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Original article

Quantitative Analysis of Synthetic Food Dyes in Libyan Confectionery Using UV-Vis Spectroscopy

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Abstract
The use of synthetic colors as food colorants in confectionery is increasing despite the potential health risks associated with their consumption. They make foods and beverages more attractive and are often used as alternatives to natural colors due to their colorfastness, consistency, and low cost. Therefore, it is necessary to constantly monitor the concentrations of these dyes in food and confectionery due to their health-related concerns. The investigation was conducted using a quartz cell and distilled water as a solvent, over a wavelength range of 400 nm to 800 nm. Twelve hard candy samples were collected from the Libyan market, containing four colorants: Sunset Yellow (E110), Ponceau 4R (E124), Allura Red AC (E129), and Brilliant Blue (E133). The results showed that the concentrations of synthetic colorants in the candy samples ranged from 14.74 ± 0.69 mg/kg to 215.03 ± 6.42 mg/kg. Among the detected dyes, E110 was found in five samples, with concentrations ranging from 14.45 ± 0.68 mg/kg to 120.77 ± 3.91 mg/kg. E129 was present in multiple samples, with concentrations ranging from 43.28 ± 0.24 mg/kg to 215.03 ± 6.42 mg/kg. E133 was identified in four samples, with concentrations between 21.13 ± 1.12 mg/kg and 127.87 ± 0.64 mg/kg. All concentrations were below the Codex Alimentarius limit of 300 mg/kg. E124 was detected in two samples, with concentrations of 38.96 ± 0.78 mg/kg and 143.59 ± 4.25 mg/kg; one sample (9(124)) exceeded the permitted level of 100 mg/kg.
Keywords. Ultraviolet/Visible Spectroscopy, Synthetic Colorant. Sunset Yellow (E110), Ponceau 4R (E124).

Introduction

Color is everywhere. Take a look around both man-made and natural teens [1]. By simply observing our surroundings, we can see the vital role that color plays in our everyday lives. Since the earliest civilizations, humanity has shown a deep fascination with colors. The man of the cave, whose cave is embellished with colorful creatures, paintings, and passages of the Egyptian, Greek, and Roman civilizations. Color permeates all aspects of our lives. Influencing our moods and emotions, and so on [2]. Colorants are applied in many applications, such as food, textiles, pharmaceuticals, and cosmetics, where colors are used as food additives added to many foods, such as ice cream, candy, jelly, yogurt, milk, and juice. There are two types of colors in general. Colors that are both natural and synthetic.

Color is the distinctiveness of a particular food, influencing its selection and perceived quality. Changes in food color typically indicate a change in quality [3]. Synthetic colorants, chemically produced substances, are used to enhance the color of food and drinks due to their brightness, stability, and affordability. Unlike natural colorants, they have complex aromatic structures. Synthetic colors in high concentrations in food may cause health problems, side effects, and toxicity in the medium and long term. Some studies suggest potential links between artificial food colorings and health issues like hyperactivity in children, allergic reactions, and even carcinogenic effects in certain conditions. As a result, their use is regulated by food safety authorities, such as the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), which set acceptable daily intake levels. And ensure that only approved colorants are used in food production. Therefore, there are analytical techniques that have been used in the identification and recognition of synthetic foods, such as. Thin Layer Chromatography TLC, Ultraviolet-visible spectroscopy UV-VIS, High-performance liquid chromatography HPLC, and Capillary electrophoresis EC. The results are compared with the Acceptable Daily Intake (ADI) from the Food and Agriculture Organization of the World Health Organization (FAO/WHO).

Synthetic dyes are widely used and regulated due to health concerns. Simple and reliable methods like UV-VIS spectroscopy are commonly used to measure their concentrations in food due to their simplicity, low cost, and rapid execution. Herndon and Pierce (2023) applied this technique using a Thermon Fisher Scientific Genesys 20 spectrophotometer to analyze 12 samples of various powdered drink mixes containing Tartrazine (E102), Allura Red AC (E129), and Brilliant Blue FCF (E133). Their findings revealed strong linear correlations between absorbance and concentration, with coefficients of determination (R^2) of 0.99875 for E102, 0.99695 for E129, and 0.99405 for E133.

