Research Article

Identification & spectrophotometric quantification of dyes in the selected confectionery items available in the local markets of Peshawar, Pakistan

Fazia Ghaffar^{1*}, Faiza Tauqeer² and Rozina Rauf¹

1. Department of Food & Nutrition Sciences, College of Home Economics, University of Peshawar, KPK 25120-Pakistan

2. Department of Textiles & Clothing, College of Home Economics, University of Peshawar, KPK 25120-Pakistan

*Corresponding author's email: faziaghaffar@uop.edu.pk

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Abstract

The study aimed at analyzing the food colours added to various confectionery items vended at the local market of Peshawar. Samples of different types of candies, chocolate beans, fruit jellies, lollipops, sweetened and coated aniseeds and papadhs were analyzed for identification and quantification of the added synthetic food colors. Although majority of branded items contained permitted colors however some items were found to contain non-permitted colors. About 22% items were found with non-permitted colors. Ponceau 4R (20.9%), sunset yellow (18.7%), tartrazine (16.7%), and brilliant blue (14.6%) were the most common dyes identified. Majority of the branded items contained different dyes within the permissible limits however, some non-branded items contained higher concentrations of Carmoisine (375.0 ± 0.9 and 364.8 ± 0.68 mg kg⁻¹) against the permitted limit of 50 mg kg⁻¹. Tartrazine was also found to be higher in non-branded items. The concentrations of naphthol yellow (312 ± 10.43 and 812 ± 21.07 mg/kg) was found to be higher in the non-branded items. The concentrations of brilliant blue FCF were quite higher in some items. The use of green FCF and green S is a matter of food safety as these dyes are phased out in some developed countries. It is concluded that constant vigilance is needed to ensure that local manufacturers comply with the food laws and regulations of food colors. Constant supervision is direly needed not only in terms of non-permitted food colors but also in the control and limits of permitted food colors. Moreover, mass awareness regarding consumption of colored food items is also needed particularly among young children.

Keywords: Concentrations; Confectionery items; Non-permitted colours; Permitted colours; Permissible limits; Synthetic food dyes

Introduction

Food manufacturers add a variety of additives to food products to enhance taste, appearance, flavor and other attributes [1-4]. Colour is a vital constituent of food probably because it is the first quality being

perceived by the senses [5, 6]. Food colour is any dye, pigment, or any other substance that is added primarily for restoring or adding colour during processing [7]. These colours are also used to enhance or optimize the colour of the end products [8]. Food

colours can be divided into four classes i.e. natural, natural identical, synthetic, and inorganic colours [7]. These colours can also be categorized as permitted and non-permitted food colours [9].

In recent years many synthetic dyes have been increasingly used to substitute natural colours or to achieve certain desirable qualities such as improved appearance, high colour intensity, colour stability and uniformity [10-14]. Compared to natural colours, these synthetic dyes are low in cost, resistant to light, temperature, oxygen, pH changes, and can be used without further processing [15]. Most of these synthetic dyes are the derivatives of coal tar and are synthesized from aminobenzene compound as azo group [16]. These dyes are widely used in many foods such as bakery products, confectioneries, jellies and beverages [17].

These colourants have been shown to cause intoxication and can cause a number of allergic reactions such as migraine, blotting, nettle rash, itching, blurred vision, rhinitis, and water retention [18]. These dyes are also associated with carcinogenicity, immune system disorders, decreased WBC and lymphocyte count and vitamin B6 deficiency [19]. They also cause anemia, sleep disturbances, liver and kidney ailments and hyperactivity in children [2, 10, 11, 20-22]. The use of non-permitted colours and the indiscriminate use of permitted colours are not safe and can pose a serious public health risk among different age groups [23-24].

A large number of food laws exist in Pakistan but most of them deal with the control of production, distribution, supply of food, profiting and hoarding. The current study aimed at the identification and quantification of food colours used in local confectionery items commonly consumed by children.

Materials and methods

A sample of forty-eight different types of branded and unbranded confectionary items were collected randomly from the local whole sale market "Peoples Mandi" of

Peshawar City. The sample consisted of hard candies, soft chewable candies, chewing gums, fruit jellies, coloured aniseeds, lollypops, papadh, and soft chocolate candies covered with hard coloured sweetened coatings. The samples were divided into two categories branded and unbranded i.e. the one that fulfilled all the requisite information of some company name and a label (branded) and the items that either were sold loose (unpacked) or were packed by the sellers or though carried the name of the brand but no trade name and a label (non-branded).

