CHARACTERISTICS	Tetrapyrrole derivatives	Chlorophylls and heme colors
	Isoprenoid derivatives	Carotenoids and iridoids Purines, pterins, flavins, phenazines, phenoxazines, and betalains
	N-heterocyclic compounds different from tetrapyrroles	Purines, pterins, flavins, phenazines, phenoxazines, and betalains
	Benzopyran derivatives	Anthocyanins and other flavonoid pigments.
	Quinones, Melanins	Benzoquinone, naphthoquinone, anthraquinone

CHEMICAL STRUCTURE OF THE COLORANTS	Chromophores with conjugated systems	Carotenoids, anthocyanins, betalains, caramel, synthetic pigments and lakes.
	Metal-coordinated porphyrins	Myoglobin, chlorophyll, and their derivatives

CLASSIFICATION OF SYNTHETIC FOOD COLOURS[13]

Table 2: Classification of synthetic food colours.

SI. No	Colour	Types	Color Shade	Chemical class	Emperical Formula
1	Red	Ponceau 4R	Strawberry Red	Mono azo	$C_{20}H_{11}N_2O_{10}S_3Na_3$
		Carmoisine	Red	Mono azo	$C_{20}H_{12}N_2O_7S_2N_2$
		Erythrosine	Bright Pink/Red	Xanthene	$C_{20}H_8O_5I_4Na_2$
2	Yellow	Tartrazine	Lemon Yellow	Mono azo	$C_{16}H_9N_4O_9S_2Na_3$
		Sunset Yellow	Orange	Mono azo	$C_{16}H_{10}N_2O_7S_2Na_2$
3	Blue	Indigotine carmine	Royal Blue	Indigoid	$C_{16}H_8N_2O_8S_2Na_2$
		Brilliant Blue FCF	Turquoise Blue	Triarylmethane	$C_{37}H_{34}N_2O_9S_3Na_2$
4	Green	Fast Green FCF	Sea Green	Triarylmethane	$C_{37}H_{34}N_2O_{10}S_3Na_2$

DIFFERENCE BETWEEN NATURAL AND SYNTHETIC FOOD COLOURS

Food colours can be synthetic, synthesized equally to the natural, or naturally derived. Although most of the natural food colours are derived from plants, some others are obtained from animals or even ores. contrarily to synthetic dyes, the consumption of natural food colourants can have significant benefits; they are in demand for their reliability, functionality, biological potential and health benefits. Natural colours are obtained from nature, and can be isolated by more or less complicated extraction processes, whereas synthetic dyes are chemically synthesised. The synthetic food colours do not occur in nature due to chemical structures, but have the advantage of prevailing in the form of powders, pastes or granulates, and are soluble in water. Nevertheless, some synthetic colour additives may present health problems, namely allergenic problems, which in children can cause hyperactivity and even mutagenic and/or carcinogenic pathologies. In order to surpass the health side effects of the synthetic dyes, their replacement with natural food colourants is frequently an option.

NATURAL COLOURS

The term ‘natural’ is associated with not altered/unchanged, not created by human, but produced by nature. A natural colour is a substance that is a substance that is produced from a plant, animal, mineral. The colours do not add any nutritional value to the foods. They are added to restore colours that are lost during food processing. Natural food colours used in the food industry are a wide range. Some of the most commonly used carotenoids, anthracyanins, betalains and chlorophylls.

SYNTHETIC COLOURS

Very often, to achieve high colour intensity, more stable and uniform colour, synthetic colours are used in food. They have some advantage for use in food, such as a light and pH stability, at the same time with low cost. These properties often make them preferred in food production. In food production, during processing and storage, foods tend to lose their natural colours and require the addition of colour, which is seen as a technological necessity.[14]

Table 3: Applications of Natural and Synthetic colours.[15,16]