The study also showed that powdered drink mixes containing E129 exhibited higher concentrations of synthetic dye compared to other samples. Furthermore, the data indicated a variation in dye concentrations between drink mixes containing Red 40 and those without it, which was influenced by both the type and number of dyes present in each sample [4].

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In a separate study (Alghamdi, et al., 2021), the Spectrophotometric technique was applied for the determination of sunset yellow (E110) in soft drinks and ice cream samples of the local Saudi markets. Produced the relative standard deviation (RSD%) of 0.042%. A concentration range of 1×10^{-5} to 1×10^{-4} mol/L was used to establish the calibration curve. For the (E110) dye to result in a linear relationship with a 0.984 correlation coefficient R2 for six measurements (n=6). limit of Detection (LOD) was calculated to be 5.77×10-8 mol L-1(0.026 ppm) [5]. (Lawal, A .al et al., 2021) determined the concentration of E110 used as a coloring in ten different brands of sweets using a spectroscopic technique. An appropriate buffer solution (pH 7.0) was used. Primary cross-sectional data were obtained through trial testing procedures in ten different samples of confectionery products (A, B, C, D, E, F, J, H, I, G) purchased (Katsina Central Market, Nigeria) by a T60 UV-Visible spectrophotometer. The calibration curve had a correction coefficient of R2 =0.9997. The results indicate the concentration values for Samples A, B, C, D, F, G, H, and J are within the acceptable permissible range. Nevertheless, samples E and I exhibited concentrations of 52.311 ± 0.178 mg/L and 87.887 ± 0.018 mg/L, respectively, which were above the established permissible threshold [6].
The same researchers (Lawal, A., Suleiman, N., and Abdul Karim, S. 2020) estimated the tartrazine concentration in the same way and with the same device for five different samples of beverages (powder) and soft drinks (K, K). D, A, B, E) Purchased from the central market located in Katsina (Katsina State – Nigeria). The analyte concentrations were found to be at, which highlighted that the concentration values for samples K, B, and E are; 74.304 ppm, 67.364 ppm, and 84.118 ppm respectively, and were Within the permissible limit of 100 mg/kg in non-alcoholic drinks as published by Codex Alimentarius International Food Standards Committee 2008, adopted by the National Agency N Food and Drug Administration and Control (NAFDAC). UK Food Standards Agency (2008) [7].
According to Ghaffar, F. (2020), the study aimed to analyze the food colors added to various confectionery items sold in the local market of Peshawar. Samples of different types of candies were examined to identify and quantify the added synthetic food colors using a UV 1700 spectrophotometer (Shimadzu, Japan). While most branded items contained permitted colors, some products were found to include non-permitted dyes. Approximately 22% of the items tested contained non-permitted colors. The most commonly identified dyes were Ponceau 4R (E124) (20.9%), Sunset Yellow (E110) (18.7%), Tartrazine (E102) (16.7%), and Brilliant Blue (E133) (14.6%). Although the majority of branded products contained dyes within permissible limits, several non-branded items showed elevated concentrations of Carmoisine (E122), with levels of 375.0 ± 0.9 and 364.8 ± 0.68 mg/kg, exceeding the permitted limit of 50 mg/kg. Tartrazine was also found in higher concentrations in non-branded items. Additionally, Naphthol Yellow was detected at significantly high concentrations (312 ± 10.43 and 812 ± 21.07 mg/kg) in non-branded products. The concentrations of brilliant blue FCF were quite high in some items [8]. (Karen E. Stevens, 2006). This article presents a simple technique for analyzing the absorption spectra by a Spectronic 20 spectrophotometer for six samples, which was an easy method for determining colorants in school [9]. (Samuella B, et al. 2004). This paper focuses on the development of a simple spectrophotometric method for quantitative determination by a UV-2401 spectrophotometer to determine the concentration of food dye E129, E102, and E133 in 9 samples of Various Powdered Drink Mixes. The results were good, and the concentration of the colorants was found [10].

Methods

Study design

An empirical analytical study will be conducted. The concentrations of four different artificial dyes (Sunset Yellow, Ponceau 4R, Allura Red AC, and Brilliant Blue) will be determined in candy samples using a UV-Vis spectrophotometer under standard conditions. For samples containing more than one dye, a chromatographic column separation method will be used to isolate the individual components.

