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Estimation of Tartrazine, sunset yellow and fast green-FCF artificial food colors in milk-sweets by normal spectrophotometer and a UV-VIS spectrophotometer

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Abstract
Several available sweets are being adulterated with artificial food colors and this study was aimed at evaluating the extent of adulteration in the milk-based sweets with respect to three synthetic colorants. Further, normal and UV-VIS spectrophotometer were compared for their efficiency in determining the color concentration. A total of 40 Milk based sweets from local shops were collected that were visually confirmed of color addition. Each sweet was taken in duplicate and the color was extracted by centrifugation and double filtration technique. Known artificial food color standards for Tartrazine, Fast Green FCF and Sunset Yellow were taken in known concentration gradient and standard curve was plotted. Further, the concentration of color was estimated based on the standard curve initially by normal spectrophotometer followed by a UV-VIS spectrophotometer. Among the sweets that were analyzed, Tartrazine was added in excess beyond the maximum permissible level with a mean 517mg/kg conc as per the UV-VIS results. Comparatively, UV-VIS spectrophotometry showed a better estimation efficiency with more turbid extracts that were erred in the normal spectrophotometer. Fast green FCF and Sunset Yellow were estimated to be below the MPL with a mean of 39.39mg/ml and 3.84mg/ml respectively. This paper offers an insight into the fate of local sweet shops where most of the sweets with colorant adulteration go unnoticed many a times. Further, other than the three color standards evaluated, many other synthetic colorants could also be estimated by other techniques of estimation in addition to this spectrophotometric evaluation.

Keywords: Artificial food colors, synthetic food color adulteration, spectrophotometry, UV-VIS spectrophotometer

1. Introduction

A property that greatly enhances the acceptance of food is its color. The more pleasing the color is to the eye, the more would be its acceptance. A myriad of synthetic colors is being used owing to their low cost and a higher color intensity than the conventional natural food colors in many areas (Dilrukshi *et al*. 2019) [11]. A significant loss of the natural food color during its processing and preparation largely allows the addition of synthetic food colorants to compensate the loss and thus these food colors fall under one of the most important classes of food additives. These synthetic food colors on reaction with the food most often renders them toxic to the human body especially in a cumulative fashion. Most of the people experience a range of allergies due to these added artificial food colors (Oveysi *et al*. 2003; Ashfaq *et al*. 2002) [21, 2].

Nevertheless, the lethal effects of such artificial food colors on human health were often emphasized urging people to curb the use of food colors in excess. Effects were reportedly severe in the children age group when foods with excess colorants were consumed (Minioti *et al*. 2007) [18]. The azo functional group (N=N) and the aromatic ring structure are responsible for the effect of food colors (De Andrae *et al*. 2014). The range of health complications due to synthetic food colors include hyperactivity in children, Decreased WBC and lymphocyte count, asthma, sleeping disorders, vitamin B6 deficiency, carcinogenicity and idiosyncrasy (Farzianpour *et al*. 2013; Hinton *et al*. 2000) [13, 15]. Inhibition of dopamine uptake by the nerve endings, sleep disturbances, glossitis, Eczema, ear infections, adrenal damage and utricaria are a few other complications attributed to a high level of artificial food colors consumption (Rao *et al*. 2005; Chandra, 1997) [23]. Numerous synthetic food colors are being used extensively added to foods especially milk-based sweets, Ponceau 4R, Sunset Yellow, Fast Green FCF, Carmoisine, Tartrazine to name a few (David A Katz, 2009; Chatwal and Anand, 1989) [8, 7] (Fig 1) and the details of these synthetic food colors is given in the Table -1.

Table 1: Artificial food colours as per PFA (Prevention of food adulteration) Act.

Sl. No.	Food colour	Colour shade	Colour index	Chemical Class	Empirical Formula
1	Tartrazine	Yellow	19140	Mono azo	C16H9N4O9S2Na3
2	Sunset Yellow	Orange	15985	Mono azo	C16H10N2O7S2Na2
3	Fast Green FCF	Sea Green	42053	Triarylmethane	C37H34N2O10S3Na2