NATURAL		SYNTHETIC	
EXAMPLES	**APPLICATION**	**EXAMPLES**	**APPLICATION**
Carotenoids	Beverages, Dairy Products, Bakery Products, Fruit Preparation.	Carmoisine	Carmoisine, a red to maroon shade in applications, is admired for its usage in add beverages, ice cream, sweat meant and allied. We are offering a wide gamut of carmoisine color and the compositions of $C_2OH_{12}N_2Na_2O_7S_2$.
Curcumin	It can be used in condiments such as butter, bakery products, sauces, canned food, pickles, mustard, seasonings, relish, hot peppers, snacks, baked goods, salad dressing, oils, margarine,	Tartrazine	Tartrazine, a synthetic lemon yellow azo-dye, is used as a wide variety of foods including desserts and candies, soft drinks, condiments and breakfast cereals with a great quality, reliability and service. The main composition of tartrazine is

	frozen desserts, cheeses, pies, cakes, candies, beverages, frosting, cereal, fruit preparation, convenient food, meat, seafood, and soups.		$C_{16}H_9N_4Na_3O_9S_2$.
Caramel	Caramel color is mostly used in soft drinks and alcoholic beverages. It can also be added to drugs, cosmetics, and food including confectionery, bakery products, dairy products, desserts, meat, seafood, vinegar, sauces, gravies, soups, snack food, and fruit preparations, and convenient food.	Erythrosine	Erythrosine gives a pink to reddish pink shade in applications. It is commonly used in candies, popsicles and cake – decorating gels. Erythrosine food color pigments are widely appreciable in different products with composition $C_2O_H6_{I4}Na_2O_5$.
Anthocyanins	Deserts, Ice-Creams, Beverages, confectionary, fruit preparations, baker's jam, and non-standard jellies and preserves, sherbets, ices, pops, raspberry, yogurt, gelatin desserts, candy, and bakery fillings and toppings.	Allura Red	A dark red dye that is used in sports drinks, candy, condiments and cereals.
Chlorophyll	Confectionery, instant food, soft drinks, soups, beverages, soaps, cosmetics, ice cream, jellies, desserts, beverages, dairy products, fruit preparation, bakery products, sauces, snack food, seasonings, and convenience food.	Sunset Yellow	An orange-yellow dye that is used in candy, sauces, baked goods and preserved fruits.
Annatto	For coloring dairy products such as cheese, and butter.	Brilliant Blue	A greenish-blue dye used in ice cream, canned peas, packaged soups, popsicles and icings.
Paprika	Paprika is used to color meat products, confectionery, vegetable oils, snacks, surimi, seasonings, sauces, meat product, soups, bakery products, salad dressings, marinades, processed cheese, fruit preparations, convenient foods, and canned goods.	Indigo Carmine	A royal blue dye found in candy, ice cream, cereal and snacks.

MATERIALS AND METHODS

APPARATUS

Volumetric flask, pipette, measuring cylinder, mortar and pestle, beakers, test tubes, test tube holder, water bath, glass slides, watch glass, spatula, funnel.

SAMPLE COLLECTION

The samples were collected from retail shops near school areas in Thrissur district.

i ii

Figure 1: (i) Palak murukku sample; (ii) Tomato murukku sample

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Figure 2: (i) Red velvet cake sample; (ii) Mango cake sample.

Figure 3: Green, Red, Yellow, Orange colored Tutti frutti.

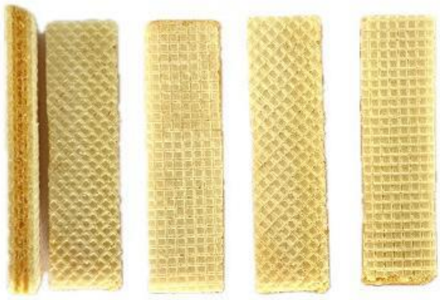

Figure 4: Wafer sample.

Figure 5: Orange, Green, Yellow, Red colored Lollipops.

CHEMICALS AND INSTRUMENTS

Standard Synthetic Colors

The standard synthetic colors used are Ponceau 4R (E-124) for red, Tartrazine (E-102) for yellow, Brilliant Blue FCF (E-133) for green and Sunset Yellow FCF (E-110) for orange.

Figure 6: Standard colors used.

Chemicals

Petroleum ether, ammonia, methanol, butanol, acetic acid, ethanol, distilled water.

Instruments

The instruments used were UV–Visible spectrophotometer – 1900 (Shimadzu), Micro centrifuge, Electronic Balance – (Wensar ISO 9001:2000 Certified).

SAMPLE PREPARATION

The confectionery sample types used were categorized as tutti frutti, wafer, lollipop, cake and murukku.