Standards and reagents

Standard food dyes ponceau 4 R C-I 16185 (E 123, FD & Red No.2), Carmoisine-C-I 14720 (E 122 EXT-D & C Red No. 1), Tartrazine C-I-19140 (E 102 FD&C Yellow No. 5), Sunset Yellow FCF C-I-15985 (E 110 FD & C Yellow No. 6), Brilliant blue FCF C-I 42090 (E-133 FD & C Blue No. 1 and D&C Blue No. 4), Green S.L.I. 44090 (E-142 Lissamin Green, Acid Brilliant Green BS, and Wool Green BS) were purchased from Sigma Aldrich. All the other solvents and reagents were of analytical grade.

Sample preparation

Approximately 10 grams each of the grinded sample was thoroughly mixed in 50ml of 2% ammonia in 70% alcohol solution. The mixture was allowed to stand for an hour. In order to settle down starch and sugars in the samples the solutions were warmed on water bath for 3-5 minutes. The aliquot was then centrifuged at 1500 rpm and the upper separated layer was allowed to evaporate on hot water bath. The residue was diluted later with 30ml of acetic acid solution for spectrophotometric determination.

Colour extraction

The pretreated samples were acidified with 2.0 ml of 2M glacial acetic acid, an equal amount of distilled water. A 5.0 cm length conditioned pure sheep wool thread was immersed in each sample. These were kept in a water bath for 1 hour for the colour to be adsorbed onto the wool. The wool

threads were later removed from the solutions and washed with running tap water. Afterwards the threads were soaked again in 2.0 ml of 2M ammonia solution with an equal amount of distilled water and kept in a water bath until the wool threads

were decolorized. The wool threads were removed and the solutions were concentrated by evaporation in a water bath. Some of the extracted colours along the confectionery samples are presented as (Figs. 1-20) here.

Figure 1. Unbranded confectionery samples

Figure 2. Chewing gum extracted carmoisine

Figure 3. Hard candy extracted colour ponceau 4R

Figure 4. Hard candy extracted colour ponceau 4R

Figure 5. Fruit jelly extracted colour tartrazine

Figure 6. Fruit jelly extracted colour tartrazine

Figure 7. Candy sunset yellow FCF

Figure 8. Candy extracted colour quinoline yellow

Figure 9. Chewy balls ponceau 4R

Figure 10. Fruit jelly ponceau 4R

Figure 11. Chewy candy carmoisine

Figure 12. Papdh extracted colour nephthol vellow

Figure 13. Aniseeds extarcted colour sunset yellow FCF

Figure 14. Fruit Jelly extracted colour sunset yellow FCF

Figure 15. Fruit jelly extracted colour tartrazine

Figure 16. Aniseeds green FCF

Figure 17. Papdh extracted colour green FCF

Figure 18. Chewy balls extracted colour brilliant blue FCF

Figure 19. Choco beans extracted colour brilliant blue FCF

Figure 20. Soft candy extracted colour tartrazine

Identification and quantification of colours

Preparation of standard curves

Stock solutions of the standards were prepared by dissolving 10mg/1000 mL of pure standard in deionized water. A series of working solution were prepared i.e. 0, 1, 2, 3, 4 and 5 mL from stock solution into 50 mL volumetric flasks and the volume was made with buffer solution to make 0 mg/50 mL (blank), 0.1 mg/50 mL, 0.2 mg/50 mL, 0.3 mg/50 mL, 0.4 mg/50 mL and 0.5 mg/50 mL solutions of known concentrations.