Fig 1: Various sweets with added food colors

Recently, the synthetic food colors are being slowly replaced by the natural food colors considering the consumer`s health like cucurmin, β-carotene and anthocyanins. Colorants like lutein, lycopene and β-carotene are being employed for the yellow, red and orange colors in the foods which are also being linked with possible health benefits (Rodriguez-Amaya *et al*. 2010; Shen *et al*. 2014) [24, 27]. However, these natural food colors are comparatively more expensive and unstable, further allowing an excess use of artificial food colors (Yoshioka *et al*. 2008) [29].
In this present study milk-based sweets were studied for their artificial food colors concentration. The concentration of three food colors *viz*., Sunset Yellow, Tartrazine and Fast Green FCF was estimated by spectrophotometric analysis. Chromatographic analysis was followed conventionally for estimation of synthetic food colors in sweets that showed promising results. Reportedly, UV-VIS spectrophotometry was considered one of the most.
Reliable method of identifying synthetic food colors as all the colorants has been characterized by this method (Sayar and Ozdemir, 1996; Santos *et al*. 2010) [26, 25]. Henceforth, the concentration of three different food colors was estimated by spectrophotometric analysis comparatively between normal spectrophotometry and UV-VIS spectrophotometry and conclusions regarding the load of synthetic food colors in the milk-based sweets were drawn.

2. Materials and Methods

All the local sweet shops were initially surveyed and a few shops were selected for collection of milk-based sweets with added artificial food colors. Various sweets were collected randomly from different shops with orange, green, red and yellow color and were further processed immediately to extract the color from them.

2.1 Color standards

Food color standards Tartrazine, Sunset Yellow, Fast Green FCF were taken to construct the standard curve by taking various known concentrations of each color and reading their absorbance in a spectrophotometer and a UV-VIS spectrophotometer separately. The standard curve was further used to calculate the unknown concentration of food colors in the collected sweet samples.

2.2 Extraction of color

The food colors were extracted as per the procedure described by Bachalla *et al*. (2016) [4-5] with slight modifications. Briefly, 2 grams of sweet sample was taken carefully and was dissolved in 10ml of distilled water in a sterile test tube. The sample was gently mixed well and was made into a fine mixture. This mixture was centrifuged for 15 minutes at 10000 rpm and the supernatant was collected which was filtered through a Whatman filter paper No.31 ET. The so obtained filtrate which was slightly turbid was further filtered through a 0.4µm syringe filter and the final filtrate was collected. Into a clean tube, 0.5 ml of this final filtrate was taken and the solution was made up to 3 ml with distilled water. The color was extracted from all the sweets using the same technique and all the color extracts were preserved for further analysis (Fig 2).

Fig 2: Color extracts from the sweet samples

2.3 Spectrophotometric analysis

Spectrophotometric analysis of the extracted food colors was performed based on the procedure followed by Bachalla *et al*. (2016) [4-5] with a few changes to it. Initially, the standard food colors were read in a spectrophotometer and UV-VIS spectrophotometer at various concentrations and a standard curve was plotted using the obtained results. The absorbance was recorded for Tartrazine, Sunset Yellow and Fast Green FCF at 425nm, 482nm and 625nm wavelength respectively (FAO). The absorbance values for each of the extracted food colors were recorded in triplicates in a spectrophotometer and a UV-VIS spectrophotometer and the concentration of the added food color was estimated based on the values from the plotted standard curve using standard food colors.

3. Results

The artificial food colors that were extracted from all the sweets and the known color standards were subjected to normal spectrophotometry and UV-VIS spectrophotometry separately. The concentration of the color added in each sweet was estimated based on the standard curve plotted using the absorbance values of the known standard synthetic food colors.

The absorbance values for the artificial food color standards Tartrazine, Sunset Yellow and Fast Green FCF obtained in the UV-VIS spectrophotometer are given in the table 2. The absorbance values that were obtained in the normal spectrophotometer are given in the table 3.

Table 2: Absorbance values for food colour standards in UV-VIS spectrophotometer

Tartrazine		Sunset Yellow		Fast Green FCF	
Conc. (millimolar)	**Absorbance**	**Conc. (millimolar)**	**Absorbance**	**Conc. (millimolar)**	**Absorbance**
200	0.934	200	0.242	0.05	0.139
175	0.436	175	0.283	0.1	0.888
150	0.738	150	0.939	0.25	3.135
125	0.784	125	3.311	0.5	3.010
100	2.767	100	1.133	1	1.172
70	1.387	70	1.030	5	0.399
45	0.594	45	0.424		
20	1.001	20	0.979		
10	0.722	10	1.276		
05	1.220	05	0.481		

