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ORIGINAL ARTICLE

Identification of Synthetic Dyes in Traditional Juices and Beverages in Shiraz, Iran

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ABSTRACT

Background: Food dyes are a group of additives which are added to food products to provide a desirable appearance that can have various side effects and complications. This study investigated the frequency of dyes used in traditional juices and beverages in Shiraz, southern Iran.

Methods: In this experimental study totally, 70 random samples of juices and beverages with different flavors were prepared from Shiraz, Iran. After the dye extraction and purification using thin layer chromatography based on the retention factor (R_f) of the corresponding color, the samples were analyzed and identified.

Results: Sixty percent of the samples did not have any dye, 32.85% contained legally permitted synthetic dyes and 7.15% consisted of 2 unauthorized synthetic dyes. The highest frequency of consumption of artificial colors was observed for pomegranate-flavored juices and the most common use of artificial color was the simultaneous combination of two colors, carmoisine and sunset yellow.

Conclusion: It was shown that in some traditional juices and drinks, artificial dyes are still used illegally, which is a warning sign for public health, especially for the children. Therefore, it is necessary to provide more supervision and continuous control over the supply products and to deal seriously with units breaking the law to minimize the use of artificial dyes.

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Introduction

Dyes are an important category of food additives widely used and are divided into two groups of natural and artificial ones (1, 2). Synthetic dyes are compounds used to provide a desirable appearance in food and to make it more attractive and appetizing for the consumer, to protect vitamins from light, to compensate the natural color of food, and sometimes to hide the defects and the frauds during the production and storage processes (3-5). According to previous researches, various side effects have been reported for synthetic dyes. They are food additives that are resistant to aerobic conditions; but in the intestine, due to the microbial flora, they are converted into aromatic amines that can cause various side effects and complications in the body,

including, frequent headaches, carcinogenesis, neurological and genetic disorders (6), attention deficit hyperactivity disorder in children (ADHA) (7-11), anaphylactic reactions, urticaria, allergies, weakened immune system, serious damage and disorders in fat metabolism, and decrease in IQ and vitamin levels (2, 12).

Therefore, the use of artificial dyes in food industry is under strict control around the world. The list of authorized food dyes varies from country to country (6, 13, 14). In Iran, permitted synthetic dyes for use in food industry include quinoline yellow, sunset yellow, brilliant blue, indigotin, Ponzio 4R, azorbin (carmoisine), and allurard (15). Due to the mentioned side effects, rules have been set for the permissible limits of daily consumption of products that are allowed to be used as artificial colors (15). In addition, the use of permitted food dyes in some products such as ice creams, beverages, juices, desserts, and traditional products has been prohibited (16-18).

In Iran, the quantities and types of additives used in industrial products are monitored. But in traditional products, due to less supervision, more problems are created in these products. Manufacturers, regardless of the dangers of using artificial dyes, they still add unauthorized dyes to attract more customers. The researches conducted in different cities of Iran confirmed the unauthorized use of these substances in some traditional food products. Due to the existing concerns about the use of these dyes, especially in traditional products, the present study was conducted to evaluate the frequency of use of unauthorized dyes in traditional juices and beverages in Shiraz, Iran.

Materials and Methods

In this experimental study, 70 samples of traditional juices and drinks with different flavors were randomly selected from 16 traditional juice shops in different parts of Shiraz, southern Iran. According to Table 1, the juice samples with different flavors were purchased in 200 mL packages and were transferred to the laboratory. Then, they were analyzed to identify and to evaluate the type of dye used. The isolation and color detection were performed by thin layer chromatography (TLC) (19).

All chemical materials and solutions, including glacial acetic acid, concentrated ammonia, n-butanol, and 96% alcohol were purchased from Merck Company, Germany. Standard synthetic dyes including tartrazine, quinoline yellow, sunset yellow, brilliant blue, indigotin, Poncio 4R, azorbine (carmoisine), and allura red, made by Sigma USA, were used. Moreover, silica gel plates with dimensions of 20×20 with an aluminum coating (Merck company, Germany) were used.

To extract the dye from the food by white wool, 20 mL of the samples were diluted with distilled water to a total volume of 100 mL. Then, 1 mL of 100% glacial acetic acid was added. A piece of white wool, 30 cm long, was thrown into it and placed on a boiling water bath for one hour so that the synthetic dyes absorb the wool fibers. Afterward, the wool was thoroughly washed with cold water to remove any other nutrients. The dyed wool was poured into a 25 mL container with 20 mL of distilled water and 1 mL of concentrated ammonia that was added to it. Then, the dish was placed on a boiling water bath and after separation of the dye, the wool was removed from the solution. The dye solution was placed in a boiling water bath until dried completely. TLC method was used to determine the type of dye.