Spectroscopic analysis

The absorption maxima and percent concentration of dyes in the samples were determined by UV 1700 spectrophotometer (Shimadzu Japan). The absorption maxima of each standard were obtained between 190-1100 nm. The spectra and λ max value of samples and standards were compared with each other. The color value (color intensity) was measured from the UV absorbance. A plot of absorbance versus concentration of the known solutions (expressed as the concentration in mg of colour per 50 mL of solution) by the Beer-Lambert Law plot. Later the concentrations were converted to mg/kg⁻¹ and the means and standard deviations were calculated with Microsoft Excel

Results and discussion

Food colours, traditionally, are used to improve the appearance of the final product. In young children food selection is strongly affected by bright colours of food products. Research on the colour additives is a fundamental need these days since such additives are frequently used by young children. Among the low-income populations and rural areas lack of awareness and low literacy tend to motivate children to buy comparatively low cost, unpacked and unbranded chewing gums, candies, lollipops, papad and other such sweetened confectioneries. Determination of the quality and quantity of colorants are the main requisites for the health of these

young children. The current study revealed that 27% type of branded and 21% of unbranded selected samples (Graph 1) contained synthetic colours of some type. These findings are comparable to the findings of a study carried out at Karachi and Sri Lanka [16, 25].

Summary of the dyes identified (Table 1) showed that confectionery samples consisted of approved colours ponceau 4R, carmoisine, tartrazine, sunset yellow, quinoline yellow, brilliant blue FCF. Some banned dyes such as naphthol yellow, fast green FCF, and green S were also identified within the samples collected.

In order to identify further the distribution of the permitted and non-permitted colours among the selected items (Graph 2 & 3) indicated that 4% candies, 4 % fruit jellies, 2% chocolate beans and 01 % chewing gums contained non-permitted/banned colourants. Similar findings were also found by Saleem *et al.* who identified 11% of food samples and 4% of beverages with non-permitted colours in Karachi city [16]. Such dyes are unfit for human consumption and shall be worked out further based on time series studies in order to identify the type of colours used. In another study conducted at Allahabad, India non-permitted Metanil yellow and malachite green in cake and ice cream in the local samples were identified [2]. Similarly, among 70 food items in Arak, Iran, 80% of the samples contained artificial colour additives that are banned as per by the National Iranian Standards [10]. Another study conducted by Farzianpour *et al.* showed that 6.52% of the pastry, poolak, and rock candy samples at Shahr-e-kord City, Iran, contained forbidden artificial food colours [11]. In a study conducted at Rawalpindi Cantt it was found that among 73 samples of sweetmeats and confectioneries, 46.57% contained nonpermitted colours [6]. The current study, therefore, creates a dire need to assure food safety and quality in the use of food colours in confectionery items locally.

The percent distribution of different synthetic dyes within the studied samples showed that highest percentage of items contained ponceau 4R (20.9%) followed by sunset yellow (18.7), tartrazine (16.7), brilliant blue FCF (14.6), and carmoisine (12.5). the samples containing fast naphthol yellow, green FCF, green S were 4.2%, 6.3%, and 2.08% respectively. The presence of these banned colours though were found to be low but they may pose some serious health hazards to young children. Naphthol yellow S is an organic compound that is a dye. It is a derivative of 1-naphthol. At one time it was a popular food colorant but was delisted in 1959 in the U.S. It is used in microscopic or electron microscopic examination of cells and tissues to give contrast and to highlight particular features of interest, such as nuclei and cytoplasm. Both green FCF and Green S are banned internationally due to their chronic toxicity, genotoxicity, and carcinogenicity [27].

The content of red dyes determined (Table 2) showed that ponceau 4R was highest in hard candies with a liquid filled center (153 mg kg⁻¹) followed by other hard candies. All these concentrations were within the permissible limits. The concentration of carmoisine was higher in the fruit jellies followed by chewing gums. Non-branded showed very higher concentrations in all the items. The concentrations of yellow dyes (Table 3) showed quinoline yellow being the most common dye within the branded items while tartrazine was within the permissible limits in the non-branded items. Non-branded Choco beans and papadhs contained naphthol yellow. It is concluded here that although majority of the items contained well within the

permitted limits of dyes, their uncontrolled and unwise use in the non-branded items will put children at a higher risk of urticaria, angioedema, asthma, atopic dermatitis, food intolerance, genotoxicity, cytotoxicity, and mutagenicity [28, 29].

The concentrations of orange coloured dyes among the analyzed samples (Table 4) revealed sunset yellow being the most common dye used in the branded items. The concentration of the dye in these samples were well within the permitted standard of 300 mg/kg. Among the non-branded items naphthol yellow was found in aniseeds and papadhs in higher concentrations. As mentioned earlier naphthol yellow had been phased out quite earlier and the use of this dye in such item is a matter of serious food safety that need to be dealt with strict measures.