Table 3: Absorbance values for food colour standards in normal spectrophotometer

Tartrazine		Sunset Yellow		Fast Green FCF	
Conc. (millimolar)	**Absorbance**	**Conc. (millimolar)**	**Absorbance**	**Conc. (millimolar)**	**Absorbance**
200	2.018	200	2.499	0.05	1.874
175	1.995	175	2.472	0.1	1.887
150	1.972	150	2.360	0.25	2.053
125	1.972	125	2.360	0.5	1.974
100	1.972	100	2.323	1	2.127
70	1.951	70	2.288	5	2.451
45	1.931	45	2.256		
20	1.893	20	2.171		
10	1.884	10	2.059		
05	1.858	05	2.040		

The standard curves were plotted based on these absorbance values and the standard curves for these food color standards for normal spectrophotometer and UV-VIS spectrophotometer are given in the Fig 3 and Fig 4 respectively.

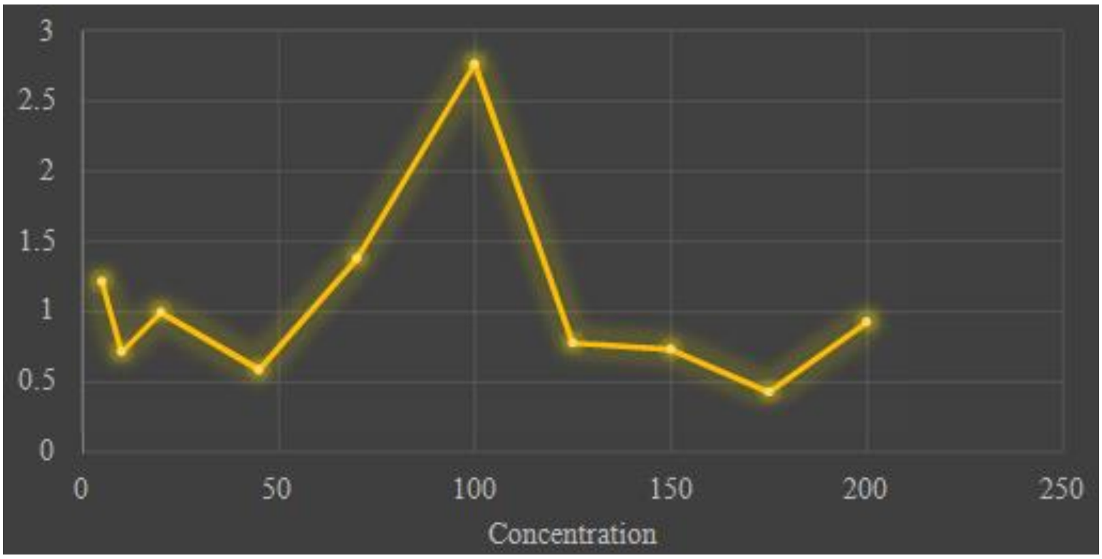

Fig 3 a): Standard curve for Tartrazine – UV-VIS

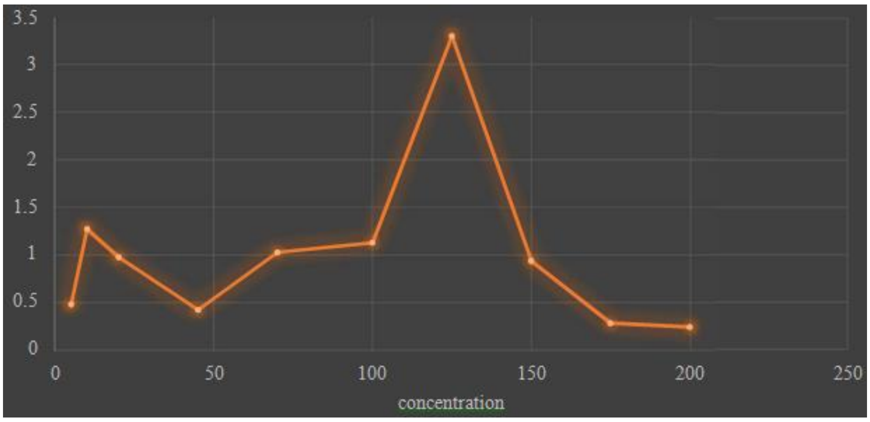

Fig 3 b): Standard curve for Sunset Yellow – UV-VIS

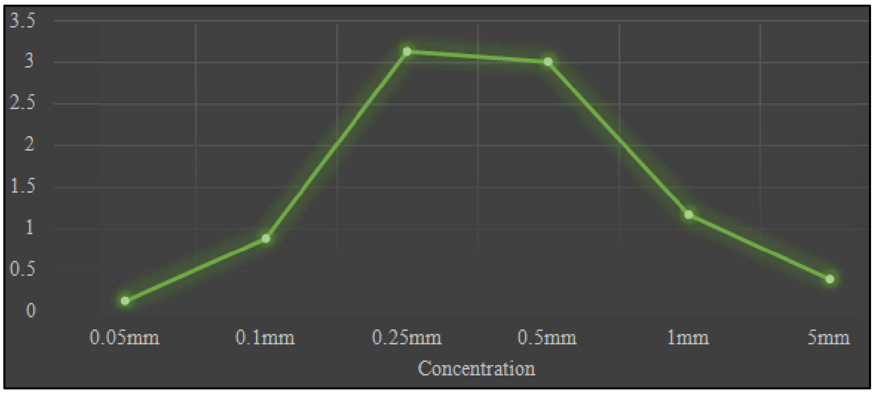

Fig 3 c): Standard curve for Fast Green FCF – UV-VIS

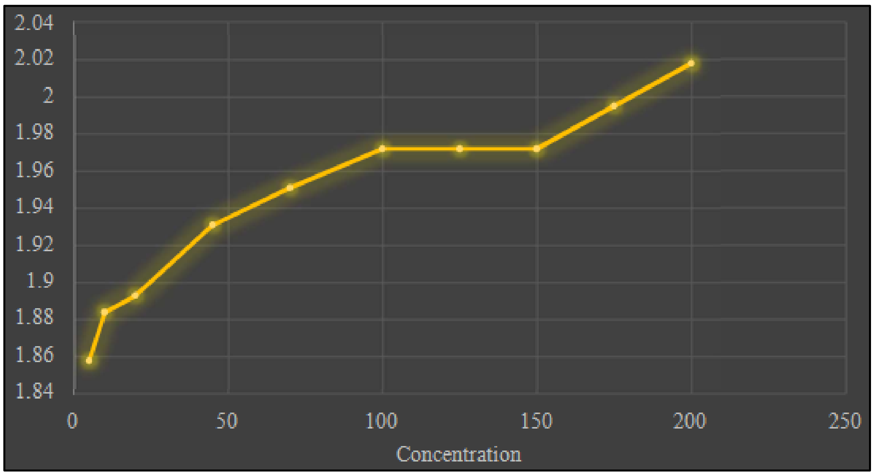

Fig 4 a): Standard curve for Tartrazine – Normal spectrophotometer

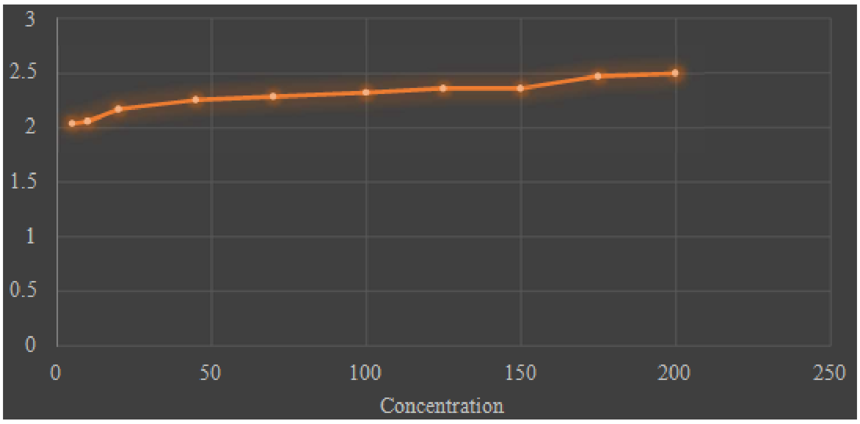

Fig 4 b): Standard curve for Sunset Yellow – Normal spectrophotometer

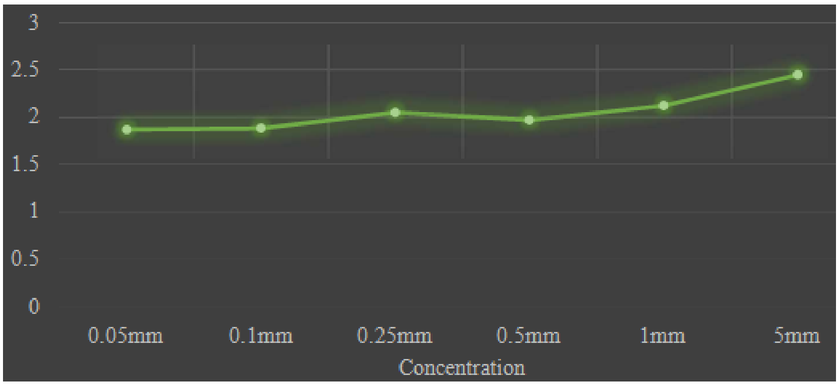

Fig 4 c): Standard curve for Fast Green FCF – Normal spectrophotometer

The absorbance values were recorded for the extracted colors from the milk-based sweets both in a normal spectrophotometer and a UV-VIS spectrophotometer and based on the standard curves plotted for each food color standard, the color concentration of the unknown samples was estimated and are given in the table 4.

Table 4: The concentration of the added food colour in the sweets estimated by both UV-VIS and normal spectrophotometry

