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Assessment of Synthetic Dyes in Food Stuffs Produced in Confectioneries and Restaurants in Arak, Iran

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Background: The artificial food colors tartrazine (E102), sunset yellow (E110), quinoline yellow (E104), ponceau 4R (E124), allura red (E129), azorubine (E122) used in foodstuffs were tested.
Objectives: This study aimed to determine the amount of artificial dyes in cookie, ice cream and saffron aqueous solution obtained from different confectionaries and restaurants of this city.
Materials and Methods: A total of 70 samples of different foodstuffs including cookies (n = 20), ice cream (n = 20) and saffron aqueous solution (n = 30) used and served in confectioneries and restaurants in Arak city, Iran, were collected in February 2013. The samples were tested using Thin Layer Chromatography (TLC) method. Using artificial colors is not permitted according to the national standard of Iran.
Results: The results showed that several products did not meet the standards. Fifty-six out of the 70 (80%) samples examined in the study, namely sunset yellow (60%), tartrazine (57.1%), quinoline yellow (44.28%), azorubine (28.57%), ponceau 4R (8.57%) and allura red (2.85%) failed to meet the standard and health codes.
Conclusions: Considering the high level of artificial colors found in the samples of this study, preventive measures are essential.

Keywords: Food; Restaurant; Food Safety

1. Background

Natural color additives (e.g. carmine, annatto extract, turmeric, saffron and beta carotene) are usually presumed to be color additives derived from plant or animal origins by derivation or other physical procedure (1). But in recent years, many artificial colors, chiefly azo dyes have been widely used as color additive and substitute for natural color. These colors are used to hide defects, make food more attractive, improve superficial features of the products, and also to replace natural color additives that cannot stand the preparation process (2). Furthermore, natural dyes are unstable and easily degraded during food processing. Comparing with natural colors, artificial colors show various preferences such as high consistency to light, oxygen and pH, color monotony, low pollution, relatively lower production costs, etc. Therefore, synthetic food colors are used instead of natural colors in many foods such as drinks, candies, and sweets (3). But some of these coloring materials and their metabolites can pose potential health risk to human beings and may even be carcinogenic, particularly if consumed in large amounts (4). Therefore, there is a necessity to control the content of the synthetic dyes in food. The synthetic food dyes have been strictly controlled by the legislation throughout the world; for example Food and Agricultural Organization (FAO) and World Health Organization (WHO), The U.S. Institute of Standards and Industrial Research of Iran (ISIRI) have set the national legal limit for using any type of artificial color in products produced in confectioneries and restaurants (5, 6).

2. Objectives

Considering that there is no information about the presence of artificial dyes in foodstuffs produced in restaurants and confectioneries in Arak city (Iran) this study aimed to determine the amount of artificial dyes in cookie, ice cream and saffron aqueous solution obtained from different confectionaries and restaurants of this city.

3. Materials and Methods

3.1. Sample Collection

Arak city was divided into five districts and the sam-

ples were collected equally from each district in February 2013. A total of 70 samples of foodstuffs, including saffron aqueous solution (n = 30), cookie (n = 20) and ice cream (n = 20) were taken randomly from different confectioneries and restaurants in this city.

3.2. Chemicals and Reagents

Samples of six synthetic food colorants (tartrazine (E102), sunset yellow (E110), quinoline yellow (E104), ponceau 4R (E124), allura red (E129), azorubine (E122)) were purchased from Merck.

- Glass capillary tubes, chromatography tank and lid.
- Stationary phase: Thin Layer Chromatography (TLC) plate-silica gel (20 × 20 cm; Merck, Darmstadt, Germany).
- Mobile phase: distilled water, n-butanol (C_4H_9OH), ammonia (NH_3) and ethanol (C_2H_6O).

3.3. Color Extraction

Synthetic dyes were isolated according to method described by Farzianpour (7). In this method, liquid foods were used directly, while solid and semi-solid foods were dissolved in water before the color isolation. About 10-15 g of the macerated sample was weighed, poured into an Erlen-mayer flask and then mixed with 100 mL of 2% ammonium in alcohol 70%. After 24 hours, supernatant liquid was picked and the precipitate was removed. Then, the solution was poured into a becher and placed on a Bain-Marie bath and brought to boil until 80% of its water evaporated. In the following, 100 mL distilled water and 1 mL acid was added and the separation step was continued using white wool. The dry color was then collected and dissolved in few drops of water and stored in a stoppered glass bottle for further analysis (7).

3.4. Thin Layer Chromatography Analysis

Mobile phases: distilled water, butanol, ammonia and ethanol 5:10:8:5 (v/v) were poured into the chromatography tank to a depth of about 2 cm. Then the TLC plate was covered with a lid.

