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Synthetic Colorants in Traditional Sweetmeat (Laddu) Available in Dhaka City, Bangladesh

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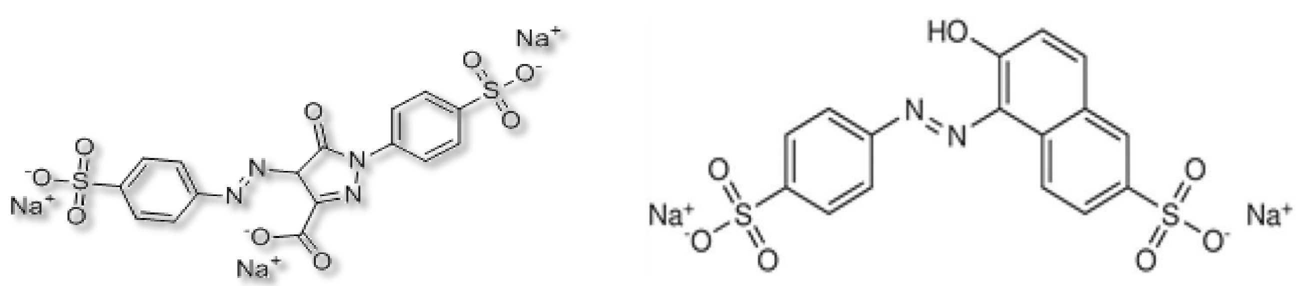
Abstract

Laddus are very popular sweets all over the South Asian countries including Bangladesh. Commercially accessible sweets (Laddus) contain a variety of Yellow 5 and Yellow 6 dyes, which are hazardous when consumed in excess. In this study, the amount of Yellow 5 and Yellow 6 in twenty-five Yellow-colored Laddus from various sweet shops in Dhaka, Bangladesh, was determined using a UV-visible spectrophotometer at 425 and 482 nm, respectively. Quantification was done using external calibration curves of the standard Yellow 5 and Yellow 6 and the calibration curves showed excellent linear correlation co-efficient (R^2) of 0.999 and 0.998 for them, respectively. Of the twenty-five samples, eleven had Yellow 5 in the range of 0.915-6.790 $mgkg^{-1}$, and eight had Yellow 6 in the range of 0.223-4.315 $mgkg^{-1}$. The rest of the samples did not contain any Yellow 6 as well as Yellow 5.

Keywords: Food colorants, Laddu, Yellow 5, Yellow 6, UV-Visible Spectrophotometer

I. Introduction

Bangladesh has a long tradition for the use of sweetmeat as dessert for different occasions. Usually, there are many different types of sweetmeats available, and Laddu is one of them. Laddus are ball-shaped non-dairy based sweets of sweetmeat companies which are made mainly from flour and sugar and topped with some other small ingredients. Different types of natural ingredients such as saffron, almond, sultana, etc. are used in Laddu to increase the flavor, taste, and colour. There are many traditional and famous shops available in Bangladesh for the production of delicious sweetmeat and they use special recipes to make it attractive and lucrative[1]. As saffron and many other natural ingredients are highly expensive and sometimes commercially unavailable, many synthetic color additives such as Yellow 5 (Trisodium 5-hydroxy-1-(4-sulfonatop-henyl)-4-[(E)-(4-sulfonatophenyl)diazenyl]-1H-pyrazole-3-carboxylate) and Yellow 6 (disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate) are used as coloring agents in food products (Fig. 1) [2].



Yellow 5 **Yellow 6**

Fig. 1. Structures of target color additives

Yellow 5 known as tartrazine, is a synthetic Yellow azo dye and is primarily used as a coloring agent in food products, drugs, and cosmetics. However, the dye has adverse effects on human health including hypersensitivity, mutagenic, carcinogenic, skin eczema, and immunosuppressive effects[3-4]. Yellow 6 (sunset Yellow) is another colorant food additive commonly used in many food products such as apricot jam, custard powder, citrus marmalade, orange soft drinks, sweets, squashes, ice-creams, etc. It also has adverse effects on human health. It is considered that exceeding the acceptable daily intake (ADI) can cause allergic reactions, diarrhea, migraines, gastric distress, skin swelling, nettle rash, vomiting, and other symptoms in a person[5].