The study sample

The study sample was taken from different types of sweets. Twelve samples were collected randomly from local markets in different places in the city of Al-Khoms.

Instruments

A UV-Vis spectrophotometer model 6300 (Jenway, Hong Kong) was used, which is a microprocessor-controlled instrument operating in the visible range from 320 to 1000 nm with a bandwidth of 10 nm. Additional laboratory equipment included a monochromator, electronic balance, quartz cuvettes, pipettes, test tubes, standard flasks, mortar and pestle, and a chromatographic column. Hydrophilic PTFE syringe filters (FILTER-LAB, Spain) with a pore size of 0.45 μm and a diameter of 25 mm were also used.

Materials and Reagents

Standards of Sunset Yellow (≥85%), Ponceau 4R (≥80%), Allura Red AC (≥80%), and Brilliant Blue (≥80%) were purchased from Glentham Life Sciences, UK. Additional chemicals used in the study included distilled water, methanol, and silica gel.

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Preparation of stock solution
Individual stock solutions of Sunset Yellow, Ponceau 4R, Allura Red AC, and Brilliant Blue were prepared at a concentration of 1000 mg/L (equivalent to 1000 ppm) by dissolving 250 mg of the reference dye powder in 250 mL of distilled deionized water. Working solutions were subsequently obtained by appropriate dilution of the stock solutions. All stock solutions were freshly prepared prior to use.

Preparation of a working solution
A 100 mL aliquot of the standard stock solution (1000 mg/L) was pipetted into a 500 mL volumetric flask and diluted to the mark with distilled water to prepare a 200 mg/L solution. Subsequently, a serial dilution technique was employed to obtain a set of working solutions with progressively lower concentrations. To initiate the dilution process, 10 mL of the 200 mg/L solution was transferred into a clean test tube containing 20 mL of distilled water, yielding a final volume of 30 mL with a concentration of 66.66 mg/L. This procedure was repeated sequentially: 10 mL from each prepared tube was transferred into the next tube containing 20 mL of distilled water, maintaining a consistent final volume of 30 mL.
The resulting concentrations obtained through serial dilution were: 66.66, 22.22, 7.4, 2.47, 0.823, 0.274, 0.0913, 0.0304, 0.0101, and 0.00337 mg/L. Each tube was thoroughly mixed after dilution to ensure homogeneity.

Preparation of Sample Solution
Candy samples were ground into a fine powder using a mortar and pestle. Accurately weighed portions of 5 grams were transferred into 50 mL conical flasks and dissolved in distilled water. The mixtures were gently heated on an electric hot plate to ensure complete dissolution. The resulting solutions were poured into chromatographic columns packed with silica gel to separate the colorants. The mobile phase—either distilled water or 75% methanol—was carefully added by allowing it to flow along the inner walls of the glass columns. Solvents were added as needed throughout the experiment. The tap was opened to initiate elution, and the separation of colorants was based on the polarity of the molecules, with non-polar components migrating faster than polar ones. The eluates were collected into evaporating dishes and evaporated to dryness. The resulting dry residues were reconstituted in 50 mL of deionized water. The final sample solutions were filtered using hydrophilic PTFE syringe filters (pore size: 0.45 µm, diameter: 25 mm; FILTER-LAB, Spain) to obtain clear, ready-to-analyze solutions.

Procedure
For the standard working solutions, aliquots were measured and transferred into 1.0 cm path length quartz cuvettes. The absorbance of the colorants was measured against a blank solution of distilled water using a UV-Visible spectrophotometer. The maximum absorbance wavelengths (λ_{max}) were recorded at 483, 507, 504, and 630 nm for Sunset Yellow, Ponceau 4R, Allura Red AC, and Brilliant Blue, respectively (4,5,6,7,8,9,10). Each measurement was repeated three times for all working standard solutions, and the average absorbance values were calculated. These averages were then used to plot calibration curves, which served to determine the analyte concentrations in the samples.
Subsequently, samples were prepared for pH and UV absorbance measurements using the spectrophotometer. Quartz cuvettes with a 10 mm optical path length were employed for all measurements.

