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Existence of Synthetic Dyes in Foodstuffs: A Retrospective Study of Food Safety Concerns

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HIGHLIGHTS

- Measuring the use of permitted and unauthorized food colorings in food is of particular importance in human health (food safety).
- Quinoline yellow, sunset yellow, carmoisine, and tartarazine were the most widely used synthetic dyes in this study.
- About 30% of the collected samples, except fruit juice, had synthetic colorants.

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Acronyms and abbreviations
TLC=Thin Layer Chromatography

ABSTRACT

Background: The use of additives, especially food colors, has attracted the attention of food industries. The purpose of this study is to retrospectively investigate the use of food colors in food products offered in Urmia, Iran.

Methods: In this study, 451 samples of different types of food were collected from Urmia (April 2019-February 2022). To identify the color type, the Thin Layer Chromatography method was used. The samples included a confections (dry sweets), saffron noghl, saffron halva (a type of confectionery originating from Persia and widely spread throughout the Middle East), saffron-flavored rice and chicken, fruit ice cream, traditional saffron-flavored ice cream, fruit drinks, and saffron solutions. These products were produced from establishments engaged in the production and distribution of food and confections, restaurants, halva manufacturing workshops, traditional ice cream production workshops, and coffeehouses. Statistical analysis of data was done using SPSS software (IBM SPSS statistic 16, USA).

Results: Within the group of synthetic dyes analyzed, quinoline yellow, sunset yellow, carmoisine, and tartarazine were identified as the most commonly used synthetic colorants in the examined food products. Findings also showed that rice and saffron chicken samples had the highest average frequency of tartarazine used among the studied food groups (41.66±1.88%). The highest mean frequency of yellow quinoline consumption was also observed in samples of saffron halva (57.27±3.86%), various types of dry sweets (55.50±0.7%), and saffron noghl (52.50±3.53%). Sunset yellow had a low frequency in all groups except saffron ice cream and fruit drinks (p=0.086). The findings showed that the highest average frequency of carmoisine synthetic color consumption was related to saffron ice cream (25±1.41%) and fruit drinks (23.80±2.54%) among the studied food groups.

Conclusion: The findings indicated that approximately 30% of the tested samples, except fruit drinks, contained synthetic colorants.

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Introduction

Eating healthy food is one of the important factors in promoting and maintaining human health. Scientific evidences to date indicate that there is a strong connection between food health and the incidence of chronic diseases; therefore, continues monitoring, and control of food is of great importance to improve the health of community (Nishida et al., 2004; Shang et al., 2023). The current body of evidences thus far imply that adhering to a nutritious dietary regimen diminishes the likelihood of developing prevalent chronic illnesses that are associated with one's eating habits, such as diabetes, cardiovascular disease, and certain types of cancer (Dragoev, 2024; Neuhouser, 2019). Although various approaches are available for evaluating eating patterns in large-scale surveys, those that classify dietary habits based on predefined scoring systems or indices, such as the Healthy Eating Index, may hold the highest merit due to their ability to provide a standardized measure that can be uniformly utilized in numerous research investigations (Neuhouser, 2019; Tuormaa, 1994). The increasing use of additives in food products can be attributed to various reasons, including improving the appearance, taste, and durability of food products, which attracts customers. According to numerous research studies, a range of health ailments, including asthma, Attention Deficit Hyperactivity Disorder (ADHD), cardiac complications, cancer, obesity, and various other diseases can be attributed to detrimental additives and preservatives. Certain food additives have the potential to disrupt hormone levels and impact growth and development, thereby contributing to the prevailing issue of childhood obesity (Ahmed et al., 2021; Trasande et al., 2018). In comparison with adults, children are more prone to exposure from such dietary constituents. Women, during pregnancy and breastfeeding, often resort to the use of certain food additives that do not entirely ensure safety. Hence, we must adopt specific precautions to avert the consumption of perilous compounds in case they harm our well-being (Sambu et al., 2022; Tuormaa, 1994).