Yellow 5 has a modest acute toxicity with an LD_{50} (median fatal dosage) value of more than 2000 $mgkg^{-1}$ body weight[6]. Several investigations have indicated that Yellow 5 does not produce genotoxicity at locations of interaction in the gastrointestinal tract[7-8]. Additionally, in numerous other rat

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developmental neurotoxicity tests utilizing a combination of colors, including tartrazine as the test drug, no impacts on reproductive parameters were seen[9-10]. According to the exhaustive review by Elhkim et al., a small fraction of people may be at risk for developing intolerant reactions to tartrazine at levels reachable through typical food consumption[11]. Due to intolerance reactions, Yellow 5 has been found to cause migraines, asthma, and other cutaneous disorders (such as urticaria) in a very small percentage of people[12]. Regarding a few other artificial food colors, Sunset Yellow FCF (for coloring food) has drawn attention due to a purported impact on children's behavior. For a long time, Yellow 6 has been linked to the possibility of allergic or pseudo-allergic reactions (PAR), either on its own or in combination with other colours.[13] According to Sasaki et al., sunset Yellow did not result in a statistically significant rise in DNA damage in colonic cells[6].With maximum values ranging from 50 to 400 mgkg^{-1}, the Codex GFSA (General Standard for Food Additives) provides allowances for sunset Yellow FCF in a variety of foods and beverages[14]. With a comparable range in Australia and New Zealand, the MPLs in food and beverages in Europe range from 50 to 500 mgkg^{-1}[14]. Orange juice samples in Bangladesh were found to contain 0.83-1.66 mgmL^{-1}of Yellow 6[15].

In addition to being prohibited in Austria and Norway, Yellow 5 has also received warnings about potential negative effects from other European nations[16]. Other nations, such as the UK, have mandated that food manufacturers label products containing Yellow 5 (E102) and Yellow 6 (E110) with a warning that reads: "May have an adverse effect on activity and attention in children." Because of this, several businesses, including Kraft, have started adding paprika and beta carotene in goods like their mac and cheese in certain nations (to maintain the Yellow appearance)[17]. As a result, research on these compounds, which are used as additives in different sweets (Laddu) in Bangladesh, has gained importance in processed food adulteration. Several methods for determining the presence of 3-40 color additives in food products have been documented[18-21]. The majority of them are LC techniques for water-soluble foods like juice drinks and confectionery because color additives may be accessed directly with minimal sample preparation. In continuing our research on food quality and safety, an easy, inexpensive, and less time-consuming method for estimating Yellow 5 and Yellow 6 in Laddu using a UV-visible spectrophotometer was followed.

II. Materials and Methods

Sample collection

A total of twenty-five (Code no. S1-S25) sweetmeat samples (Laddu) were purchased from various shops in Dhaka city, particularly near schools, densely populated residential areas, and roadside tea stalls. All the samples were stored in the refrigerator until use.

Chemicals and reagents

n-Hexane (Merck KGaA, Darmstadt, Germany), absolute ethanol (Merck KGaA, Darmstadt, Germany), and distilled deionized were used to carry out the experiment.

Standard calibration curves

The primary standard solution (100 mgL^{-1}) of Yellow 5 and Yellow 6 was prepared separately by dissolving 0.01 g of Yellow 5 and Yellow 6 in distilled deionized water (100 mL) in a 100 mL volumetric flask. The working standard solutions of Yellow 5 and Yellow 6 (2, 4, 5, 10, 20, 25, and 50 mgL^{-1}) were prepared by diluting with distilled deionized, from the primary standard solution. Individual absorbances of the solutions were measured at the wavelength of 425 nm for Yellow 5 and 482 nm for Yellow 6 by a double beam Ultraviolet-Visible spectrophotometer (Shimadzu, UV-1800, Kyoto, Japan) and calibration curves were drawn by plotting absorbance vs concentration graph (Fig. 2). The overlain UV-visible spectrum of Yellow 5 (left) and Yellow 6 (right) standard solutions are shown in Figure 3. The absorption spectrum of Yellow 5 and Yellow 6 in different samples are shown in Fig. 4.