S. No.	Sweet sample	Concentration UV-VIS (mg)	Concentration Spectrophotometry (mg)
Orange sweets (n=17)			
1	Orange khova	1.58	3.84
2	Jangree	0.904	3.03
3	Orange Khoa -2	2.17	3.84
4	Orange sweet	1.35	3.84
5	Halwa	0.497	2.66
6	Orange Khoa-3	2.53	3.84
7	Maroon sweet	3.34	3.84
8	Halwa-2	1.13	3.70
9	Besan Laddu	0.904	3.84
10	Diamond sweet orange	1.53	3.52
11	Jangree-2	0.497	2.57
12	Halwa-3	0.769	2.53
13	Halwa-4	0.588	1.35
14	Orange Khoa- 4	0.090	
15	Brown Khoa	3.30	3.84
16	Chocolate Sweet	1.80	3.84
17	Besan Laddu	0.678	3.70
Green Sweets (n=7)			
18	Green Diamond	11.64	39.99
19	Green Middle	11.16	24.42
20	Green topped sweet	13.83	39.99
21	Green topped sweet -2	7.19	9.2
22	Green date sweet	9.05	20.78
23	Green top sweet -3	7.27	12.69
24	Green sweet	8.12	14.22
Yellow sweets (n=16)			
25	Diamond Sweet – yellow	518.97	16.56
26	Yellow mysore pak	518.86	16.56
27	Diamond sweet yellow -2	518.97	16.56
28	Besan Laddu – 2	518.64	2.67
29	Brown Diamond	518.97	16.56
30	Mysore Pak	518.86	16.56
31	Diamond sweet Yellow	518.97	16.56
32	Laddu -1	518.38	16.56
33	Laddu -2	517.63	16.56
34	Yellow Khoa	518.97	16.56
35	Yellow Date Sweet	518.97	16.56
36	Yellow Top Sweet	518.38	1.33
37	Small Jangree	516.77	1.17
38	Jangree-1	518.27	2.5
39	Jangree-2	511.79	0.908
40	Cake	511.81	1.068

Note: All the extractions and estimations were done in triplicates

A total of 40 milk-based sweets were evaluated in this study (Orange-17, Green- 7 and Yellow-16) and the color from the sweets was extracted by repeated centrifugation and double filtration. The maximum permissible level (MPL) for each of the synthetic food color were noted and the values attained in this study were compared to the MPL. This revealed