An important group of additives that are widely used in food products is colors. Food coloring is used to make food colorful and indicates the quality of many food items in terms of health and hygiene (Baala vignesh et al., 2020; Marklinder et al., 2013). Color is an important element in food industry and increases the appetizing value and subsequent acceptance towards foods and beverages. On the other hand, synthetic food colors are increasingly used by food manufacturers due to low cost, color stability, and improved appearance (Cobbold, 2020). Undeniably, some foods and beverages may contain illegal synthetic colors, and some food producers may also overuse some authorized synthetic colors or unidentified colors including a textile dye (De Mejia et al., 2020; Dey and Nagababu, 2022). The consequences may result in health problems such as cancers and allergic reactions. Hence, implementation of regulatory procedures and awareness programs for both consumers and food manufacturers can be considered as a practical approach to minimize the consequences (Gholami et al., 2021; Mirzay-Razaz et al., 2022). Colors are divided into three groups: mineral colors or non-edible synthetic colors (these compounds are not found in nature and are made artificially, they are not usually used in food industry), edible natural colors (of plant origin such as chlorophyll, carotenoids, tannins, anthocyanins), and synthetic or artificial food colors (such as quinoline yellow, sunset yellow, ponceau R 4) (Vikram et al., 2015).

Due to the high cost of extraction and low efficiency, instability in environmental conditions, and pH, nowadays synthetic colors are used more than natural colors (Luzardo-Ocampo et al., 2021). The utilization of synthetic colors offers certain benefits as compared to natural colors with regards to color permanence, luminosity, and steadfastness. However, it is important to note that the application of these colors can potentially result in adverse health effects, including toxicity and even carcinogenicity in humans (Durazzo et al., 2022). The empirical evidence gathered by researchers suggests that the utilization of synthetic pigments is associated with detrimental consequences, including the manifestation of allergies such as asthma and urticarial, an elevated likelihood of developing cancer, impairment to the liver, and kidneys, an increased probability of experiencing miscarriage, diminished cognitive focus, and intelligence quotient in young individuals, the occurrence and exacerbation of issues in children with hyperactivity, a decline in the efficacy of the immune system, and disturbances in sleep patterns (Kleinman et al., 2011; Kobylewski and Jacobson, 2012). Studies have discovered a notable correlation between the ingestion of synthetic pigments in a range of food items and the magnitude of the ailment in children afflicted with hyperactivity (Farzianpour et al., 2013; Yamjala et al., 2016).

Therefore, as a result of the escalating consumption of these compounds by the producers of conventional products and the negligence of this particular cohort towards the nature of the utilized dye and the resulting consequences from the application of these pigments, an inquiry shall be conducted in the sufficient amount on variety of food pigments (both natural and synthetic) used in the comestible items available in the city of Urmia. This investigation shall encompass an array of products, including assorted confections (dry sweets), saffron noghl, saffron halva, saffron-flavored rice, and chicken, fruit ice cream, traditional saffron-flavored ice cream, fruit drinks, and saffron solutions.

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Materials and methods

Sample collection

In this study, 451 samples of different types of food were collected randomly in Urmia city from April 2019 to February 2022. These sample include dry sweets from local market (214), Saffron noghl of Urmia (40), saffron halva of Urmia (54 samples), saffron ice cream from local market (10 samples), fruit ice cream (18 samples), saffron rice, and chicken from restaurant (58 samples), fruit drinks (20 samples), and saffron solutions (37 samples) in two consecutive samplings. Also, health officials inspected 310 confectioneries, 102 halva manufacturer, and 125 restaurants. Food production and supply centers such as confectioneries, restaurants, halva production workshops, traditional ice cream production workshops, and coffee shops were randomly sampled and sent to the laboratory for control and monitoring.

Chemicals and solutions

All materials and chemical solutions used in the experiment were prepared and used from Merck and Sigma Aldrich, Germany and UK, including seven synthetic dyes: tartarazine (E102), sunset yellow (E110), quinoline yellow (E104), pensio 4 R (E124), allura red. (E129), carmoisine (E122), brilliant blue (E133), hydrochloric acid (HCl), ammonia, capillary glass tube, hamilton syringe, degreased white wool, 20×20 silica gel plate, n-butanol, and acetic acid.

