Journal of Human Environment and Health Promotion
Print ISSN: 2476-5481 Online ISSN: 2476-549X

Assessment of Unauthorized Artificial Food Colorants in Confectionery Products in Zanjan, Iran

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ARTICLE INFO

Article type:
Original article

Article history:
Received: 27 September 2025
Revised: 12 November 2025
Accepted: 29 November 2025
Available online: 9 December 2025

https://doi.org/10.61882/jhehp.737

Keywords:

Amaramth
Brown chocolate dye
Confectionery products
Synthetic color additives
Tartrazine

ABSTRACT

Background: Unconsciousness and misuse of synthetic and non-permitted food colorants are considered widespread fraud and a serious threat to the health of the community, especially young children. The purpose of this study was to determine the status of three non-permitted food colorants in confectionery products in Zanjan.

Methods: As a cross-sectional study, 150 samples of Confectionery products (candies, polki, pastilles, chocolate confectioneries such as Smarties, dragées, freezer pops, and cakes) were randomly sampled from different supply centers in Zanjan, during the summer of 2020. Color analysis was performed by the thin-layer chromatography (TLC) method. Finally, the retention value (RF criterion) was used to detect color type.

Results: In this study, among all samples were collected from the supply centers including confectionaries and supermarkets, 47 samples (31%) contained at least one or three banned yellow synthetic dyes including tartrazine (E 102), amaranth (E 123) and chocolate brown HT E 155) with a misuse rate 42%, 31% and 20%, respectively. Tartrazine had the highest misuse rate among the studied dyes.

Conclusion: The study concludes that unauthorized synthetic food colors-specifically tartrazine, amaranth, and chocolate brown-are widely present in confectionery products in Zanjan, Iran, with 80% of samples containing at least one of these additives.

1. Introduction

Food additives, regardless of their nutritional value, are compounds with natural or synthetic origin that are added to the food products to prevent microbial spoilage, flavor improvement, color recovery, improvement of organoleptic properties of food and beverages and the creation of commercial identity for the product (Bearth et al., 2014; Martínez et al., 2022). The most widely used additives are food colorants (5%), preservatives (5%), processing improvers (close to 20%), sweeteners (30%), and flavoring agents (40%) (Martínez et al., 2022; Izzat, 2017). Colorants are one of the key ingredients to enhance the appetizing qualities of food, so they are widely used in the food industry. Food dyes were used to achieve color uniformity in final products, the appearance and visual appeal of foods and beverages, to shield vitamins from light, and occasionally to conceal the defects and the frauds during the production and storage processes (Gholami et al., 2021).

The governmental regulations are approved by the Food and Agriculture Organization (FAO), the World Health Organization (WHO), and the Iranian National Standards Organization (INSO) to confirm the safety and limitations of artificial food colorants' consumption in products produced by confectionery producers and restaurants. Food dyes are classified on the basis of origin, solubility (soluble and insoluble), and coating capacity (Martínez et al., 2022; Rezaei et al., 2015). Natural pigments of plant roots, including anthocyanins, betanin, beta-carotene, and chlorophyll, have high sensitivity to changes in pH, heat, and light.

Risk assessment is done in animal studies and toxicology to confirm or reject new food additives. However, the periodic

How to cite: Shahimoridi, Y., Hassanzadazar, H., Aminzare, M., Taran, J., & Hashemi, M. (2026). Assessment of Unauthorized Artificial Food Colorants in Confectionery Products in Zanjan, Iran. *J Hum Environ Health Promot*, *12*(1), 52-57.