The TLC plate was marked using a soft lead pencil. Then, baseline 3 cm from the bottom of the plate was marked along with the positions, where the spots will be applied and labeled. At least 1.5 cm was left between spots.

Spot the solutions were examined. Then a clean capillary tube was dipped into the solution and tested by applying a small drop to the plate. Clean capillary tubes were used for each solution. Then the spots were left to dry. The plate was placed in the TLC tank carefully to prevent the solvent from splashing around. The TLC plate was removed from the tank and laid on a clean piece of paper. Mark the position of the water front and leave the plate until it has dried.

3.5. Calculations

The identities of the separated spots were recorded. For each spot of food coloring, retention factor (Rf) was calculated using:

Rf = (distance moved from the baseline by the food coloring)/(distance moved from the baseline by the water front)

The position of each spot was taken from its center.

4. Results

In this study, a TLC method for the determination of synthetic colors which were added to saffron aqueous solutions, cookie and ice cream was introduced. Table 1 lists percentages of food color additives identified in saffron aqueous solutions, cookie and ice cream consumed in the restaurants and confectionaries of Arak. Among the 70 food items which were analyzed, 56 samples (80%) contained artificial color additives that are banned in foods by the national Iranian standards; namely, sunset yellow (60%), tartrazine (57.1%), quinoline yellow (44.28%), azorubine (28.57%), ponceau 4R (8.57%) and allura red (2.85%) (Figure 1). Fourteen samples of 70 analyzed samples (20%) did not contain any artificial food color additives. The mostly used artificial colorants in products of the confectionaries and restaurants were the sunset yellow followed by tartrazine, quinoline, azorubine, ponceau 4R and allura red. Distribution of the amount of synthetic dye in samples of saffron aqueous solution, cookie and Ice cream is presented in Table 1. Among the products, cookie samples represented the first group of contaminated products with unauthorized colorings (Table 2) followed by saffron aqueous solutions (86.66%) (Table 3) and ice cream (50%) (Table 2).

5. Discussion

The results indicated that approximately 80% of the samples were non-consumable due to application of artificial colorings. The colorings and additives, which were added to foodstuffs, had unknown hazards for consumers.

Table 1. Distribution of the Amount of Synthetic Dye in Samples of Saffron Aqueous Solution, Cookie and Ice Cream [a]

Type of Sample	Artificial Colors	Without Artificial Colors
Saffron aqueous solutions	86.66	13.33
Cookie	100	0
Ice cream	50	50
Total	80	20

[a] Data are presented as %.

Table 2. Distribution of the Amount of Synthetic Dye in Cookie and Ice Cream

NO.	Type of Color											
	Azorubine		Allura red		Ponceau 4R		Quinoline Yellow		Sunset Yellow		Tartrazine	
	Ice cream	Cookie	Ice cream	Cookie	Ice cream	Cookie	Ice cream	Cookie	Ice cream	Cookie	Ice cream	Cookie
1		+						+	+	+	+	+
2		+						+		+		
3		+								+		
4										+	+	+
5	+	+				+	+	+	+	+		
6								+			+	+
7									+	+	+	+
8		+						+		+		
9							+	+			+	+
10												+
11						+				+	+	+
12		+						+		+		
13								+	+	+	+	+
14								+		+		+
15	+	+					+		+	+		
16								+		+		+
17								+				+
18						+			+	+	+	+
19		+								+		+
20								+		+		+
Total	10%	40%	0%	0%	0%	15%	15%	60%	30%	80%	40%	70%

Table 3. Distribution of the Amount of Synthetic Dye in Saffron Aqueous Solutions

No.	Type of Color					
	Azorubine	Allura Red	Ponceau 4R	Quinoline yellow	Sunset yellow	Tartrazine
1	+			+	+	+
2						
3					+	
4	+				+	+
5				+	+	
6				+		+
7	+				+	+
8				+	+	
9				+		+
10						
11			+		+	+
12				+	+	
13	+	+		+	+	+
14	+			+	+	+
15					+	
16	+			+	+	+
17				+		+
18		+	+		+	
19	+				+	+
20				+	+	
21			+	+	+	+
22	+			+	+	+
23					+	+
24				+		
25	+			+	+	+
26						
27	+				+	+
28				+		+
29						+
30						
Total	33.3%	6.6%	10%	53.3%	66.6%	60%