To identify the extracted dye by thin layer chromatography, after drying the contents of the container, one or two drops of distilled water were added and the samples were stained with a capillary tube on a silica gel plate. For staining, the first 3 cm was separated from the edge of the chromatographic paper, and staining was conducted at 1.5 cm intervals on the relevant points. After drying of the stains, the plate was placed in a solvent tank containing a mixture of n-butanol-acetic acid-distilled water in volume ratios of 50:25:30. When the solvent rose to about 70% of the plate, the tank lid was removed, and the plate was removed from the solvent. This test was performed simultaneously for standard dyes. In the next step, the size of the colored sample (Rf) was compared to the standard color movement and the color type was identified.

Results

After analyzing and identifying the dyes in 70 samples, most of the samples did not have artificial dyes and only in 40% of the samples, artificial food colors were observed (Table 1). As shown in Figure 1, 39.3% of the samples contained carmoisine dye

Table 1: Status of dyes existed in the samples of traditional fruit juices and beverages.

Types of dyes used	Number of samples (%)
Authorized synthetic dyes	(32.85%) 23
Unauthorized artificial dyes	(7.15%) 5
No artificial colors	42 (%60)
Total	70 (%100)

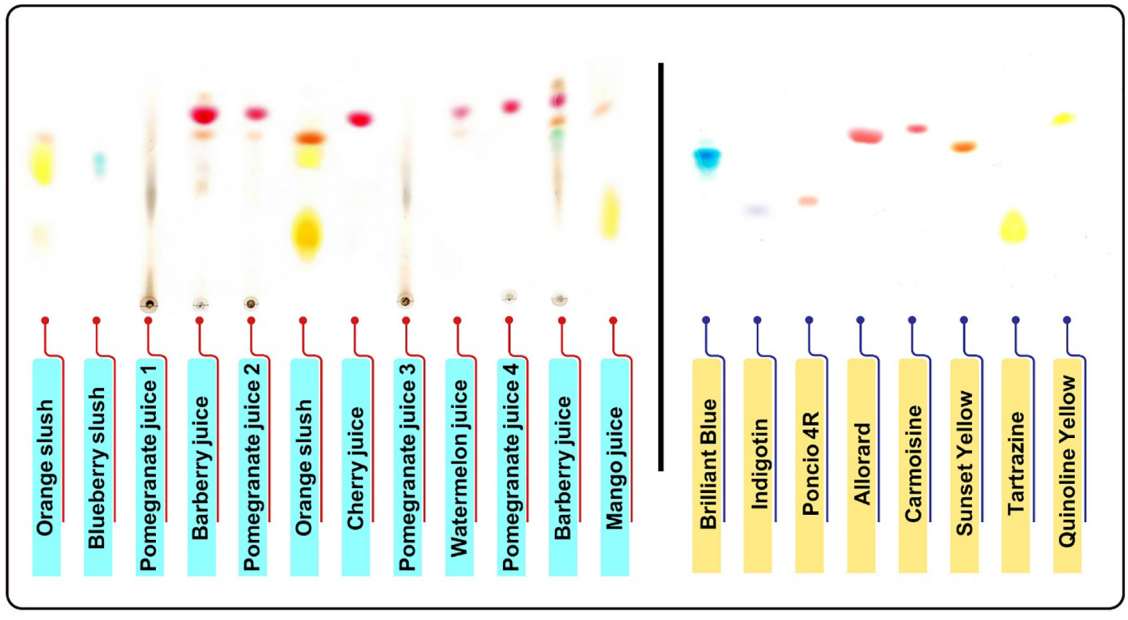

Figure 1: Chromatography of several juice and beverage samples with different flavors and standard colors.

in combination with other synthetic dyes, 21.43% consisted of carmoisine dye alone, 14.28% had brilliant blue, 14.28% possessed simultaneous combination of quinoline yellow, tartrazine and sunset yellow, 3.57% comprised sunset yellow, 3.57% were included with tartrazine and sunset yellow, and 3.57% contained brilliant blue and quinoline yellow.