Preliminary Treatments

5.0 g of sample was weighed, to which petroleum ether was added in quantity enough to remove the oil content from it. The oil-removed sample was heated in a water bath for two to three minutes to allow the starch to settle. Then add 10 ml of 2% ammonia in 70% alcohol was added to oil-removed sample, the above mixture was warmed in water bath for about 2-3 mins for the starch to settle down. The resulting colorful liquid was centrifuged at 30,000 rpm for 15 minutes. Supernatant liquid layer was collected and evaporated using a water bath.

COLOR EXTRACTION

2 ml of 2M glacial acetic acid was added to the pretreated sample to slightly acidify it and then added 2 ml of distilled water. To concentrate the color intensity, boil it on a water bath for a few minutes before diluting it with water. Move it to a glass cuvette, then measure the color's absorbance at the longest wavelength.

Figure 7: Test tubes containing extracted colors.

IDENTIFICATION OF EXTRACTED COLORS

Chromatographic Analysis

To identify the extracted colors, thin layer chromatography was used. This technique deals with separating and identifying artificial food coloring in candies. The adsorption concept is the foundation of TLC. Stronger adsorption results from increased polarity and vice versa. A single drop of ethanol was added to a few drops of distilled water to dissolve the extracted color samples and color standards. They prepared mobile phases for color identification.

- Mobile phases preparation

For red color identification:
ethanol : n-butanol : distilled water (9:2:1).
For yellow color identification:
n-butanol : ethanol : distilled water : ammonia (10:5:5:2).
For orange color identification:
n-butanol : ethanol : distilled water : ammonia (10:5:5:2).
For green color identification:
n-butanol : acetic acid : distilled water (4:1:5)

- Retention Factor (R_f)

In chromatography, the proportion of the sample in the mobile phase at equilibrium is known as the retention factor (R_f). After recording the distinct color spots, it was computed. Each spot's location was determined by measuring it from the center. It was computed using the subsequent formula/

$$Rf = \frac{\textbf{distance of the colour spot travelled from the baseline}}{\textbf{distance of the solvent travelled from the baseline}}$$

The R_f values of the standards and samples were contrasted.

- Developing chromatograms

A thin-layer chromatographic plate was positioned vertically in a beaker of mobile phase after spots of samples and standards were affixed to the bottom of the plate in accordance with each color. By means of capillary action, the solvent migrated higher on the plate, bringing sample spots with it. After the solvent reached the top of the plate, it was taken out and inspected so that the colors and migration distances of the standards and samples could be compared to identify the samples.[17]

UV-Visible Spectrophotometric Analysis

Using a UV-visible spectrophotometer, unknown colors were verified. All of India's approved synthetic food coloring standards were applied in this investigation. One drop of methanol and distilled water were used to create a color standard of 0.0001 M. After being scratched, the unidentified color spot on the TLC silica plate was dissolved in two drops of methanol and five drops of distilled water. After the upper liquid layer was removed, a UV-visible spectrophotometer was used to measure the λ_{max} (also known as lambda max or λ_{max}, which is the wavelength of the absorption spectrum where the absorbance is maximal) for each color standard and sample falling between 280 and 800 nm. The spectra and λ_{max} values of the standards and samples were compared. 14 days following the TLC analysis, the reproducibility test was conducted.[18]

RESULTS AND DISCUSSION

The identification of synthetic food colors in confectioneries is crucial to ensure compliance with food safety regulations. This study employed Thin Layer Chromatography and UV-Visible spectrophotometry to detect and quantify synthetic food colors, specifically Red, Yellow, Orange and Green in confectionery products. The use of these methods offers a rapid, sensitive and cost-effective method for analyzing these colorants. The results of this study provide valuable insights into the presence and concentration of synthetic food colors in confectioneries. This section presents the findings of the study, including the R_f values, absorbance and concentrations of the identified synthetic food colors.

THIN LAYER CHROMATOGRAGHY

Table 4: TLC reports of various samples.

