Original Article



Zahra Gholami¹, Roya Firouzi², Marzieh Rashedinia^{2,3*}

¹Food and drug control laboratory, Shiraz University of Medical Sciences, Shiraz, Iran.

²Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

³Food and Supplements Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Food colorants are addictive substances that are creating a good and attractive appearance in foods. Synthetic food dyes have been strictly controlled by the legislation in the world. Artificial food colorants may cause harmful effects on human health. As the safety of substances in some traditional food products is questionable, this study was conducted to evaluate the type and frequency of the food colorants in traditional confectionery, syrup and fruit juice, in Shiraz, Iran. Totally 806 samples were collected from 2017 to 2019. Analysis of samples was done using the thin layer chromatography method according to the Iranian national standard. Results showed that 51.61% of samples did not have any additive color, and 48.39 % of samples contained artificial colors that should not be used in sweets and are not permitted for use according to the Iranian national standards. Tartrazine and Quinoline Yellow were the most frequently used as non-permitted food color. Therefore, about half of the samples are non-consumable. Use synthetic food dyes in a high percentage of supplied products in unauthorized places needs necessity to increase supervision of relevant officials and raise consumer awareness about the permitted food colors and adverse and toxic effects of forbidden colors.

Keywords: Food color, Traditional Confectionery, Traditional Beverage, TLC.

1. Introduction

Food colorants are addictive substances that are creating a good and attractive appearance in foods (1). The best additive dye to foods and sweets is the one that does not change the quality and taste of the food and only affects their appearance (2).

Two types of dyes used in food are synthetic or natural ones. The natural colors that are extracted from plants such as chlorophyll, carotenoids, tannins and anthocyanins (3). They are harmless to humans or have low health adverse effects, but the food industries are less tending to use natural colors because of disadvantages, which they are easily decomposed and more expensive (4). The benefits of using artificial colors in comparison to the natural food colors are stability over the heat, light and oxygen, and resistance to microbial contamination (5). Artificial colors are divided into five groups including azo, triaryl methane, quinolone, xanthene, and indigoid compounds on the basis of their chemical structures (6). Previous studies have side effects of synthetic food colorants on human carcinogenesis, hyperactivity in children, diarrhea, abortion, asthma, decreased immune response in children, and immune deficiency (6). Synthetic food dyes have been strictly controlled by the legislation in the world. The list of authorized food dyes varies by country and is

Corresponding Author: Marzieh Rashedinia, Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran. Email: Rashedinia@sums.ac.ir

set according to daily consumption of products (7). The Institute of Standards and Industrial Research of Iran (ISIRI) have set the national legal limit for using any permitted food additives and food colors in products. The list of permitted artificial colors according to the Iranian national standards No. 740 are includes; yellow colors (Quinoline Yellow, Sunset Yellow), red colors (Azorubine, Ponceau 4R, Allura Red) and blue colors (Indigotine, Brilliant Blue) (8). Although, use of permitted synthetic food colors is forbidden in some products such as beverages, juices, desserts, ice creams, and traditional products (9, 10).

Most of the permitted food additives used in industrial products are monitored, while use of synthetic color is more common in traditional food products due to less control in local sale market, for attracting more attention of customers. Some studies showed that the use of unauthorized substances in some traditional food products in other different cities of Iran. This study was conducted to evaluate the type and frequency of the unauthorized food colorings in traditional confectionery, syrup and fruit juice, in Shiraz, Iran.

2. Materials and methods

All Chemicals and reagents including standard of synthetic dyes obtained from Sigma USA. N-butanol, ammonia, 96% ethanol, glacial acetic acid and silica gel plates were purchased from Merck Company, Germany.