Preparation of samples and color extraction

A total of 15 g from each specimen were weighed and subsequently dissolved in 100 ml of a 2% ammonia solution. After 24 h, the supernatant solution was separated and transferred to container. Then, the sample was subjected to a bain-marie (Memmert, Germany) until approximately 20% of the original sample remained. During the purification phase, 5 ml of the sample was dissolved in 100 ml of double distilled water, and to acidify the mixture, 1 ml of HCl (37%) was added into the specimen. Next, several white wool fibers were employed to absorb the dye. The wool fibers were positioned within the sample receptacle, subsequently subjecting the sample receptacle to elevated temperatures within a bain-marie. One h later and after the achievement of full dye absorption, the fibers were relocated to a different receptacle. Then, 1 ml of ammonia (65%) and 50 ml of double distilled water were added into the sample. This mixture was subjected to a boiling process using a bain-marie. After 1 h and making sure that the color is separated from the fibers to the alkaline environment, the sample was placed in the oven (Memmert, Germany) at 37 °C for 4 h to dry. All laboratory procedures were carried out according to the instructions of the general department of laboratories with standard number 740 (De Andrade et al., 2014; Ntrallou et al., 2020).

Determining the type of color by Thin Layer Chromatography (TLC) method

To identify the color type, the TLC (Macherey Nagel, Germany) method was used. A silica gel plate (Macherey Nagel, Germany) with dimensions of 20×20 was used for chromatography. First, from the top side of the plate, about 3 cm, it was marked. On this horizontal line, the number of samples and the standard of seven authorized synthetic dyes including quinoline yellow, sunset yellow, carmoisine, pensio 4 R, allura red, and brilliant blue and one unauthorized synthetic color tartarazine with 1.5 cm intervals with a pencil was marked. The becher containing the dried sample was soaked with one to several drops of distilled water in such a way that the dried color at the bottom of the container dissolves in it, and a drop of the solution inside the becher was stained with a micropipette on the marked area of the sample. After staining the samples, the stains prepared in the same way from eight standard colors in normal butanol were stained on the specified points. Then, the plate was placed inside the tank containing the solvent from the stained part. To prepare the chromatography tank solution for color separation, 10 volumes of normal butanol, five volumes of acetic acid, and six volumes of distilled water were poured into the chromatography tank and mixed. The lid was close at least 4 h before the chromatography until the solvent in the tank was saturated. After placing the silica gel plate, when the solvent rises to about two-thirds of the plate, the plate was removed from the tank and moved under the hood to dry, and then the movement of the stain was compared and sample stains was detected with standard dye stains of the synthetic dye used in the samples (Moghadasi et al., 2019).

Statistical analysis

Statistical analysis of data was done using SPSS software (IBM SPSS statistic 16, USA). The comparison between the average frequencies of colors used in the mentioned products was performed using one-way ANOVA with Duncan's test post a probability level of 5% ($p<0.05$). Kolmogorove-Smirnov test was used to evaluate the normal distribution of data in each group, and then, the mean of data was compared with t-test. p-value of less than 0.05 was considered significant. Moreover, Excel 2010 software was used to draw graphs. The results were reported as the mean±Standard Deviation (SD) of three replicates.

Results

In this study, 451 samples of different types of food in the supply level of Urmia city, including types of dry

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sweets, saffron noghl, saffron halva, traditional saffron ice cream, fruit ice cream, saffron chicken, fruit drinks, and saffron solutions were studied, and the frequency of permitted and unauthorized food colorings were investigated in these products. The findings showed that the amount of natural saffron color was respectively higher in ice creams, saffron chicken and rice, saffron halva, saffron solutions, and saffron noghl than the rest of food products (Table 1). These results demonstrated that the consumption of saffron color in food was more than ($p<0.05$) other colors, which can be due to the nativeness, and abundance of this spice in Iran. The highest and the lowest consumption of synthetic food colors in Urmia city was reported in fruit drinks and fruit ice creams, respectively.

The results of this study showed that among the unauthorized food colors, the use of tartarazine color was higher ($p<0.05$) first in chicken and saffron rice (41.67%) and then in saffron ice creams (25%), while it was less in saffron noghl (5%) than other food products. Also, the highest consumption of quinoline yellow color was observed in saffron halva and the lowest consumption was reported in saffron ice cream. After quinoline yellow, sunset yellow, and then carmoisine had a higher consumption frequency in the examined foods products (Table 2). Pensio 4 R was not observed in any food group, and the consumption of allura red and brilliant red was lower than the rest of the permitted food colors (Figure 1).