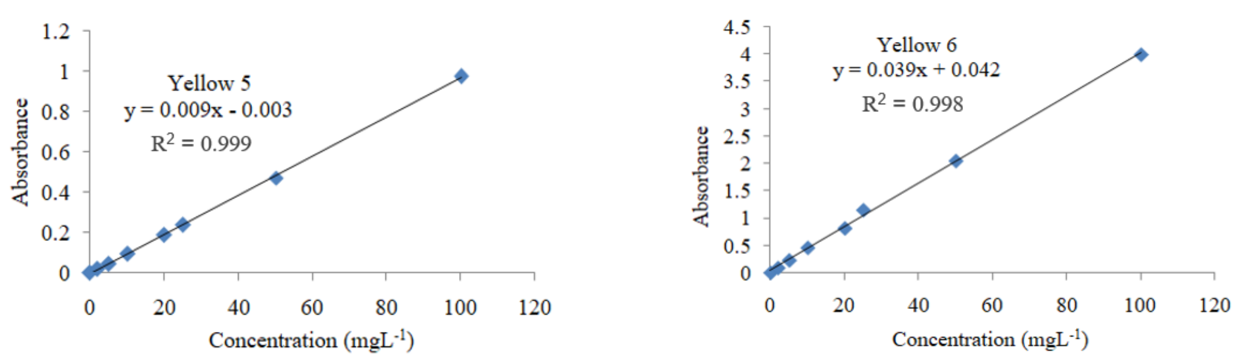


Fig. 2. Standard calibration curve of Yellow 5 (at 425 nm) and Yellow 6 (at 482 nm)

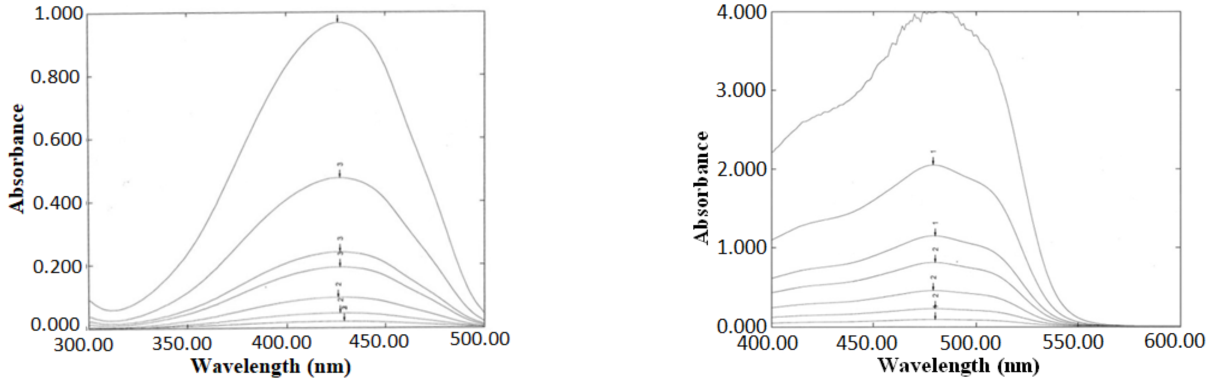
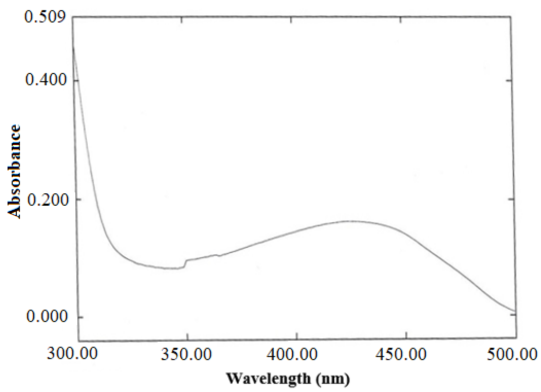
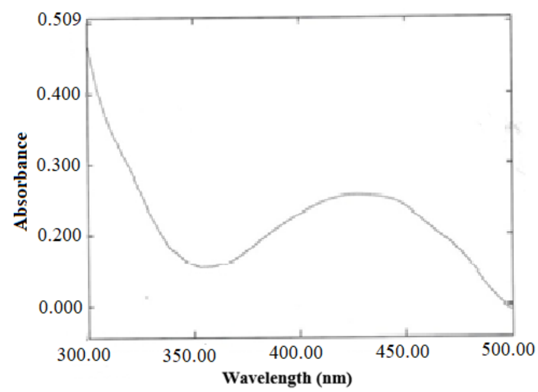