evaluation of the food additives has an important role to confirm or eliminate food additives used previously (Bearth et al., 2014). According to the results of scientific research, updating the list of allowable food colorants is done every single year and some of them that have potential health risks are eliminated from the authorized list (Farzianpour et al., 2013). Tartrazine (E 102) as a di-azo dye, Amaranth (E 123) as a mono-azo dye and chocolate brown HT (E 155) as a di-sulfonated Azo dye are the banned synthetic dyes in IRAN food industry (INSO, 2019). Food safety experts agree about the uncertainty of the potential risks of food additives. However, from the point of view of safety in food supply, the viewpoints of food experts and consumers are not necessarily consistent, and there are major concerns regarding the effects of food additives on human health (Scotter, 2011). Dyes used as food colorants may be accompanied with various complications on the respiratory system (asthma, urticaria), brain system (Hyperactivity and decreasing the IQ of children), Blood parameters (reduced number of white blood cells and lymphocytes), immune system (Anaphylaxis and suppression of immune system), digestive problems, skin allergies and in some cases of hepatic problems and some complications such as fetal abortion, carcinogenesis and vitamin B6 deficiency especially if used excessively or from invalid sources (Farzianpour et al., 2013). For these reasons, the use of some azo dyes is less likely to be recommended (Zahedi et al., 2020). However, there are some reports of the use of unauthorized industrial dyes in a variety of foods. Alipour (2016) reported that the consumption rate of unauthorized food colorants reaches 52 to 80 percent in restaurant foods, candies, cakes, ice cream, and beverages. Gholami et al. (2021) reported that 7.15% of beverages had 2 unauthorized synthetic dyes. Economic criteria, limited knowledge about the consequences of using illegal synthetic food dyes in food production, and inadequate monitoring by supervisory organizations are important factors in using the synthetic additives. Therefore, it is necessary to determine the status of unauthorized food dyes used in the supplied food in Zanjan (IRAN) using the TLC method, given their potential adverse effects on public health, particularly among children.

2. Materials and Methods

This cross-sectional study was conducted in the summer of 2020. A total of 150 samples (determined based on the results from previous studies), including confectionery products Pastille (n = 40), Freezer pop (n = 20), Candies (n = 20), dragées chocolate confectioneries (Smarties) (n = 20), dragées (n = 20), and cakes (n = 15) and chocolates (n = 15) (Figure 1) were provided randomly from the local market of Zanjan City (Zanjan, Iran) based on cluster sampling method and transferred to the chemistry laboratory in the school of public health of Zanjan University of Medical Sciences. All used chemicals were purchased from Sigma-Aldrich (Missouri, USA). All the samples were prepared according to the Iranian national standard NO. 740 to analyze and verify the presence of Tartrazine, amaranth, and chocolate brown food dyes by the TLC method.

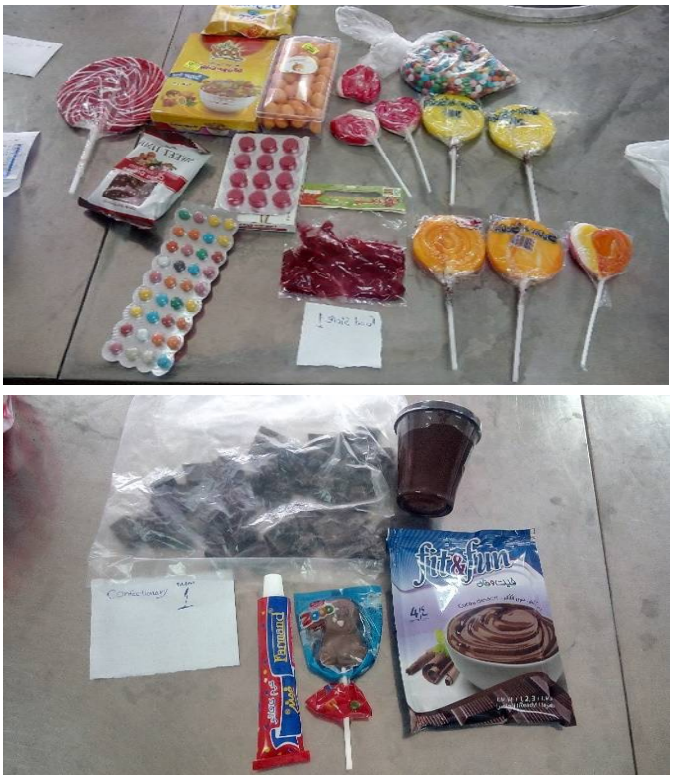

Figure 1. Confectionery products (pastille, Smarties, dragées, freezer pops, and cakes)

2.1 Defatting of the samples

In order to extract color from food, it is necessary to remove starch materials from the environment and then proceed to dye separation. For this purpose, initially 10 to 15 g of the milled sample were weighed and mixed with 100 mL of the mixture solution of ammonia (2% W/V) in alcohol (70%) added in an Erlenmeyer flask (250 mL). After 24 hours, the upper phase of the solution was separated, and the dried starch was removed. The upper phase solution was transferred to an Erlenmeyer flask (250 mL), and then reached the boiling point in a Bain-Marie tank till 80% of the water evaporated (until the solution no longer smells ammonia). Then, 100 mL of distilled water and 1 mL of glacial acetic acid were added to the solution, and the extraction step by white wool (Farzianpour et al., 2013).