Linearity and calibration standards
The linearity of the method was assessed using ten different concentrations of each synthetic colourant: 66.66, 22.22, 7.4, 2.47, 0.823, 0.274, 0.0913, 0.0304, 0.0101, and 0.00337 mg/L. However, not all concentrations were included in the construction of the calibration curves. The lowest concentrations were excluded because they were below the detection limit of the UV-Vis spectrophotometer, while the highest concentrations (66.66 to 22.22 mg/L) were omitted due to absorbance values exceeding the instrument's linear range. The UV-Vis Spectrophotometer 6300 (Jenway, Hong Kong) used in this study operates within an absorbance range of –0.300 to 1.999 A, with a resolution of 0.001 A. This high resolution allows for precise detection of small changes in absorbance, which is particularly advantageous when analysing samples with low analyte concentrations.
Calibration curves were plotted with dye concentration on the x-axis and absorbance on the y-axis (Figure 1). The coefficients of determination (R^2) obtained were 0.9999 for E110, 0.9987 for E124, 0.9987 for E129, and 0.9983 for E133 (Figures 1–4), indicating excellent linearity. Additionally, the limits of detection (LOD) for E110, E124, E129, and E133 were found to be 0.1445, 0.656, 0.464, and 0.620 mg/L, respectively. The corresponding limits of quantification (LOQ) were 0.437, 1.990, 1.406, and 1.884 mg/L. These values confirm the high sensitivity and reliability of the method. The strong linearity can be attributed to the high purity of the standard materials and the use of the serial dilution technique, which helps minimise experimental errors.

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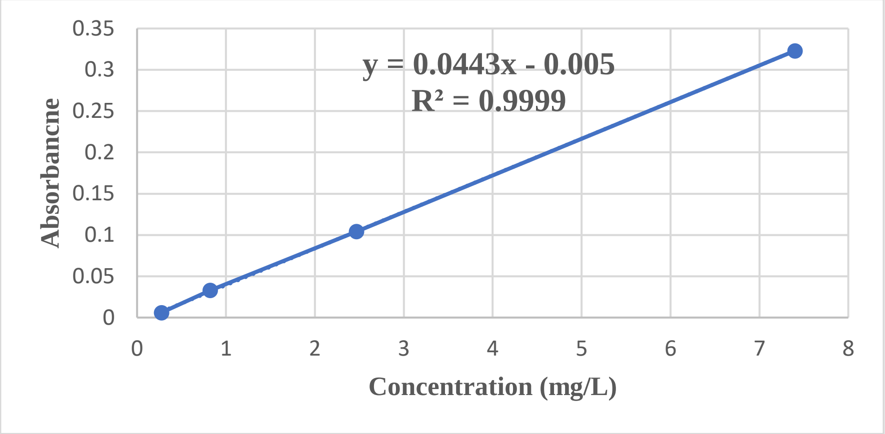

Figure 1. Calibration curve for Sunset yellow FCF (E110)

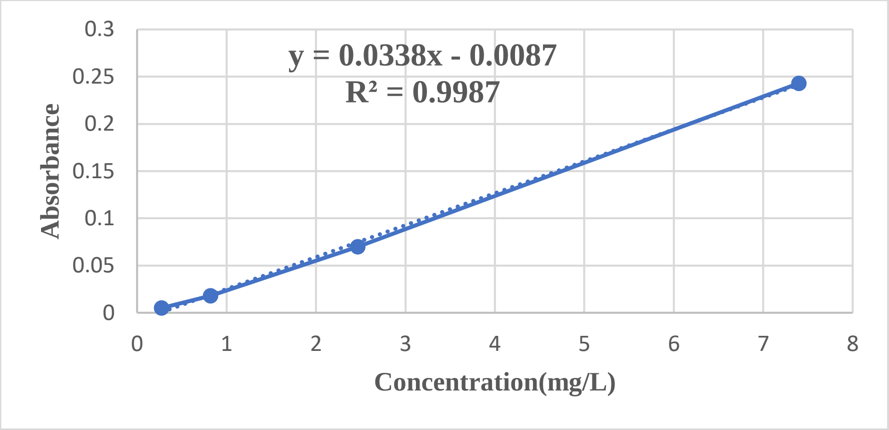

Figure 2. Calibration curve for Ponceau 4R (E124)