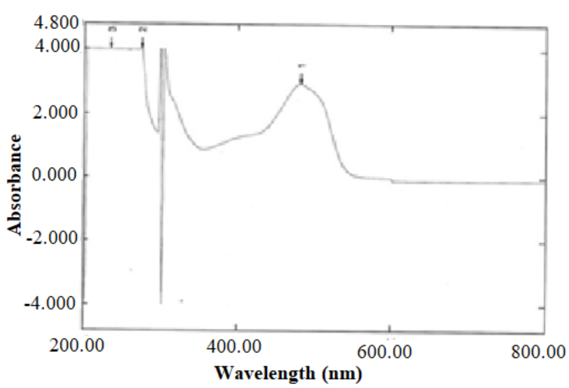
Fig. 3. Overlain UV-visible spectra of Yellow 5 (left) and Yellow 6 (right)



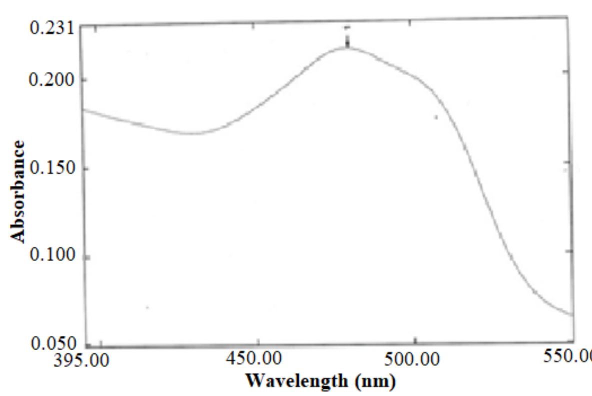
(a)



(b)



(c)



(d)

Fig. 4. UV-visible spectra of Yellow 5 and Yellow 6 in samples: Yellow 5 in (a) S5 and (b) S7; and Yellow 6 in (c) S1 and (d) S2

Sample preparation

Sweetmeat samples (100g) were dissolved in n-hexane (120 mL) in a beaker, (500 mL) and filtered, and the residue was taken in another beaker and distilled deionized (200 mL) was poured into it. The mixture was sonicated for 10 minutes and centrifuged at 3000 rpm (Hanil Science Industrial Co. Ltd., Model-Combi 514 R).The supernatant was separated and partitioned with n-hexane (120 mL). Then, the clear aqueous part (60 mL) was collected and reduced to 20 mL by a rotary vacuum evaporator (Heidolph, Germany) which was then poured into a beaker containing 80 mL of absolute alcohol making 80% ethanol. The mixture was allowed to stand for 15 minutes to macro molecules e.g., starch to be precipitated and removed by centrifugation followed by filtration. The supernatant was evaporated with added water by a rotary vacuum evaporator. The ethanol-free solution was reconstituted with 5 mL water and filtered through a 0.22 µm membrane filter to get the particle-free clear solution (Fig.5).