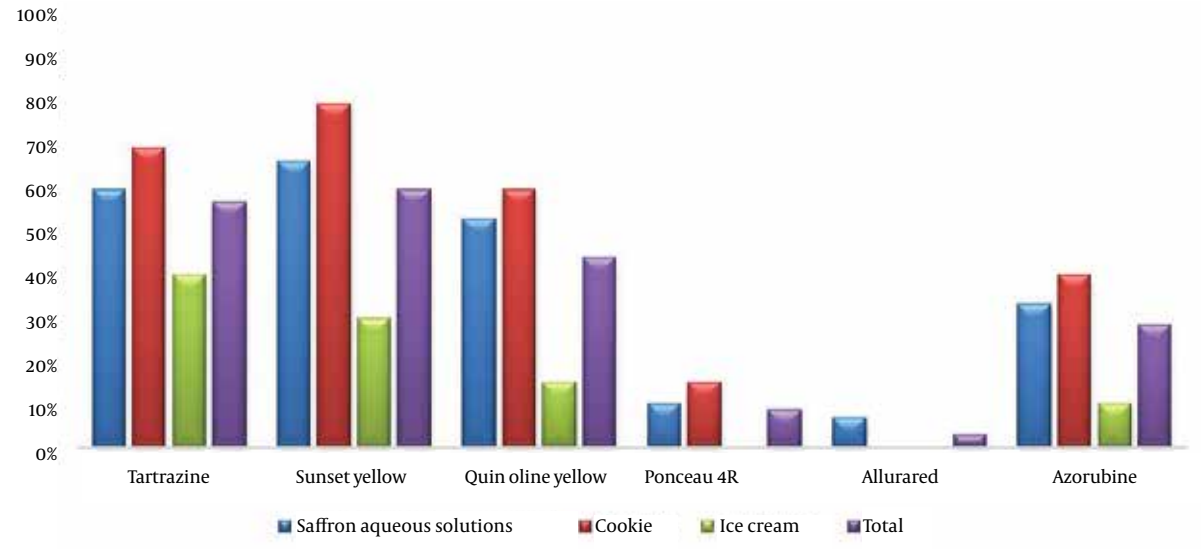

Figure 1. Types of Colors Observed in the Samples

The results were nearly consistent with Arast et al. who reported artificial color in 48% of different confectionary products (8). Soltan Dallal et al. also pointed out high contamination in 336 juice samples produced in Tehran, Iran, so that synthetic colors were detected in 89% of the samples (9).

In following studies, 93.2% of synthetic color in dried sweet samples were non-consumable according to national standard of Iran (10); 48.47% of synthetic colors were found in pastry, poolak (a type of coin-shaped candy) and rock candy (7), 47.56% in variety of ready to eat foods (11).

Tartrazine was found in 57.1% of the samples which were examined. Regarding the toxic effects caused by tartrazine, hyperactivity induction in children, creating hives, and red skin rashes are notable (10). Moreover, it must be reminded that children constitute the main target group in majority of the studies on foodstuffs, while their higher physiological susceptibility is neglected. Sunset yellow, as far as the results showed, causes no significant damage to DNA in colonic cells (12). Nevertheless, it was found in 60% of the samples which were examined. It should be noted that the samples, which contained either permitted or forbidden artificial colors were not approved for consumption, since according to the guidelines of the ministry of health and national standard of Iran (5, 6), using any type of artificial color in confectionaries and restaurants is forbidden. This law is set because these manufacturing units do not have hygiene manager and they do not have the production license and health code required for using artificial colors (7). These manufacturing units are allowed to use natural colors and colors of plant origin such as saffron (10). Colors are responsible for various complications such as asthma, urticaria, abortion, carcinogenicity, anaphylactic reactions, idiosyncrasy, sleeping disorders, hypertension, weakening of the immune system, decreased WBC and lymphocyte count and vitamin B6 deficiency (13, 14). The toxic effects, which are attributed to artificial color (especially tartrazine) are hyperactivity induction in children, creating hives, and red skin rashes (10). It seems that providing food safety training for manufacturers and restaurant owners could play a major role in reducing the use of artificial color content in manufactured products. Based on the results, it was shown that different food color additives, namely, tartrazine, sunset yellow, allura red, quinoline yellow, ponceau 4R, allura red and azorubine, are widely used in several foodstuffs. In light of these results, it is necessary to extent quality control of restaurant and confectionery products by detecting the synthetic colors added. This is more important for specific groups of people, especially children who consume high amounts of synthetic food colors. The modification of the existing food color standards, strengthening of the food control system in the country and promotion of awareness for the application of good manufacturing practices in food industries are also recommend.

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Authors' Contributions

Study concept and design, analysis and interpretation of data, statistical analysis and study supervision: Mohammad Rezaei; acquisition of data: Fatemeh Safar Aba-

di, Ruh Allah Susan Abadi and Hossein Roostaei; drafting of the manuscript: Fereshteh Karimi; critical revision of the manuscript for important intellectual content and administrative, technical, and material support: Mahmood Alimohammadi.

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