2.2 Purification and extraction of dye

The sample container was placed in the water vapor bath for an hour, and a piece of white wool fiber (40 cm) was left in it. In acidic conditions, the studied artificial dyes have a negative charge, and wool protein has a positive charge. Therefore, dyes are well absorbed by wool. (Figure 2). After assuring the full absorption of the dye, wool fibers were transferred to another container (Heydarzade et al., 2021; Amiri et al., 2020). The specimens were washed with cold water using forceps. After that, the washed wool was transferred to a beaker, followed by 30 mL of distilled water and 1 mL concentrated ammonia (65%) (Aalipour & Mahdavi,

2016). The dye transferred to the alkaline solution after 30-60 min. Colored solution was placed on indirect heat (Bain-marie tank) for concentration and at least 80% drying (Asadi et al., 2019).

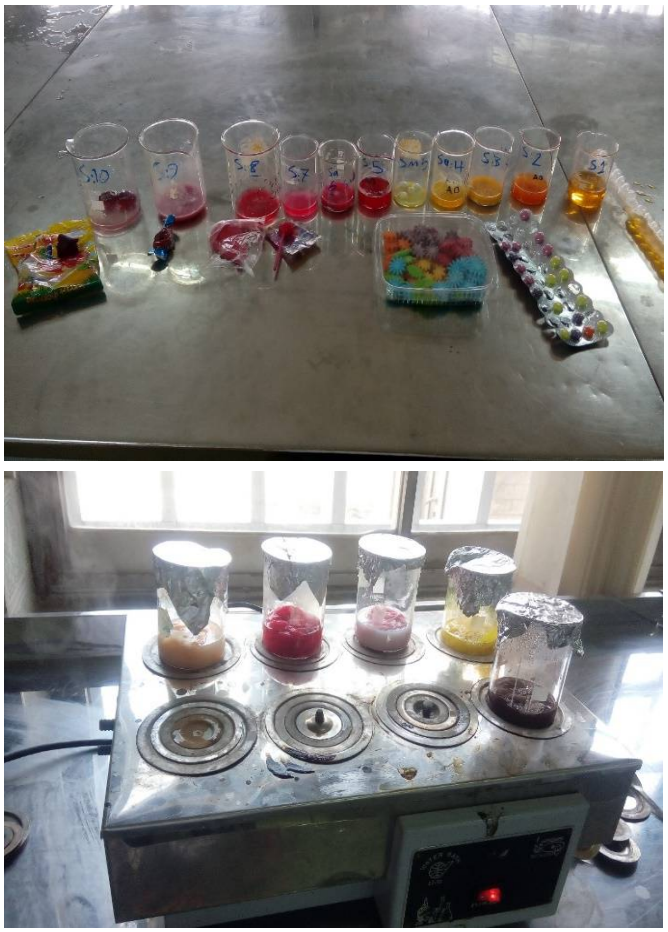

Figure 2. Purification and extraction of dye

2.3 Thin-layer chromatography (TLC)

Dyes were isolated from prepared samples using the TLC method. After washing and drying the chromatography tank, a mixture of glacial acetic acid, butanol, and distilled water in a ratio (6, 5, 10 v/v) was added to the tank as solvent. After spotting the sample solutions (Dotted at a distance of 3 cm from the edge of the chromatography paper, and the diameter of each color blot must not exceed 2 to 3 mm) on the TLC paper, the spotted paper was hung vertically in the chromatography tank. The extracted color was detected after short-term contact of the tank solvent with the spot plate at specified points. the specification of each sample under the marked stain is written and dried by an air dryer. For this purpose, the RF index was used.