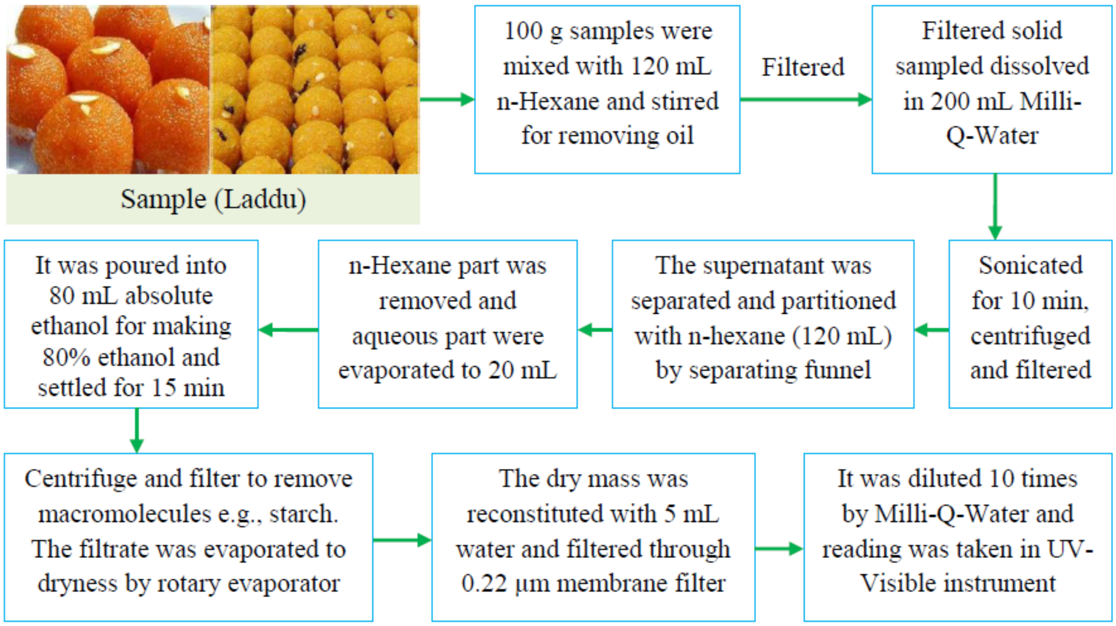


Fig. 5. Flow diagram of the sample preparation method

Determination of Yellow 5 and Yellow 6

The UV-visible absorption spectrum of the clear solution was measured at 425 and 482 nm by double beam spectrophotometer for the presence of Yellow 5 and Yellow 6, respectively. Using the standard calibration curve, the amount of Yellow 5 and Yellow 6 in sweet samples (Laddu) was quantified. The authentication of the color additives was also done by running the additives through thin layer chromatography (TLC, Mobile Phase Butanol:Ethanol: in50:25:25) along with the samples (Figure 6).