that all the green and orange sweets were added with only little quantities of the synthetic food colors i.e., Tartrazine and Fast Green FCF taking both the spectrophotometric readings into consideration. However, the color extracts being subjected to simple extraction procedures like centrifugation and filtration, the extract was slightly turbid and based on this present study, though all the recordings were taken in triplicates, slight complication was observed in normal spectrophotometer owing to the turbidity of the color extract. Though a preliminary filtration by a Whatman filter paper No. 31 ET was done followed by a syringe filtration by a 0.4 µm filter followed by a 0.2 µm filter was done to extract the food color, the readings were better read in an UV-VIS spectrophotometer compared to the normal spectrophotometer that erred a few samples which were thought to be comparatively more turbid for the instrument to pass the light. MPL for Tartrazine as per European Food Safety Authority (EFSA) is 50-500 mg/kg widely and for Sunset Yellow maximum limit is 0.04% by weight. As per FSSAI, Maximum Permissible Level (MPL) for these food colors i.e., Tartrazine, Fast Green FCF and Sunset Yellow is 100mg/kg (Food Safety and Standards Regulations, 2009-FSSAI). Considering these regulations, the attained values in this study were correlated to identify the most common adulterated food color. Looking at the graphical representation, Sunset Yellow and Fast Green FCF were added well below the Maximum Permissible Level whereas Tartrazine was added in very high quantities, higher than the MPL issued by the regulatory authorities which indicate an adulteration of these sweets with Tartrazine (Fig 5, Fig 6 and Fig 7).