This study was carried out on unbranded confectionery products and different types of sweets including; confections, cookies, candy Noghl, helva, Zoolbia and Bamieh, traditional syrup and fruit juices. Totally 806 samples were collected randomly from confectioneries and juice local sale markets in Shiraz city from 2017 to 2019. Subjects were evaluated for natural and synthetic dyes by using Thin layer chromatography (TLC) method. The experiments including 3 steps; Step 1) Sample Preparation: elimination of starch fat and protein from samples using ammonia alcohol. After 24 hours the supernatant was gently removed and dried on water bath. Step 2) Extraction color with white wool and acetic acid on boiling water bath for one hour. Then wash the wool under cold water. The wool was then immersed in distilled water and concentrated ammonia were added, after 20 minutes the wool was removed and allowed to dry. Step 3) Spotting the sample extracted color and standard dyes on TLC. The solvent tank was prepared and saturated with n-butanol, acetic acid and distilled water 50:25:30 volume ratios. When the solvent was raised to about 70 % of chromatography paper, the plate was removed from the solvent. The plate was dried and the retention factor (Rf) of samples compared with standard colors. Finally, the type and name of color were reported (8).

3. Results

In this study, 747 samples of confectionery products, including sweets, candy, helva and zoolbia, Bamieh also, 59 samples of traditional syrup and fruit were analyzed for the presence of artificial food colorings (Table 1). As shown in table 1, 416 samples (51.61%) did not have any additive color, and 390 samples (48.39 %) contained artificial colors that should not be used in sweets and are not permitted for use according to the Iranian national standards. The 200 samples (24.81%) contained authorized artificial food color and 190 samples (23.57%) contained unauthorized artificial food color. Mainly, food colorants have used in 59 of 59 types of beverages (100%.) and 331 of 747 types of sweets (44.31%) (Table 2).

The type and frequency of artificial food

Table 1. The status of color used in sweets and traditional beverages					
	Samples in year	Samples in year	Samples in year	Total	
	2017	2018	2019		
No artificial color	111 (46.83%)	191 (59.31%)	114 (46.15%)	416 (51.61%)	
Authorized artificial color	61 (25.73%)	63 (19.56%)	76 (30.76%)	200 (24.81%)	
Unauthorized artificial color	65 (27.42%)	68 (21.11%)	57 (23.07%)	190 (23.57%)	
Total	237	322	247	806	

Table 2. Number of observed products in rood category containing artificial rood colors.					
Food category	Sweets	Syrup and fruit juices	Total		
Products containing artificial food coloring	331/747 (44.31%)	59/59 (100%)	390/806 (48.38%)		
N/total studied (%)					

Table 2. Number of observed	products in food	l category co	ntaining a	rtificial	food colors.

colorants were encountered and exhibited in Table 3. Tartrazine (48.71% of colored products) was the most frequently used as a non-permitted food color and Quinoline yellow (32.05% of colored products) among the permitted food color.

The overall pattern use of unauthorized oral synthetic dyes indicate that Tartrazine is most commonly used in confectionery products and also, Quinoline yellow, Sunset yellow, Carmoisine, Brilliant blue and Alura red, permitted artificial colors were used in beverages. Based on the National Standard of Iran, and criteria of the Iran Ministry of Health and Medical Education, use of every artificial dye are unauthorized in the confectionery and juice, consequently, 48.38% of total samples of this study were non-consumable.

4. Discussion

This study showed that synthetic colors were found in a major percentage of evaluated products. More than 48% of confectionaries and fruit juice, that contained forbidden and permitted artificial food colors. Therefore, about half of the samples are non-consumable, because of according to the guidelines of the Iran Ministry of Health, using any type of artificial color in confectionaries and beverages has banned and producers are only permitted to use natural and herbal dyes (9, 10). Nowadays, there are many food additives that are used in food products to more attract consumers, most of which are colors, whereas in many countries significant amounts of food products are nonconsumable due to the use of illegal additives each year (5, 11).

Most producers consider using of artificial colors rather than natural colors, due to high costs, low color value, and instability in changes pH condition of natural ones (12). The distribution of different synthetic colors within the studied samples showed that Tartrazine (E102) and Quinoline Yellow (E104) were most frequently used as a forbidden, and permitted artificial color, respectively. Similar findings were found by various surveys demonstrated that, Quioline Yellow and Sunset Yellow were the most common permitted coloring used in the confectionery in different cities of Iran and the most frequent use of unauthorized artificial yellow paint was observed in Sohan, poolaki and helva samples (2, 13-15).