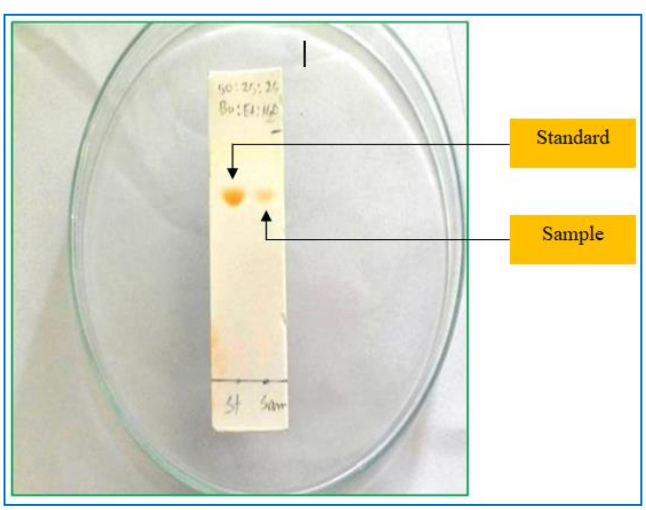


Fig. 6. Authentication of the color additives by TLC

III. Results and Discussion

Analysis of Yellow 5 and Yellow 6 in twenty-five sweetmeat samples (Laddu) was carried out by UV-visible spectrophotometric method. The absorption maxima of Yellow 5 and Yellow 6 were 425 and 482 nm, respectively and these two wavelengths were selected for their analyses. Several samples contained detectable amounts of these colorants, indicating that they were used in the preparation of these sweets. It is worth noting that the levels of these synthetic colorants varied among the samples. Eleven of the twenty-five samples had levels of Yellow 5 between 0.915 and 6.790 $mgkg^{-1}$, while eight samples had levels of Yellow 6 between 0.223 and 4.315 $mgkg^{-1}$(Table 1).Sample S18 contains the highest amount of Yellow 5 (6.790 $mgkg^{-1}$) and S5 contains the lowest (0.915 $mgkg^{-1}$). The descending trends of Yellow 5 containing samples are S18 > S25 > S13 > S24 > S14 > S6 > S23 > S11 > S7 > S21 > S5. S8 contains the highest amount of Yellow 6 (4.315 $mgkg^{-1}$) and S2 contains the lowest (0.223 $mgkg^{-1}$). The descending trends of Yellow 6 containing samples are S8 > S9 > S1 > S15>S22 > S19 > S3 > S2 (Table 1). According to the Food and Drug Administration, an acceptable daily intake (ADI) for Yellow 5 and Yellow 6 are 7.5 and 3.75 mg/kg/day[22]. Based on the ADI value an adult person weight between 55 to 65 kg can intake approximately 0.50 grams of Yellow 5 and 0.20 grams of Yellow 6.

Table 1. The amount of Yellow 5 and Yellow 6 in Laddu samples

Sample ID	Yellow 5 ($mgkg^{-1}$)	Yellow 6 ($mgkg^{-1}$)	Sample ID	Yellow 5 ($mgkg^{-1}$)	Yellow 6 ($mgkg^{-1}$)
S1	BDL	3.687	S14	3.632	BDL
S2	BDL	0.223	S15	BDL	1.790
S3	BDL	0.322	S16	BDL	BDL
S4	BDL	BDL	S17	BDL	BDL
S5	0.915	BDL	S18	6.790	BDL
S6	3.545	BDL	S19	BDL	0.465
S7	1.440	BDL	S20	BDL	BDL
S8	BDL	4.315	S21	1.145	BDL
S9	BDL	3.960	S22	BDL	0.815
S10	BDL	BDL	S23	3.471	BDL
S11	2.621	BDL	S24	4.625	BDL
S12	BDL	BDL	S25	6.125	BDL
S13	5.785	BDL			

*BDL= Below Detection Limit

Table 1, presents the absorbance and the amount of Yellow 5 and Yellow 6 in Laddu samples from Dhaka City, Bangladesh. The results from Table 1 reveal the presence of synthetic colorants, specifically Yellow 5 and Yellow 6, in several of the Laddu samples. Yellow 5 and Yellow 6 are widely used as food colorants and are subject to regulatory limits in many countries due to potential health concerns. The absorbance values at 425 nm and 482 nm provide insight into the color intensity of the Laddu samples. It is noteworthy that while some samples exhibited absorbance at 482 nm, indicative of the presence of Yellow 6, others showed absorbance at 425 nm, suggesting the presence of Yellow 5. This variability in results underscores the diverse use of synthetic colorants in Laddu preparations in Dhaka City.

The presence of synthetic colorants in food products raises concerns about potential health implications. Yellow 5 and Yellow 6 have been associated with adverse health effects in some studies, and regulatory authorities often set limits on their use in food products to ensure consumer safety. The levels found in these Laddu samples should be evaluated in the context of regulatory standards to assess potential health risks. These findings underscore the importance of quality control in food production, particularly in traditional sweets like Laddu. It is imperative that food producers adhere to regulations and label products accurately to inform consumers about the presence of synthetic colorants. Consumer awareness campaigns may also be necessary to educate the public about the use of these additives and their potential health consequences.