Table 1: The distribution of synthetic and natural colors in all kinds of food

Food groups	**Number of samples**	**% of colored samples**	
		Artificial color	**Natural color (saffron)**
All types of dry sweets	214	32.2	6.54
Saffron halva	54	31.49	68.52
Saffron noghl	40	32.5	67.5
Saffron rice and chicken	58	31.03	68.96
Saffron solution	37	32.43	67.57
Fruit drink	20	50	ND
Saffron ice cream	10	30	70
Fruit ice cream	18	27.77	ND

ND=Not Detected

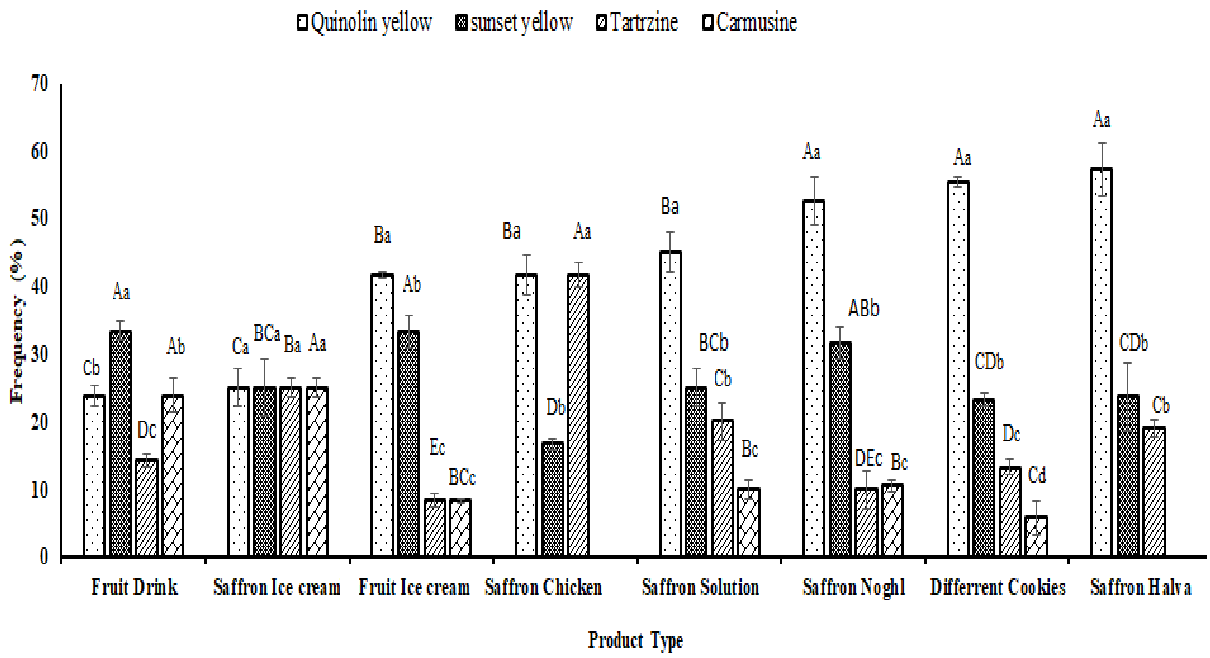

Figure 1: Comparison of the mean frequency (mean±Standard Deviation (SD)) of authorized and unauthorized synthetic colors in each and between groups. Lowercase letters (a, b, c, d) and uppercase letters (A, B, C) indicate significant differences in the frequency of synthetic colors in each and between groups, respectively ($p<0.05$)

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Discussion

The frequency of synthetic and natural colors use has been shown in Table 1. The results showed that almost one third of all tested samples except fruit drinks contained synthetic colors. In addition, synthetic coloring was not observed in 61.21% of dry sweets, 50% of fruit drinks, and 72.23% of traditional fruit ice cream (Table 1). In addition, the distribution of authorized and unauthorized synthetic colors in the studied foodstuff of Urmia was shown in Table 2.

Among the studied synthetic dyes, quinoline yellow, sunset yellow, carmoisine, and tartarazine were the most widely used synthetic dyes in the studied food groups. Quinoline yellow was identified as the most widely used food color among the authorized synthetic dyes in dry sweets, saffron halva, saffron rice, and chicken, saffron solutions, and fruit ice cream. Pensio 4R synthetic dye was not observed in any of the studied food groups, and allura red and brilliant blue synthetic dyes were detected only in the samples of dry sweets, ice cream, and traditional fruit drinks, respectively. This result indicated that tartarazine as an unauthorized synthetic dye has been used more ($p<0.05$) in rice and saffron chicken. Also, in the saffron halva, rice, and saffron chicken, synthetic carmoisine (azorbin) color was not observed (Table 2).