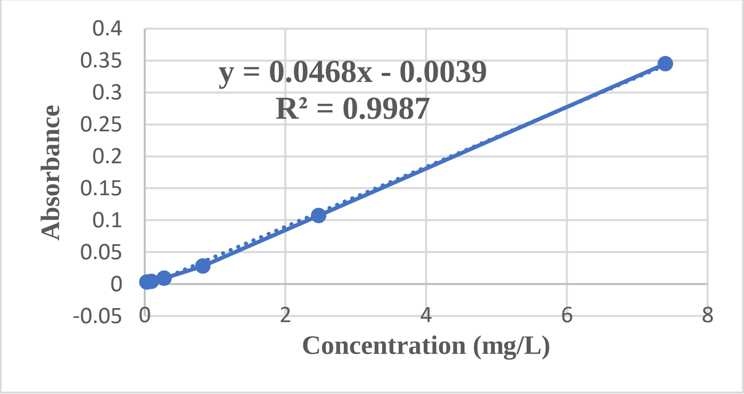

Figure 3. Calibration curve for Allura red AC (E129)

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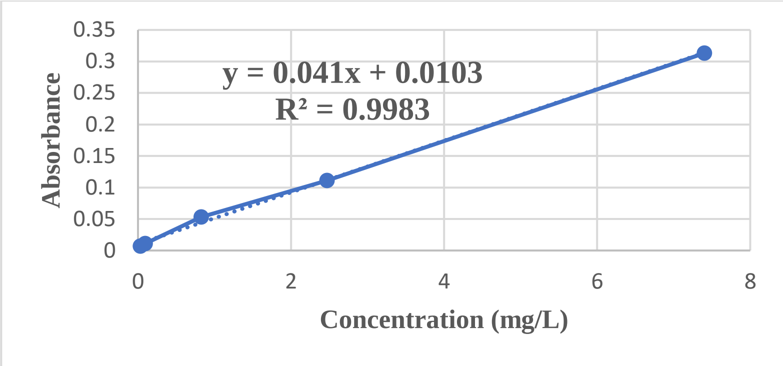

Figure 4. Calibration curve for Brilliant blue FCF (E133)

the accounts

Food colorant concentrations are found using some mathematical operations. The first sample will be taken to illustrate.

First, convert the color concentration in the sample from mg/l to mol/l.

$M= \frac{ppm*0.001}{MW}$

$M= \frac{3,45*0.001}{452.37\frac{g}{mol}}$

$M= 7.7*10^{-6}$ M

M is molarity

Ppm is Parts Per Million

MW is the Molecular weight of the colourant

Then the masses of the food colorant were calculated by using the above-computed food dye concentrations, the volume of the solutions, and the food colorant molecular weights. M is the concentration of the food colorant calculated above, V is the volume of the solution in liters, and MW is the molecular weight of the food colorant.

mass of the food colorant= $(M)*(V_L)*(MW)$

mass of the food colorant= $(7.7*10^{-6})*(0.05)*(452.37)$

mass of the food colorant $1.741*10^{-4}$ gram

The concentrations were then calculated using the computed mass of food dye in the candy, shown above, as well as the sample mass of each. These values were multiplied by 106 to obtain the concentration expression ppm.

$Ppm = \frac{\text{mass of the food colorant}}{\text{mass of sample}}$

$Ppm = \frac{1.741*10-4\ \text{gram}}{5\ \text{gram}}$

Ppm=34.25mg/kg

The previous calculations are applied to all samples to determine the sample concentration of mg/kg as shown in (Table 1) [4].