Data regarding the concentrations of green dyes (Table 5) revealed that some branded items contained green S in quite higher concentrations. Similarly, the concentrations of green FCF was found to be high in chocolate beans in a branded sample and that of green S in a lollipop sample (213.6 mg/kg). Among the non-branded items blue FCF was quite higher (210.6 mg/kg) against the permitted 100 mg/kg limit. A sample of a candy was found to contain 250.3 mg kg⁻¹ green S and 256.3 mg kg⁻¹ brilliant blue FCF against the 100 mg kg⁻¹ permitted limit in a fruit jelly. The presence of green S in the analyzed samples is a serious public health concern while the unwise use of permitted dyes is a matter of food safety law implementations. Such confectioneries are mostly consumed by young children and will contribute to compromise their health and may cause some serious diseases.

Graph 1. Percent Distribution of the Samples based on labels

Table 1. Summary of the Dyes Identified

Dye Identified	E-Number	Molecular Formula	Approval Status	Max. Permissible limit/ADI
Ponceau 4R	E 124	$C_{20}H_{11}N_2Na_3O_{10}S_3$	Approved in EU but under consideration by FDA	50mg/kg/0.7 g/kg/day
Carmoisine /Azorubine	E 122	$C_{20}H_{12}N_2Na_3O_7S_2$	Approved in EU, voluntary phase out in UK, currently banned in Canada, Japan, Norway, USA	50mg/kg /04mg/kg body weight/day
Tartrazine	E 102	$C_{16}H_9N_4Na_3O_9S_2$	Approved in USA & EU	50mg/kg/7.5mg/kg/day
Naphthol Yellow	-----	$C_{10}H_4N_2NaO_5$	Banned as food additive	-----
Quinoline Yellow	E 104	$C_{18}H_{13}NO_{5/8/11}S_{1/2/3}Na_{1/2/3}$	Approved in EU, voluntary phase out in UK	0.5mg/kg/day
Sunset Yellow	E 110	$C_{16}H_{10}N_2Na_2O_7S_2$	Approved in EU, Banned in Norway	50mg/kg/1mg/kg/day
Brilliant Blue FCF	E 113	$C_{37}H_{34}N_2Na_2O_4S_3$	Approved in USA & UK	50mg/day
Fast Green FCF	E 143	$C_{37}H_{37}N_2O_{10}S_3$	Banned in UK	
Green S	E 142	$C_{27}H_{25}N_2NaO_7S_2$	Banned in Canada, USA, Japan, Norway	50m/kg

Graph 2. Percent distribution of samples based on permissibility of dyes

Graph 3. Percent distribution of dyes within the samples

Table 2. Content of Red Dyes in the Selected Confectionery Samples

S#	Food items	Dye identified	λ max (nm)	Conc.mg kg ⁻¹	Permissible limits*
Branded items					
1.	Hard Candy	Ponceau 4 R	507 nm	125.4±1.071	300 mg
2	Hard Candy	Ponceau 4 R	507 nm	126.6±1.08	
3	Hard Candy (coloured syrup in center)	Ponceau 4 R	507 nm	153.2±0.56	
4	Hard Candy	Ponceau 4 R	507 nm	133.8±0.71	
5	Fruit Jelly	Carmoisine	516 nm	134.7±0.36	-----
6	Fruit Jelly	Carmoisine	516 nm	141.0±1.96	-----
7	Choco beans with hard coating	Carmoisine	516 nm	133.8±0.39	
8	Chewing gum	Carmoisine	516 nm	114.9±0.25	
Non - Branded Item					
9	Hard Candy	Carmoisine	516 nm	337.5±0.63	
10	Fruit Jelly	Quinoline Yellow	507 nm	144.9±0.29	100mg
11	Choco beans with hard coating	Ponceau 4 R	507 nm	158.4±1.19	50mg
12	Choco beans with hard coating	Carmoisine	516 nm	375.0±0.90	50mg
13	Aniseeds (color coated)	Carmoisine	516 nm	364.8±0.68	