In the fruit drinks group, sunset yellow had the highest average frequency of consumption and was detected in 33.33±1.41% of the samples containing color, and quinoline yellow was detected in 23.8±1.60%, and carmoisine was observed in 23.8±2.54% of colored samples.

The ever-increasing supply of products with various types, colors, flavors, smells, and textures that are produced to ensure the expectations of consumers suggested the importance of food industry science in the quality and health of food among consumers (Dias et al., 2015; Zuñiga-Martínez et al., 2022). Additives play an undeniable role among food compounds that significantly improve organoleptic characteristics. In fact, they have important advantages on the shelf-life, microbiological quality, and safety of many foods. Food coloring can be considered as one of the most impressive and enjoyable characteristics of food, which directly affects choice, and food preferences (Carocho et al., 2014; Osaili et al., 2023).

Saffron is one of the most widely used natural colors in food. Saffron is used in all kinds of food products, including meat products, sweets, ice cream, nuts, etc., in order to increase the quality (taste, color, and smell). The use of saffron as a native plant has been proved to treat diseases such as diabetes and its eye complications, cancer, etc (Ebrahimi et al., 2024; Sepahi et al., 2018, 2022). Saffron can be counterfeited in various ways due to its value, importance and high price (Rezaei et al., 2015). In similar, in our study, saffron as a natural dye used frequency in foods such as ice creams, saffron chicken and rice, saffron halva, saffron solutions, and saffron noghl ($p<0.05$). Furthermore, the highest amount of saffron color had been used in saffron ice creams.

The most common synthetic colors include tartarazine, sunset yellow, and quinoline yellow (Gholami et al., 2021; Heydarzade et al., 2021). Tartarazine and having an azo chemical structure is one of the most dangerous groups of synthetic dyes, which can be broken down by intestinal microbial flora and cause continuous headaches in adults, hyperactivity in children, and also asthma attacks or other allergic reactions after being converted into aromatic amines (Heydarzade et al., 2021). In the present study, the results showed that tartarazine was the most used synthetic dye in rice and saffron chicken (41%), while its use in saffron noghl was not significant (5%).

In the study by Gholami et al. (2021), the frequency of colors used in fruit juices and traditional drinks was investigated in Shiraz, southern Iran, using the TLC method. They randomly prepared 70 samples of fruit juices and drinks with different flavors from Shiraz city. According to their findings, 60% of the samples had no color, and 32.85% contained authorized synthetic dyes. The highest frequency of using synthetic colors was related to pomegranate flavored fruit juices, and the highest use of synthetic colors was the simultaneous combination of two colors, carmoisine, and sunset yellow (Gholami et al., 2021). In this study, the frequency of permitted and unauthorized synthetic colors in 8 food groups was investigated. Findings showed that the highest use of synthetic colors was observed in the group of fruit drinks. In this group, similar to the study of Gholami et al. (2021) the use of sunset yellow and carmoisine was higher than other colors.

Barani and Tajik (2019) investigated the simultaneous use of saffron and synthetic colors in ready-to-cook Iranian foods such as grilled chicken. A total of 160 ready grilled chicken samples including 136 samples from restaurants and 24 samples from food industries were collected from the central regions of Iran. They showed that saffron was used in only 41 samples (25.62%), and other food colors were used in 119 samples (74.38%). Among the synthetic dyes used, sunset yellow was the mostly used dye with 25.62%, following by quinoline yellow with 18.75%, and tartarazine with 10%. The consumption of tartarazine in restaurant samples was significantly higher than the samples obtained from food industry, while sunset yellow and quinoline yellow were more frequent in the samples taken from the food industry (Barani and Tajik, 2019). Moreover, the findings demonstrated that the consumption of tartarazine and quinoline yellow in chicken and saffron rice (41.67%) was higher than other food groups.

In another study from Jaffna region of Sri Lanka, the use of synthetic food colors was investigated in confectionery and beverages in the region. For this purpose, 110 random samples from 11 districts of medical officers of Jaffna region were analyzed using TLC and UV-visible spectrophotometry. According to the results, 100% of drinks and 85% of sweets contained synthetic food colors. In total, 7% of confectioneries had no synthetic food coloring and 8% of confectioneries had authorized synthetic dyes that did not match any of the permitted synthetic food colors. Tartrazine (41%) was the most used synthetic food color in confectionery and beverages (Dilrukshi et al., 2019). Accordingly, the results of this study showed that the consumption of synthetic dyes such as quinoline yellow (55.55±0.70) and sunset yellow (33.33±1.41) was high in dry sweets, and fruit drink, respectively. However, the average consumption of tartarazine in dry sweets (13.33±1.06) and fruit drink (14.30±0.98) was not statistically high.