Table 1. Concentration of synthetic color samples

		N=3		Absorbance (A)		N=3		Concentration (mg/kg)	
Samples	1	2	3	Mean	1	2	3	mean	SD
1) (110)	0.147	0.149	0.144	0.147	34.31	34.76	33.63	34.23	0.568887804
2) (110)	0.118	0.123	0.126	0.122	27.76	28.89	29.57	28.74	0.91427567
3) (124)	0.126	0.122	0.121	0.123	39.85	38.66	38.37	38.96	0.784283112
4)(110)	0.44	0.41	0.41	0.420	100.45	93.68	93.68	95.94	3.908661322
5) (110)	0.061	0.063	0.057	0.060	14.89	15.34	13.99	14.74	0.687386354
6) (129)	0.218	0.212	0.215	0.215	47.41	46.13	46.77	46.77	0.64
7)(129)	0.198	0.198	0.2	0.199	43.14	43.14	43.56	43.28	0.242487113
7) (133)	0.079	0.079	0.071	0.076	21.78	21.78	19.83	21.13	1.125833025
8) (110)	0.52	0.55	0.52	0.530	118.51	125.28	118.51	120.77	3.908661322
9) (124)	0.49	0.48	0.46	0.477	147.54	144.58	138.66	143.59	4.521474686
10) (129)	0.365	0.357	0.36	0.361	78.82	77.11	77.75	77.89	0.863963734
10) (133)	0.333	0.334	0.351	0.339	83.73	83.97	88.12	85.27	2.468204476
11) (129)	1.025	1.012	0.971	1.003	219.85	218.99	208.31	215.71	6.428758304
11) (133)	0.515	0.516	0.511	0.514	128.12	128.36	127.15	127.88	0.640650711
12) (129)	0.316	0.299	0.311	0.309	68.35	64.77	67.28	66.8	1.84
12) (133)	0.142	0.136	0.144	0.141	37.14	35.68	37.63	36.82	1.014

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Results and discussion

Synthetic colorants were separated from the candy samples using chromatographic columns packed with silica gel. In general, the separation process was effective. However, for Sample 4 containing (E110), the analysis indicated the presence of two colorants (E110 and E129). Despite this, only E110 was observed during the separation, while no visible trace of (E129) a red dye, was detected. This may be attributed to the complex matrix of the sample, including components such as emulsifiers and gelatin, which likely interfered with the chromatographic separation process and contributed to longer elution times. In Sample 7, three colorants were initially indicated (E110, E129, and E102). However, E102 was excluded from the analysis as it falls outside the scope of this study. (Table 1) shows the absorbance values of the synthetic colorants obtained using UV-Vis spectrophotometry under standard conditions at 25 °C. The recorded absorbance values ranged from 0.1467 ± 0.0025 to 1.0027 ± 0.0282, with relative standard deviations (RSD%) ranging between 0.515 and 6.051. The concentrations of the synthetic colorants in the candy samples were determined using calibration curves based on the linear relationship between absorbance and concentration, as described in previous sections of the study.

The concentration of synthetic colorants in the analysed candy samples ranged from 14.74 ± 0.69 mg/kg to 215.03 ± 6.42 mg/kg, as shown in (Table 1). Among these, five samples contained the synthetic dye E110, with concentrations ranging from 14.45 ± 0.70 mg/kg to 120.77 ± 3.91 mg/kg. Specifically, the concentrations of E110 in samples 1, 2, and 5 were 34.18 ± 0.58 mg/kg, 28.62 ± 0.92 mg/kg, and 14.45 ± 0.70 mg/kg, respectively. According to the Codex Alimentarius—a collection of international food standards established by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations—the maximum permitted level for E110 in hard and soft candies is 300 mg/kg (as of 2021). This result is similar to that reported by (Lawal, A., et al., 2021). Therefore, the concentrations of E110 detected in all analysed samples were well below the established permissible limit [11.]

The synthetic dye E124 was detected in two samples: Sample 3 (E124) and Sample 9 (E124), with concentrations of 38.96 ± 0.78 mg/kg and 143.59 ± 4.25 mg/kg, respectively. According to the Codex Alimentarius (2021), the maximum allowable concentration of E124 in hard and soft candies is 100 mg/kg. Thus, Sample 9 exceeded the permissible limit. This finding contrasts with the results reported by Ghaffar, F. (2020), in which the color concentration was within the allowable limits.

Regarding E129, its concentration in the analysed samples ranged from 43.28 ± 0.24 mg/kg to 215.71 ± 6.42 mg/kg. Sample 6 (E129) included two variants: a red candy containing only E129, and a purple candy containing both E129 and E133. The concentrations of E129 in these red and purple samples were 46.55 ± 0.64 mg/kg and 43.28 ± 0.24 mg/kg, respectively. Additional samples containing E129 included Samples 10, 11, and 12, all of which also contained E133. The E129 concentrations in these samples were 77.89 ± 0.88 mg/kg, 215.03 ± 6.42 mg/kg, and 66.80 ± 1.83 mg/kg, respectively. All values were within the Codex Alimentarius limit of 300 mg/kg for E129 in confectionery products (2017).