Color	Sample	Obtained R_f Value	Standard R_f Value
RED	Tutti frutti	0.56	0.53 - 0.57
	Lollipop	0.57	
	Red velvet cake	0.55	
	Tomato murukku	0.53	
YELLOW	Tutti frutti	0.67	0.65 – 0.70
	Lollipop	0.69	
	Mango cake	0.65	
ORANGE	Tutti frutti	0.58	0.55 – 0.60
	Lollipop	0.59	
	Wafer	0.55	
GREEN	Tutti frutti	0.43	0.40 - 0.45
	Lollipop	0.45	
	Palak murukku	0.40	

The TLC chromatograms shows that the confectionery samples contain a mixture of synthetic food colors. The R_f values of the confectionery samples match with those of the standard synthetic food colors, indicating the presence of Ponceau 4R, Tartrazine, Sunset Yellow FCF and Brilliant Blue FCF in the samples. However, the use of these colorants is regulated by food safety authorities and excessive consumption of these has been linked to adverse health effects.

UV-VISIBLE SPECTROPHOTOMETRY

Red Color

The absorbance standard solutions with varying concentrations were measured. The sample and standard findings are tabulated.

ANALYSIS OF STANDARD RED FOOD COLOR

Table 5: Linearity data of standard Red food color.

Concentration	Absorbance
10	0.199
15	0.381
20	0.568
25	0.740
30	0.917
35	1.062
Lollipop sample	3.898
Concentration	116.0086
Red velvet cake sample	4.050
Concentration	120.3764
Tutti frutti sample	0.954
Concentration	31.4109
Tomato murukku sample	0.419
Concentration	16.0373

Using the calibration curve method, a graph was created by plotting absorbance on the y-axis and concentration on the x-axis. The resulting straight line shows linearity, meaning that absorbance rises linearly as the solution's concentration rises. The standard graph can be extrapolated to determine the sample's concentration.

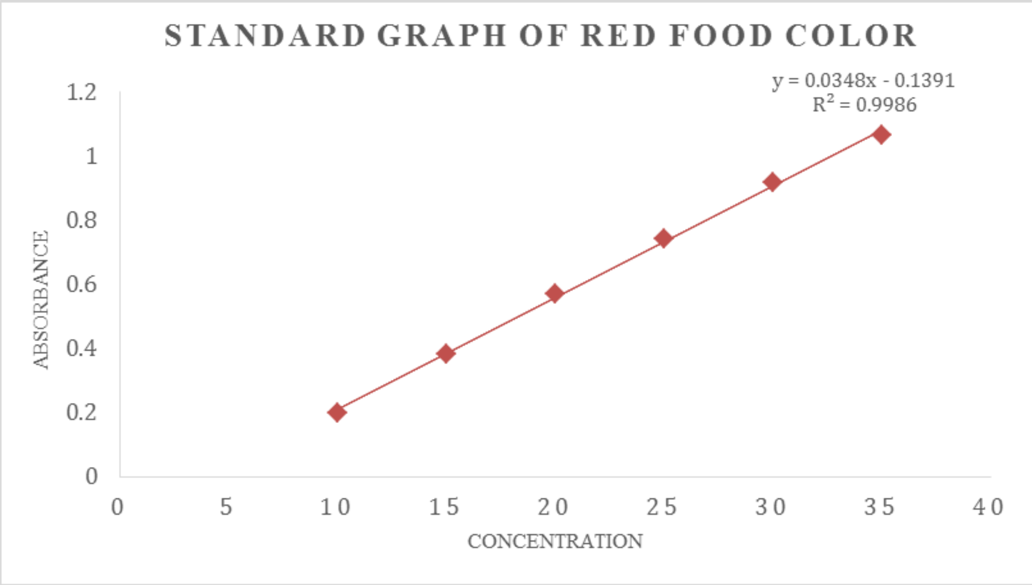

Figure 8: Calibration curve of red food color (Graph 1)

Yellow color

The absorbance standard solutions with varying concentrations were measured. The sample and standard findings are tabulated.

ANALYSIS OF STANDARD YELLOW FOOD COLOR

Table 6: Linearity data of standard Yellow food color.

Concentration	Absorbance
10	0.209
15	0.340
20	0.495
25	0.635
30	0.771
35	0.921
Lollipop sample	3.835
Concentration	123.1187
Tutti frutti sample	0.534
Concentration	19.9625
Mango cake sample	4.628
Concentration	147.9

Using the calibration curve method, a graph was created by plotting absorbance on the y-axis and concentration on the x-axis. The resulting straight line shows linearity, meaning that absorbance rises linearly as the solution's concentration rises. The standard graph can be extrapolated to determine the sample's concentration.