Fig. 7 presents valuable information regarding the presence of Yellow 5 and Yellow 6 in analyzed samples. It is evident that both additives are widely used in the food industry, as indicated by their presence in a significant percentage of the tested samples which may raise concerns about the potential cumulative exposure to these dyes through the consumption of Laddu in Dhaka city.

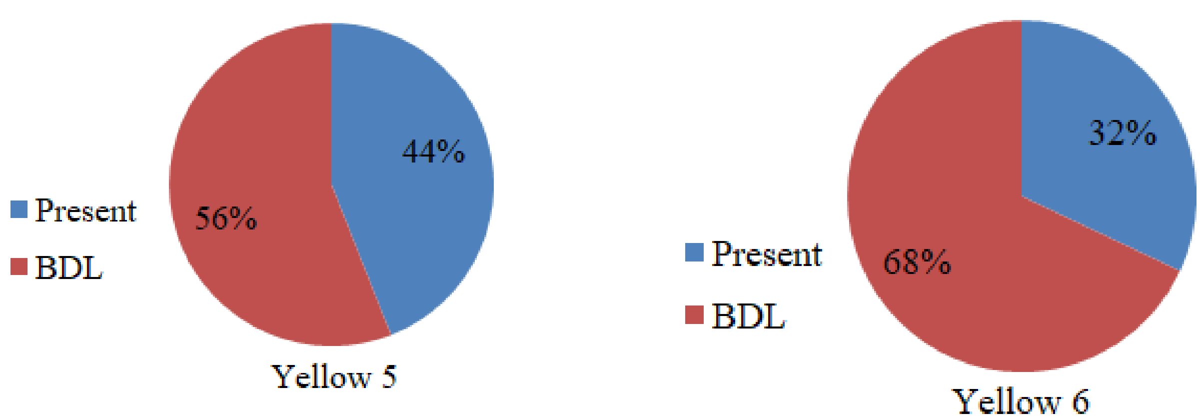


Fig. 7. Percentage of samples containing Yellow 5 and Yellow 6

This study provides valuable initial insights into the use of synthetic colorants in Laddu in Dhaka City, Bangladesh. However, further investigation is needed to determine the sources of these colorants, the reasons for their use, and their compliance with local food safety regulations. Future research should also consider the potential health risks associated with the consumption of Laddu containing these additives. These findings emphasize the importance of monitoring and regulating the use of synthetic additives in food products to safeguard public health and promote transparency in the food industry.

IV. Conclusion

Twenty-five sweet (Laddu) samples were analyzed for the quantitative determination ofYellow5 and Yellow 6 using the UV-visible spectrophotometric method. The absorption maxima for sweet samples were 425 nm for S5, S6, S7, S11, S13, S14, S18, S21, S23, S24, and S25, which was comparable to the absorption maxima of Yellow 5. The absorption maxima of S1, S2, S3, S8, S9, S15, S19, and S22, on the other hand, were at 482 nm and were identical to those of Yellow 6. Eleven of the twenty-five samples had levels of Yellow 5 between 0.915 and 6.790 $mgkg^{-1}$, while eight samples had levels of Yellow 6 between 0.223 and 4.315 $mgkg^{-1}$.These findings suggest that Yellows 5 and Yellow 6 were added as additives to commercial sweet samples. The data presented in this study provided a preliminary overview of the quantity of Yellow 5 and Yellow 6 in Laddu, which are often consumed in Bangladesh, even though the number of sweet samples evaluated was still modest. This is a quick and affordable procedure that may also be used to determine whether Yellow 5 and Yellow 6 are present in samples of sweets. The limit of these color additives used in sweets is less than the authorized limit except for S8 and S9. Therefore, they don't necessarily have adverse effecton the consumer. However, the haphazard addition of color chemicals to foods like sweets and other foods can cause allergic reactions in people and make humans more hyperactive. Sweets without any of these colors are preferable to consume.

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