As for Brilliant Blue (E133), it was present in four samples: 7, 10, 11, and 12. The corresponding concentrations were 21.13 ± 1.12 mg/kg, 85.27 ± 2.47 mg/kg, 127.87 ± 0.64 mg/kg, and 36.81 ± 1.01 mg/kg, respectively. These values are well below the Codex Alimentarius maximum permissible level of 300 mg/kg (2017). These results are in agreement with those reported by Samuella B. et al. (2004).

In conclusion, all samples complied with the international permissible limits for synthetic dyes, except for Sample 9, which exceeded the recommended level for E124, as illustrated in the corresponding (Figure 5).

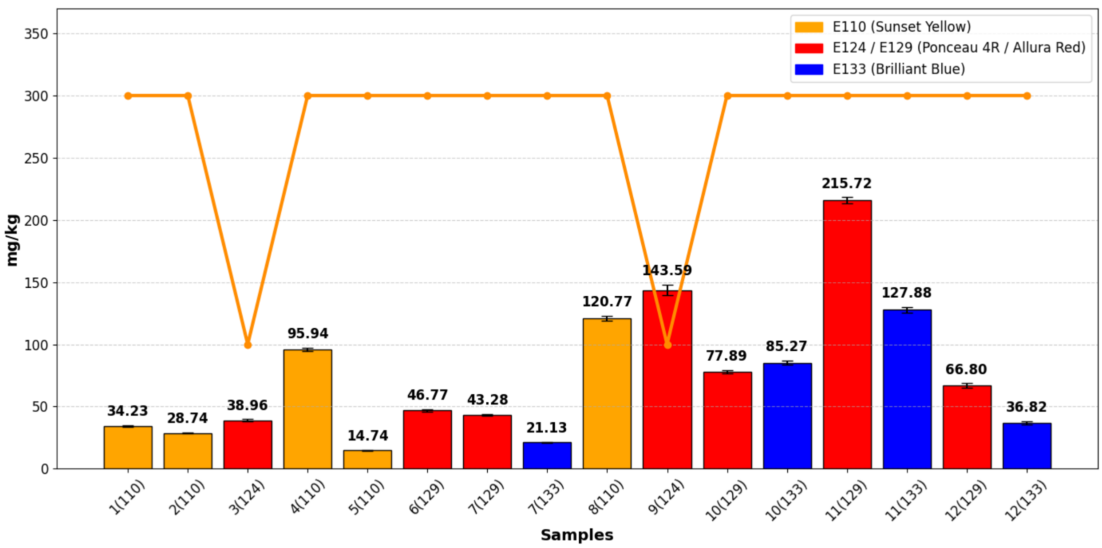

Figure 5. Concentration of colors in samples in(mg/kg)

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Conclusion

This study evidenced that the Ultraviolet/Visible (UV-Vis) spectrophotometry technique is a sensitive and reliable analytical tool for the quantification of these dyes. The study showed that the concentrations of synthetic dyes in the common candy samples collected from the Libyan market fall within the maximum permissible limits set by the Codex Alimentarius. The concentrations of Sunset Yellow (E110), Allura Red AC (E129), and Brilliant Blue FCF (E133) ranged from 14.45 mg/kg to 215.03 mg/kg, all of which are below the established limit of 300 mg/kg. However, disturbing exceedance was detected in one sample containing Ponceau 4R (E124), which had a concentration of 143.59 ± 4.25 mg/kg, exceeding the permitted level of 100 mg/kg. This finding suggests a potential lapse in regulatory oversight for some products available in the market.

Recommendations

The study recommends strengthening regulatory enforcement by intensifying inspection campaigns on imported and locally produced confectionery in Libya to ensure compliance with safety standards. It also suggests conducting broader studies that include more samples from different cities and analyzing other food products and beverages containing artificial colorants. Furthermore, the use of advanced analytical techniques, such as High-Performance Liquid Chromatography (HPLC), is advised to complement UV-VIS spectrophotometry, particularly for complex dye mixtures or borderline cases. Finally, the study emphasizes the importance of enhancing consumer awareness through public campaigns that promote reading food labels and selecting products with natural colorants.

Conflict of interest. Nil

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