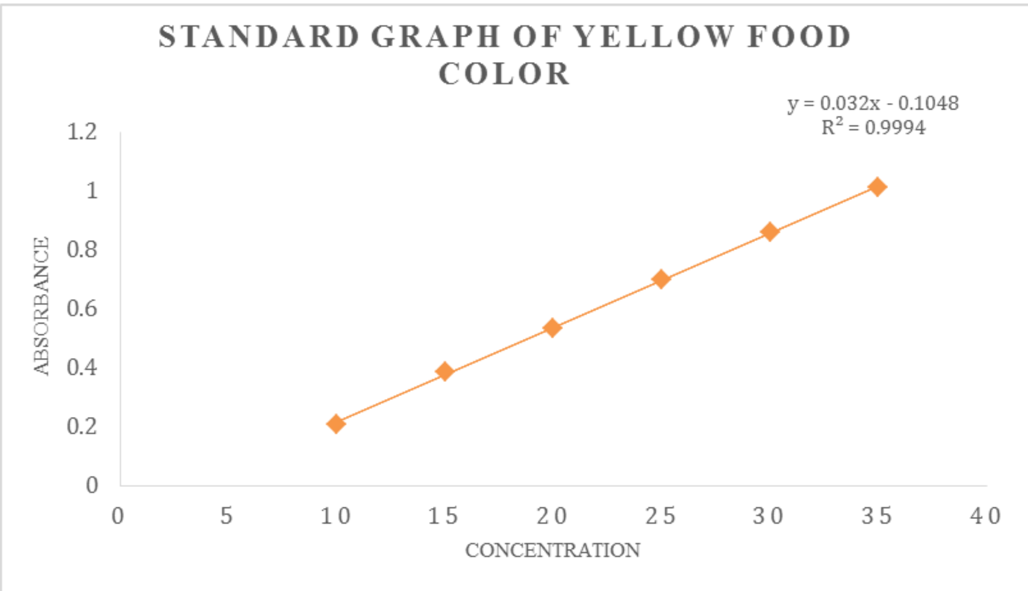

Figure 9: Calibration curve of yellow food color (Graph 2)

Orange color

The absorbance standard solutions with varying concentrations were measured. The sample and standard findings are tabulated.

ANALYSIS OF STANDARD ORANGE FOOD COLOR

Table 7: Linearity data of standard Orange food color.

Concentration	Absorbance
10	0.238
15	0.389
20	0.553
25	0.703
30	0.846
35	0.988
Lollipop sample	4.283
Concentration	137.1187
Tutti frutti sample	0.719
Concentration	25.7437
Wafer sample	0.475
Concentration	18.1187

Using the calibration curve method, a graph was created by plotting absorbance on the y-axis and concentration on the x-axis. The resulting straight line shows linearity, meaning that absorbance rises linearly as the solution's concentration rises. The standard graph can be extrapolated to determine the sample's concentration.