Retention value (RF) = Distance travelled by compounds (cm)/Distance travelled by solvent front (cm)

The dyes were matched to the standard-mentioned dyes in this study (Tartrazine, Amaranth, and chocolate brown) to specify the color type. As the final stage, by comparing the distance travelled by the uncertain stain of the sample (Rf) and the standard dye movement on TLC paper, the Dye type was identified (Figure 3). The results of this study were compared with the Iranian national standard of food dyes (Farzianpour et al., 2013; Amiri et al., 2020; Okogbenin et al., 2023).

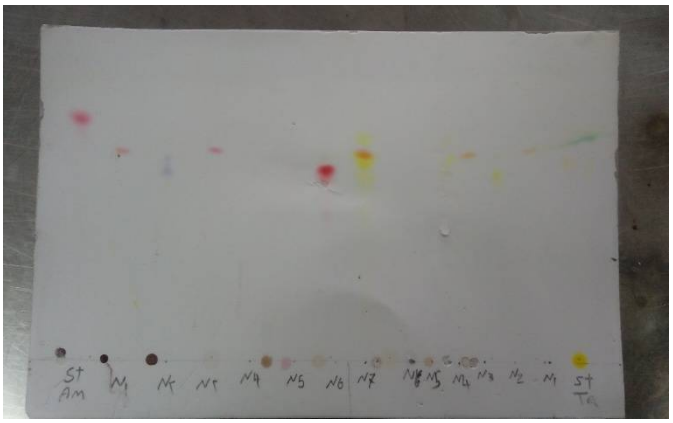

Figure 3. Comparing the distance travelled by the uncertain stain of the sample and the distance traveled by the standard dye on TLC paper

2.4 Statistical analysis

All assessments were performed in triplicate. The presence or absence of unauthorized colors has been reported in this study. Statistical analysis was performed with SPSS Version 26.0 statistical software package.

3. Results and Discussion

According to the results gathered from the confectioneries in Zanjan (Table 1), 102 out of 150 samples (68%) contained the artificial colors tartrazine, amaranth, and chocolate brown, which are considered unauthorized under Iranian national standard No. 740. Among samples collected at supply centers, including confectioneries and supermarkets, 56% of the products contained the unauthorized yellow-orange color tartrazine, 68% of the samples contained the unauthorized red-pink color (Amaranth), and 80% contained the unauthorized chocolate brown color. The greatest occurrence of tartrazine was found in 50% of candies, flakes, and decorative confectionery items, 60% of cakes, 67% of Smarties and dragées, and 46% of pastilles and soft beverages. According to the results, 60% of colored pastilles and freezer pops, 67% of Smarties and dragées, 70% of cakes and 80% of candies, flakes and confectionery decorations contained the prohibited color amaranth. The highest frequency of chocolate brown color was observed in brown candies and chocolates with 87%, brown Smarties and dragées with 87% and brown cakes with 70%. The prevalence of yellow and orange tartrazine was higher than expected. The greatest proportion of these color additives was observed in snacks and edible ices (such as freezer pops), as well as in unlabeled candies. A similarly high frequency of use was also noted in bulk confectionery ingredients, including dragées and Smarties.

Table 1. Presence rate of three synthetic food colors in confectionery products in the retail market of Zanjan city (Iran)

Samples	Tartrazine Total samples (inconsistent samples with standard No. 740)	Amaranth Total samples (inconsistent samples with standard No. 740)	Chocolate brown Total samples (inconsistent samples with standard No. 740)
Candy and Polki	10 (5)	10 (8)	15 (13) *
Smarties and dragées	15 (10)	15 (10)	15 (13)
Pastilles and freezer pops	15 (7)	15 (9)	------
Chocolates and cakes	10 (6)	10 (7)	20 (14) **
Total samples	50	50	50
Total unauthorized samples (Positive samples)	28 (56%)	34 (68%)	40 (80%)
Negative samples (without artificial colorants)	22	16	10

* It was evaluated in candies and chocolates
** it was evaluated just in cakes

Along with changes in lifestyles and the increasing access of people to ready-to-eat foods and the use of colored snacks (a small amount of food eaten between meals) by people and most children, the number of food distribution centers has increased significantly. The color stability, variety, availability, and affordability of artificial colors have led to an increasing use of these colors in various food products. The obtained results showed that 80% of confectionery products in Zanjan city contain at least one unauthorized artificial color. Among the three unauthorized colors studied, the most commonly used unauthorized colors in confectionery products were tartarazine with 56%, amaranth with 68% and chocolate brown color with 80%. Several studies have confirmed the presence and widespread use of unauthorized artificial food colors in different foods, confectioneries, and beverages in the range of 20-85% through the world, including IRAN (Soltan Dalal et al., 2007; Khosravi Mashizi et al., 2012; Suh & Choi, 2012; Saleem et al., 2013; Farzianpour et al., 2013; Rezaei et al., 2015; Rovina et al., 2017; Dilrukshi et al., 2019; Ghaffar et al., 2020; Rahmati & Karajhiyan, 2021; AbuKhader et al., 2021).