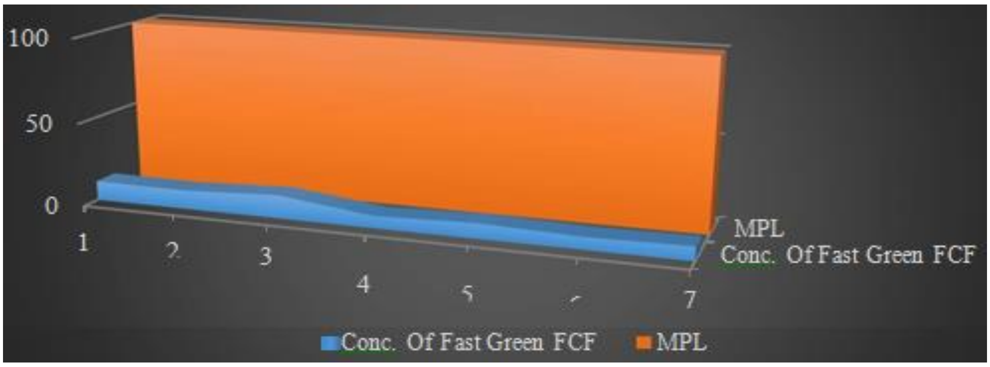

Fig 5: Concentration of Fast Green FCF in sweets by UV-VIS

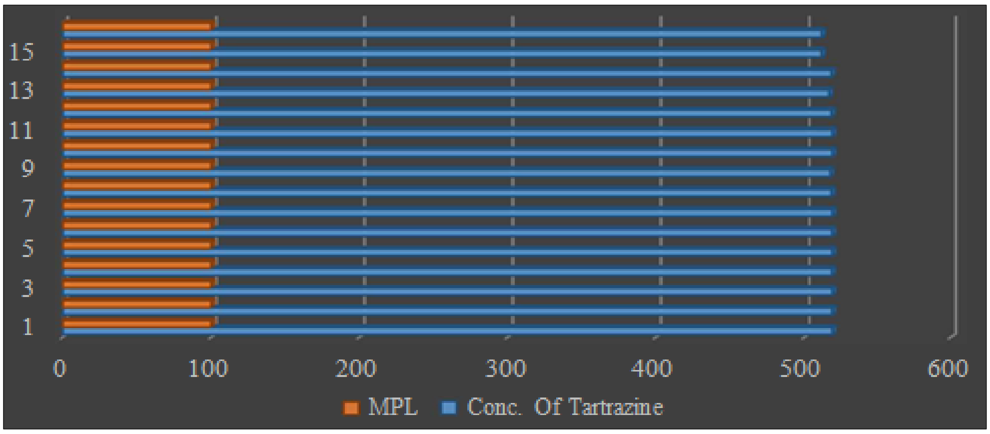

Fig 6: Concentration of Tartrazine in sweets by UV-VIS

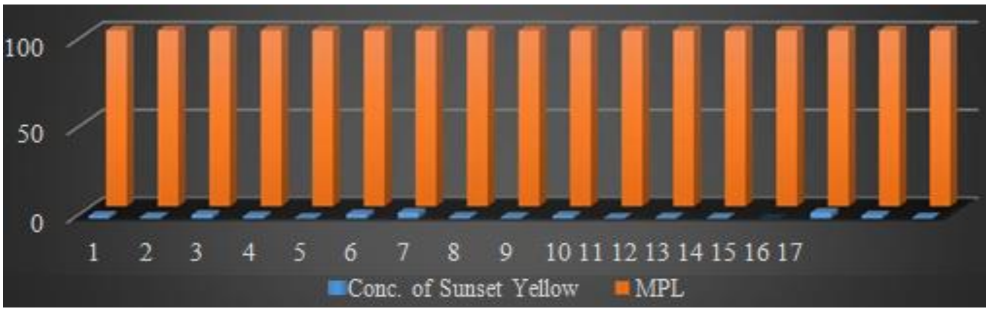

Fig 7: Concentration of Sunset Yellow in sweets by UV-VIS

Henceforth, UV-VIS spectrophotometer was comparatively better in giving out the absorbance values based on which the concentration of all the food colors was calculated which revealed that the most used food color locally was Tartrazine as most of the sweets showed a very high concentration of Tartrazine than the MPL of it. The other two food colors were well within the limit indicating less adulteration in the sweets locally.