Table 3. Content of Yellow Dyes with in the Confectionery Samples

S#	Food items	Dye identified	λ max (nm)	Conc.mg kg ⁻¹	Permissible limits*
Branded items					
1.	Hard Candy	Tartrazine	425nm	146.7±0.36	300mg
5	Fruit Jelly	Quinoline Yellow	410 nm	244.5±0.61	-----
6	Fruit Jelly	Quinoline Yellow	410 nm	181.5±0.71	-----
8	Chewy soft candy	Quinoline Yellow	410 nm	206.5±1.02	-----
Non- Branded Item					
10	Fruit Jelly	Tartrazine	425 nm	327.2±1.36	300mg
11	Hard candy	Tartrazine	425 nm	287.5±0.73	300 mg
12	Choco beans with hard coating	Naphthol Yellow	378 nm	312.5±10.43	-----
13	Papadhs	Naphthol Yellow	378 nm	812.5±21.07	-----

Table 4. Concentrations of Orange Dyes within the Selected Samples

S#	Food items	Dye identified	λ max (nm)	Conc.mg kg ⁻¹	
Branded items					
1.	Hard Candy	Sun set yellow	482 nm	381.2±0.03	300mg
2	Hard Candy (coloured syrup in center)	Tartrazine	425 nm	244.5±0.38	
3	Hard Candy	Sunset Yellow	425 nm	238.6±0.82	
4	Fruit Jelly	Quinoline Yellow	425 nm	178.9±0.51	
5	Chewy soft candy	Sunset Yellow	425 nm	119.8±0.29	
6	Fruit Jelly	Quinoline Yellow	425 nm	119.5±0.12	
7	Choco beans with hard coating	Sunset Yellow	425 nm	214.6±0.16	
8	Hard Candy	Sunset Yellow	425 nm	214.2±0.71	
Non -Branded Item					
9	Fruit Jelly	Quinoline Yellow	425 nm	300.6±1.20	300mg
10	Fruit Jelly	Quinoline Yellow	425 nm	254.5±0.71	
11	Choco beans with hard coating	Sunset Yellow	425 nm	290.5±1.84	
13	Aniseeds (color coated)	Naphthol Yellow	378 nm	219.8±0.44	
	Papadh	Naphthol Yellow	378 nm	314.5±1.36	

Table 5. Content of Green Dyes in the Confectionery Samples

S#	Food items	Dye identified	λ max (nm)	Conc.mg kg ⁻¹	Permissible limits*
Branded items					
1.	Fruit Jelly	Green FCF	624 n	327.0±1.28	400
5	Lolli pop	Green S	640 nm	213.6±0.74	-----
6	Aniseed	Green FCF	624 nm	378.2±1.05	100
8	Fruit Jelly	Green S	640 nm	141.3±0.36	400
	Fruit Jelly	Brilliant Blue FCF	630 nm	189.6±0.91	300mg
	Fruit Jelly	Brilliant Blue FCF	630 nm	141.3±0.34	100mg
	Choco beans with hard coating	Green FCF	624 nm	158.5±0.26	100
Non -Branded Item					
10	Fruit Jelly	Brilliant Blue FCF	630 nm	256.5±0.16	100 mg
11	Hard candy	Brilliant Blue FCF	630 nm	144.5±0.28	300
12	Chewing gum	Green FCF	624 nm	144.5±0.93	300
	Hard Candy	Green S	640 nm	250.3±0.25	
	Choco beans with hard coating	Brilliant Blue FCF	630 nm	210.6±.017	100
13	Hard Candy	Green S	640 nm	68.5±0.38	100
	Ani seeds	Brilliant Blue FCF	630 nm	114.5±0.96	100

Conclusion

The current introductory study found excessive use of permitted food colours in

some confectioneries while some contained nonpermitted food colours. Therefore, there is a dire need to create awareness among the

masses at different levels about the consumption of synthetic food colours and their toxic effects and health hazards particularly young children. Consequently, a systematic approach must be carried out within the country to establish rules and regulations to prevent malpractices of synthetic food colour adulteration, proper labelling of confectionaries, supervising the permissible level of permitted synthetic colours, and to guard against the use of non-permitted colours in all food items to assure food safety and quality. This study also highlights the requirement of a governing authority to introduce global regulations on food safety within the country.

Authors' contributions

Conceived and designed the experiments: F Ghaffar, Performed the experiments: F Ghaffar, F Tauqeer & R Rauf, Analyzed the data: F Ghaffar & F Inayat, Contributed reagents/ materials/ analysis tools: F Ghaffar & F Inayat, Wrote the paper: F Ghaffar.

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