Tartrazine is the most commonly used color additive in sweets, beverages, and fast foods in communities such as Hyderabad (India). There is a general belief in the customs and cultures of various Asian communities that tartrazine is a lucky and royal yellow color (Dilrukshi et al., 2019). The close similarity to saffron, the low cost, and the stability properties of tartrazine are important factors in its higher use by food manufacturers and suppliers than other synthetic colors (Amin et al., 2010). According to toxicological studies, tartrazine is more likely than other azo dyes to cause allergic reactions such as asthma, eczema, and purpura, especially among asthmatics and those who are aspirin-intolerant. Tartarazine also causes hyperactivity in children, migraines, and rhinitis (Elliott et al., 2013). Kleinman et al. (2011) confirmed a significant relationship between the consumption of artificial colors and the severity of the disease in hyperactive children. Boris and Mandel (1994) investigated the effects of artificial color consumption in hyperactive children and concluded that removing artificial colors from their diet had a positive effect on improving their disease. The genotoxic, cytotoxic, and cytostatic potential of food coloring agents (such as amaranth and tartrazine) has been investigated in other studies, and their harmful effects on human health have been confirmed (Mpountoukas et al., 2010). It is worth noting that even natural food colorants can have adverse and toxic effects on humans, so the absolute safety of any substance, especially food colorants, has not been proven (Hagiwara et al., 2003). Hence, increasing awareness among food producers and consumers about the problems caused by unauthorized colors for human health is of great importance in reducing the use of artificial colors. Providing training plans to food producers based on Iranian national standards, improving the knowledge of susceptible consumers, especially children, about the food colorants, rational consumption of the artificial or natural food colorants in food products, and continuous monitoring and quality control of colored foods by regulatory bodies are recommended to reduce the possible adverse effects of consuming artificial or natural food colorants.

4. Conclusion

Based on the findings, the study concludes that unauthorized synthetic food colors, specifically tartrazine, amaranth, and chocolate brown, are widely present in confectionery products in Zanjan, Iran, with 80% of samples containing at least one of these additives. Tartrazine was found in 56% of products, amaranth in 68%, and chocolate brown in 80%, with the highest prevalence observed in candies, cakes, Smarties, dragées, and bulk confectionery ingredients. The widespread use is likely due to the low cost, stability, and appealing visual properties of these colors, along with cultural preferences. However, these additives have documented toxicological risks, including allergic reactions, hyperactivity in children, and potential genotoxic effects. The findings highlight an urgent need for stricter enforcement of Iranian national standards, public education, particularly targeting producers and parents, and continuous regulatory monitoring to mitigate health risks from unauthorized artificial food colors.

Authors' Contributions

Yaser Shahimoridi: Project administration; Writing-original draft; Software; Methodology; Visualization; Investigation. **Hassan Hassanzadazar:** Resources; Methodology; Writing-review & editing; Supervision;

Funding acquisition; Conceptualization; Data curation. **Majid Aminzare:** Methodology; Writing-review & editing. **Jafar Taran:** Validation; Resources; Methodology. **Mahsa Hashemi:** Project administration; Resources; Investigation.

Funding

This research was funded by the Zanjan University of Medical Sciences (Grant No. A-11-940-19).

Conflicts of Interest

There are no competing interests to declare.

Acknowledgments

We extend our gratitude to Zanjan University of Medical Sciences for its support of this study.

Ethical considerations

All Ethical issues have been confirmed by Iranian National Committee for Ethics in Biomedical Research (IR.ZUMS.REC.1398.374).

Using Artificial Intelligence

We hereby declare that artificial intelligence was not used in the current manuscript.

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