Moreover, based on the results of previous studies in Shiraz city, 40% of traditional juices and drinks used artificial dyes illegally (16). Also, in Tehran city, 51% of the traditional juices contained artificial colors (17).

It seems that yellow dye is most favorable in confectioneries, since similarity to natural color of saffron and advantages on health, make producers more motivated to use this color (18).

Side effects of artificial food colorants including hyperactivity of children, asthma, skin rash, carcinogenicity, blood pressure, anaphylactic reaction, sleep disorders, immune system failure, decreased WBC as well as lymphocytes and vitamin B6 deficiency was previously reported (19,

Table 3. Type and frequency of artificial food colorants in samples.

		<u>^</u>	
Color type	Samples in year 2017	Samples in year 2018	Samples in year 2019
Tartrazine	65 (51.18%)	68 (51.9%)	57 (43.18%)
Quinoline yellow	52 (40.94%)	45 (34.35%)	28 (21.21%)
Sunset yellow	6 (4.7%)	3 (2.3%)	25 (18.94%)
Carmoisine	4 (3.15%)	6 (4.6%)	10 (7.57%)
Brilliant Blue	0	8 (6.1%)	10 (7.57%)
Allura red	0	1 (0.76%)	2 (1.52%)
Total	127	131	132

Zahra Gholami *et al*.

20).

Macioszek et al. 2004, confirmed that the two common food colors of Quinoline Yellow and Bryant Black caused the genotoxicity of human lymphocyte cells (21). Furthermore, Tartarazine causes hyperactivity in children and urticaria and skin rash (22).

According to our results, use of unauthorized colors and number of non-consumable products have not changed during these three years (2017 to 2019). It is proposed that, more attention and monitoring should be done in this regard. The official authorities should pay more attention and health inspectors follow up.

It should be noted that children may consume more colored foods than predictable by the regulatory authorities. Considering the colored sweets and candies are consumed too much in children, daily intake of artificial food colorants may exceed over ADI range. The long-term effect of using these compounds can make health problems such as idiosyncratic and anaphylactic reactions, metabolic, or affect hormones function and vitamins levels (23). Providing information on the health risk of synthetic dyes, enhancing awareness, and training food producing staff, in addition to improving food color guidelines, strengthening the country's food monitoring system, are important in reducing the use and consumption of artificial dyes in food (12).

5. Conclusion

Use synthetic food dyes in a high percentage of supplied products in unauthorized places needs the necessity to increase supervision of relevant officials and raise the consumer awareness about the permitted food colors and adverse and toxic effects of forbidden colors.

Acknowledgement

The authors thanks Deputy of Food and Drug, Shiraz University of Medical Sciences, Shiraz, Iran

Conflict of Interest

None declared.

References

1. Zahra N, Alim-un-Nisa IK, Fatima S, Khan H, Akhlaq F, Butt IF, et al. Identification of synthetic food dyes in various candies. *Pak J Biochem Mol Biol*. 2016;49:9-17.

2. Farzianpour F, Khaniki GJ, Younesian M, Ghahferkhi BB, Sadeghi M, Hosseini S. Evaluation of food color consumption and determining color type by thin layer chromatography. *Am J Appl Sci.* 2013;10(2):172.

3. Murthy HN, Georgiev MI, Park SY, Dandin VS, Paek KY. The safety assessment of food ingredients derived from plant cell, tissue and organ cultures: a review. *Food Chem*. 2015 Jun 1;176:426-32. doi: 10.1016/j.foodchem.2014.12.075. Epub 2014 Dec 25. PMID: 25624252.

4. Stich E. Food color and coloring food: quality, differentiation and regulatory requirements in the European Union and the United States. Handbook on Natural Pigments in Food and Beverages: Elsevier; 2016. p. 3-27.

5. Vinha AF, Rodrigues F, Nunes MA, Oliveira MBP. Natural pigments and colorants in foods and beverages. Polyphenols: Properties, Re-

covery, and Applications: Elsevier; 2018. p. 363-91.

6. Okafor SN, Obonga W, Ezeokonkwo MA, Nurudeen J, Orovwigho U, Ahiabuike J. Assessment of the health implications of synthetic and natural food Colourants–A critical review. *UK Journal of Pharmaceutical and Biosciences*. 2016;4(4):01-11.