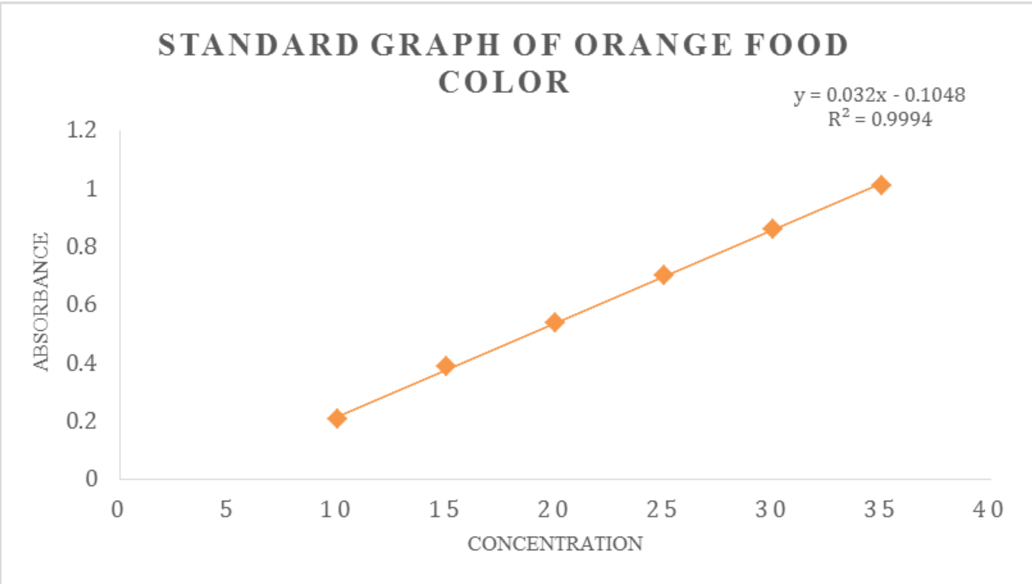

Figure 10: Calibration curve of orange food color (Graph 3)

Green Color

The absorbance standard solutions with varying concentrations were measured. The sample and standard findings are tabulated.

ANALYSIS OF STANDARD GREEN FOOD COLOR

Table 8: Linearity data of standard Green food color.

Concentration	Absorbance
10	0.205
15	0.387
20	0.535
25	0.698
30	0.859
35	1.010
Lollipop sample	5.251
Concentration	167.3687
Tutti frutti sample	0.578
Concentration	21.3375
Palak murukku sample	0.312
Concentration	13.025

Using the calibration curve method, a graph was created by plotting absorbance on the y-axis and concentration on the x-axis. The resulting straight line shows linearity, meaning that absorbance rises linearly as the solution's concentration rises. The standard graph can be extrapolated to determine the sample's concentration.

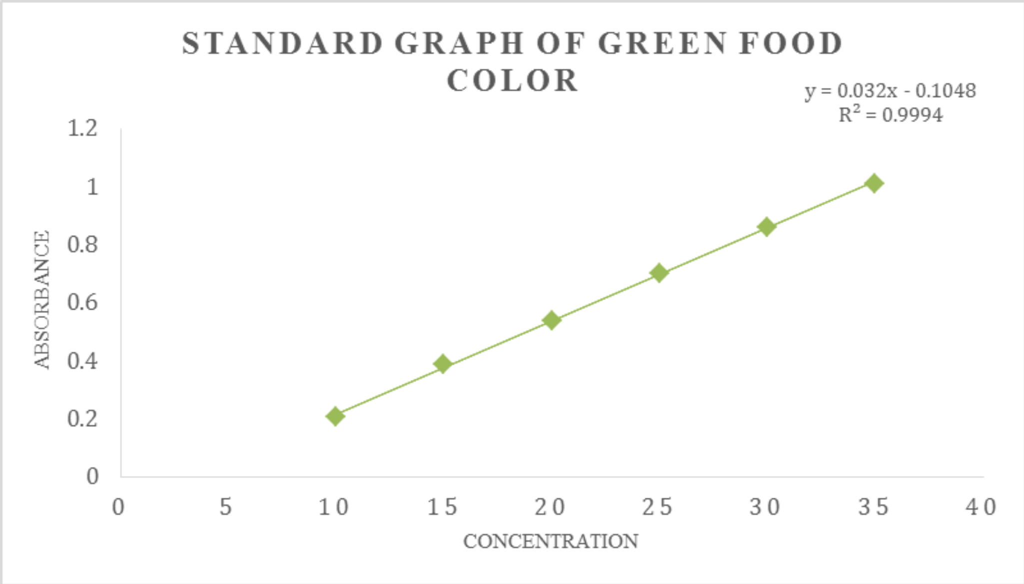

Figure 11: Calibration curve of green food color (Graph 4).

COMPLIANCE

Table 9: Comparison of sample concentration with permissible limit.

Color	Samples	Observed concentration (100 ppm)	Permissible limit set by food safety authorities
RED	Lollipop	116.0086	100 ppm
	Red velvet cake	120.3764	
	Tutti frutti	31.4109	
	Tomato murukku	16.0373	
YELLOW	Lollipop	123.1187	100 ppm
	Tutti frutti	19.9625	
	Mango cake	147.9	
ORANGE	Lollipop	137.1187	100 ppm
	Tutti frutti	25.7437	
	Wafer	18.1187	
GREEN	Lollipop	167.3687	100 ppm
	Tutti frutti	21.3375	
	Palak murukku	13.025	

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