4. Discussion

Adulteration of foods with synthetic food colors is an age old mal practice that's been happening around us for a long time. As color is the major factor that determines one`s acceptability of the food material, preparing the food in an appealing color is crucial in order to enhance their consumption. Nevertheless, use of natural food colors proved to be better than the synthetic colors which is not the case owing to the fact that the natural colors do not give out a uniformity in their distribution, are often expensive and unstable which made their usage limited. Artificial food colors emerged at this juncture fulfilling the earlier mentioned lacunae and are being used indiscriminately often ignoring the health of the consumers. Food regulation authorities like FAO, FSSAI and EFSA have been publicizing the ill effects of over consumption of these food colors which is being shadowed by our craving for a colorful food on our table. The maximum permissible levels of every food color like Tartrazine, Ponceau 4R, Sunset Yellow, Fast Green FCF, Carmoisine and many others have been allotted by the regulatory bodies and several studies were conducted in estimating the amount of adulteration of food items by these food colors based on these MPL values.

Various extraction methods have been employed before by several authors to extract the food color from the food samples. Chromatographic techniques, Centrifugation technique are a few techniques that were commonly followed. Bachalla (2016) [4-5] extracted 10 synthetic food colors from sweet samples that were analyzed by Paper chromatography. In another study, Paper chromatography was employed to study five different synthetic food colors by David A. Katz (2009) [8]. In another study on Ponceau 4R and Tartrazine, derivative spectrophotometric method was utilized in analyzing these synthetic food colors by Sedat Sayar and Yuksel Ozdemir (1996) [26]. In a study regarding the utilization of UV-VIS spectrophotometry in evaluating the food quality, Nawrocka and Lamorska (2013) [20] reported that these spectroscopic methods can successfully evaluate the food quality and also does not require elaborate sample preparation procedures that would ensure arapid analysis of the food quality. Any sort of adulteration of food could be successfully determined by these spectroscopic methods when combined with other analytical techniques like chromatography.

In another study by Arast *et al*. (2013) [1] regarding the adulteration of confectionary products, 48% of the products examined were adulterated with artificial food colors. Similar adulteration of sweets and confectionaries with synthetic food colors was reported by Farzianpour *et al*. (2013) [13]; Soltan *et al*. (2008) [28] and Ashfaq and Masud (2002) [2]. With regard to the health complications, the adverse effects of these synthetic food colors especially hyperactive instincts in children have also been reported (Feingold, 1973) [14]. In a similar study with respect to synthetic food colors, Rao and Sudershan (2008) [22] reported that Tartrazine and Sunset Yellow were used in most of the foods that were analyzed which is in agreement with this study results. Tartrazine was reported to be adulterated in 48% of the food samples analyzed in urban areas and 51% in the rural dwellings. Similar findings of high Tartrazine levels in the foods was reported by several researchers (Khanna, Singh and Krishna Murthi, 1980; Dixit *et al*. 1995; Mathur, 2000) [16, 17].

5. Conclusion

Artificial food colors have become a part and parcel of our everyday lives and most of the available foods are being adulterated with these colors. Food regulatory authorities have been enlightening the people on the adversities of prolonged uptake of such synthetic colorants and gradually people have been conscious of their health. In this study, three different synthetic food colors *viz*., Tartrazine, Sunset Yellow and Fast Green FCF were estimated in locally available milk-based sweets by two different methods, among which UV-VIS spectrophotometer resulted in more reliable results. The color extracts were read efficiently in the UV-VIS spectrophotometer instrument whereas a normal spectrophotometer erred with a few sample extracts. The results revealed that the sweet samples were adulterated with Tartrazine color at a level more than MPL issued by the regulatory authorities. The method followed in this study for the extraction of the color from the sweets and the spectrophotometric analysis require less time and could be employed for a preliminary screening of samples which can be further confirmed by spectrophotometric analysis.

6. Conflict of interest

The authors of this paper declares that there is no conflict of interest.

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