7. McAvoy SA. Global regulations of food colors. *Manuf Confect*. 2014;94(9):77-86.

8. Organization INS. Permitted food additives Food colors List and general specifications ISNO 740. 2019;6th Revision.

9. 5th. IoSaIRoIIcN. Revision 1387.

10. Institute of Standards and Industrial Research of Carbonatedfruitjuices f, fruitbasedrinks. Number 14345. . First Revision; 1390.

11. Llewellyn G, Penberthy J, Parker J. Food Color Additives in the US Food Supply: Review of Neurobehav-ioral Safety. *J Pediatr Neurol Neurosci.* 2020;4(1):55-72.

12. Rezaei M, Abadi FS, Sharifi Z, Karimi F, Alimohammadi M, Abadi RAS, et al. Assessment of synthetic dyes in food stuffs produced in con-

fectioneries and restaurants in Arak, Iran. *Thrita*. 2015; 4(1):e22776. doi: 10.5812/thrita.22776.

13. Arast Y, Mohamadian M, Noruzi M, Ramuz Z. Surveillance on artificial colors in different confectionary products by chromatography in Qom. 2013.

14. Samadi M, Alimohamadi S, Salari M, Bayat R, Shabanloo A. The survey of Types of Oral Dyes in the Pastries of Tuyserkan City. Pajouhan Scientific Journal. 2018;16(4):14-20.

15. REZAEI R, MIRLOHI M, MARACCY MR, VAHID DM. Exposure Estimation to Tartrazine through Traditional Hard Candies (Nabat and Poulaki) in Iran, Isfahan Province. 2015.

16. Gholami Z, Marhamatizadeh MH, Mazloomi SM, Rashedinia M, Yousefinejad S. Identification of Synthetic Dyes in Traditional Juices and Beverages in Shiraz, Iran. *Inter J Nut Sci.* 2021;6(1):39-44.

17. Mohammadi H, Vahedi S, Hajimahmoodi M, Nadjarian A, Salsali M, Shokrzadeh M. A Survey on the Use of Synthetic and Natural Fruit Colures in non-certified Juice and Fruit Products in Tehran, Iran. *Journal of Mazandaran University of Medical Sciences*. 2015;24(120):159-72.

18. Faustino M, Veiga M, Sousa P, Costa EM, Silva S, Pintado M. Agro-Food Byproducts as a New Source of Natural Food Additives. *Molecules*. 2019 Mar 18;24(6):1056. doi: 10.3390/

molecules24061056. PMID: 30889812; PMCID: PMC6471601.

19. Pérez-Ibarbia L, Majdanski T, Schubert S, Windhab N, Schubert US. Safety and regulatory review of dyes commonly used as excipients in pharmaceutical and nutraceutical applications. *Eur J Pharm Sci.* 2016 Oct 10;93:264-73. doi: 10.1016/j.ejps.2016.08.026. Epub 2016 Aug 12. PMID: 27531552.

20. Ghosh D, Singha PS, Firdaus SB, Ghosh S. Metanil yellow: The toxic food colorant. *Asian Pacific J Health Sci.* 2017;4(4):65-6.

21. Macioszek VK, Kononowicz AK. The evaluation of the genotoxicity of two commonly used food colors: Quinoline Yellow (E 104) and Brilliant Black BN (E 151). *Cell Mol Biol Lett.* 2004;9(1):107-22. PMID: 15048155.

22. Kamel MM, El-lethey HS. The potential health hazard of tartrazine and levels of hyperactivity, anxiety-like symptoms, depression and antisocial behaviour in rats. *J Am Sci.* 2011;7(6):1211-8.

23. Khedmati Morasa H, Mahmoudi R, Hosseinabadi Z, Mehrabi A. Evaluating the Artificial and Microbial Contamination (Pathogenic Bacteria, Molds, Yeasts) of Confectionery Products in Iran: A Systematic Review. *J Chem Health Risks*. 2020. doi: 10.22034/jchr.2020.1883030.1064

Zahra Gholami et al.