Asadnejad et al. (2018) studied the main food colors used in local foods and drinks in Tehran. Their survey was conducted to show the frequent use of color additives and products that are targeted for adulterating colors in Iranian foods and beverages. Of 1,120 collected samples, 18.86% contained synthetic color, 11.89% contained natural color, and 69.25% had no additive color. Tartrazine (E102) was the only unauthorized synthetic color used in the samples. In their study, tartarazine was the only non-permitted color in these food groups, which was mostly used in rice and saffron chicken (Asadnejad et al., 2018). In this study, 36.56% of the food products were without color, 34.01% contained synthetic color, and 29.43% contained natural color, and the use of taratarazine (41.67%) in saffron chicken and saffron rice was more than other colors.

In another study, Farzianpour et al. (2013) investigated the consumption of food colorings in Shahrekord city in the confectionary units of candy flakes (a type of coin-shaped candy) and rock candy. For this purpose, 720 samples of sweets, flakes, and rock candy were collected randomly in a period of 10 months and analyzed by TLC. The results showed that 48.47% contained food coloring and 6.52% contained authorized synthetic dyes. According to the results, there was a high consumption rate of synthetic colors (41.22%), and sunset yellow was the most used synthetic dyes (Farzianpour et al., 2013). In this study, the most and least widely consumed synthetic food coloring in Urmia city was reported in fruit drinks and fruit ice creams, respectively. Contrary to Farzianpour et al.’s research (2013), the consumption of sunset yellow in this study was lower than Quinoline yellow in all eight studied food groups

Despite the fact that natural food products have their own color intensity, storage conditions, manufacturing, and processing methods have a great influence on their final coloring. Therefore, food additives may be considered as a promising tool to mask their unpleasant characteristics (Dias et al., 2015; Laokuldilok et al., 2016). Numerous synthetic food colors have been developed to enhance the quality of food product and organoleptic properties, but over time, most of them were banned due to obvious side effects, in addition to short-term and long-term toxicity (Amchova et al., 2015). The results of this study showed that although the use of permitted synthetic food colors was observed in food groups, the average use of the unauthorized food color tartarazine in food groups was relatively lower than the permitted colors. Achieving better statistics and more clear results requires the examination of more samples from different food workshops in the city, which will be possible with more supervision by health experts. Also, continuing these studies in the near future can reduce people's concerns about the health of food supplied in the city.

Conclusion

Results indicated the abundant use of quinoline yellow compared to other synthetic colors in the food supply of Urmia city, specifically in the samples of dry sweets, saffron halva, saffron rice, and chicken, saffron solutions, and traditional fruit ice cream. Pensio 4 R synthetic dye was not observed in any of the studied food groups, and allura red and brilliant blue synthetic dyes were detected only in the samples of dry sweets, ice cream, and traditional fruit drinks, respectively. In general, compared to natural colors, the consumption of authorized synthetic dyes was more than natural colors in all the studied food groups, which can probably be due to the lower cost and economic justification of these colors compared to natural colors. Raising people's awareness, especially people related to the production of food products, can facilitate the planning and necessary management and control measures, which is an important step to protect the health of society. It seems that the most important criteria for the unauthorized use of synthetic colors are the economy, and the lack of knowledge is secondary. Therefore, by increasing the awareness of general public and related employees in food preparation, effective measures can be taken to reduce the consumption of these colors. However, other activities such as more effective inspection and decisive action by the responsible bodies with violators, controlled distribution of synthetic colors, and food labeling will help to manage the above health problem.

Authors' contributions

R.S.V., N.V.S., M.R.A., and S.S. participated in study design, drafting the manuscript, method development, and statistical analysis. S.S. and R.S.V. were involved in manuscript editing and final approval. All authors read and approved the final manuscript.

Ethical Consideration

The ethical number of this study was IR.UMSU.REC.1401.27.

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Conflicts of interest

The authors declared no conflict of interests.

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