The extent of contamination of traditional juices and beverages with artificial dyes was presented in Table 2. It was shown that pomegranate juice samples had the highest amount of artificial color, while no artificial dye was observed in cantaloupe and carrot juice samples. The results of Table 3 illustrated that artificial food dyes were allowed in a percentage of the samples, but in mango juice and orange ice product, tartrazine dye has been used, which is not permitted according to the national standard of Iran. Figure 2 demonstrates the chromatography of several samples of juice and beverages with different flavors along with standard colors. Therefore, according to the results of this study and based on the criteria of the Ministry of Health and National Standard of Iran (No. 14345, 2837, and 3964), 40% of the total samples were considered to be non-consumable.

Discussion

Consumption of traditional fruit juices and beverages has a special place for families, especially children, since ancient times. It is one of the best products for consumption in hot months of the year, due to its vitamins, especially vitamin C and antioxidants. It also contains high energy which is of particular importance. Since, according to the criteria of the Institute of Standards and Industrial Research, the use of synthetic dyes in some products such as juices and beverages is prohibited (17, 18), this study analyzed and identified the type of synthetic dyes used in juice products, and traditional drinks which served in Shiraz, Iran.

Table 2: The rate of contamination of juices and beverages with artificial dyes.

Type of juice and beverages	Total number of analyzed samples	Number of samples containing unauthorized artificial dyes No. (%)	Number of samples containing authorized synthetic dyes No. (%)
Pomegranate juice	13	-	6 (46.15)
Cherry juice	7	-	3 (42.85)
Barberry juice	10	-	(30) 3
Pineapple juice	5	-	(20) 1
Mango juice	5	(20) 1	-
Watermelon juice	5	-	(20) 1
Cantaloupe juice	7	-	-
Carrot juice	5	-	-
Blueberry slush	3	-	(100) 4
Cherry slush	4	-	(100) 4
Orange slush	5	(80) 4	(20) 1
Total	70	5 (7.15)	23 (32.85)

Table 3: Types of artificial dyes observed in different flavors of juices and beverages.

Beverage type	Dye type						
	Quinoline+ Brilliant blue	Quinoline Yellow + Tartrazine+ Sunset yellow	Carmoisine + Sunset yellow	Brilliant blue	Tartrazine+ Sunset yellow	Sunset yellow	Carmoisine
Pomegranate juice	-	-	*	-	-	-	*
Barberry juice	-	-	*	-	-	-	*
Cherry juice	-	-	*	-	-	-	*
Mango juice	-	-	-	-	*	-	-
Watermelon juice	-	-	-	-	-	-	*
Pineapple juice	*	-	-	-	-	-	-
Cantaloupe juice	-	-	-	-	-	-	-
Carrot juice	-	-	-	-	-	-	-
Blueberry slush	-	-	-	*	-	-	-
Cherry slush	-	-	*	-	-	-	*
Orange slush	-	*	-	-	-	*	-

*Contains artificial dye, -Contains no artificial dye.

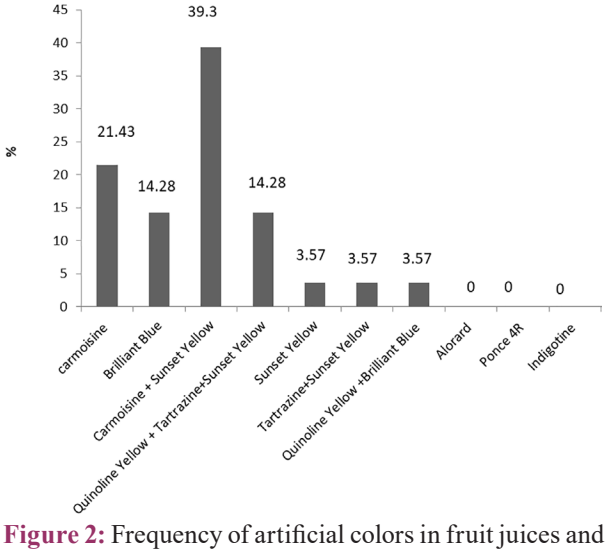

Figure 2: Frequency of artificial colors in fruit juices and traditional drinks.

Based on the results of this study, it was found that out of 70 samples of juices and traditional drinks, 28 samples (40%) contained artificial dyes. The results of this study were close to the findings of Heshmati *et al.* (2014) in which 57 samples (35%) out of 163 ice cream samples and 35 samples (25%) out of 140 samples of nuts contained artificial dyes (11). Our findings revealed that 60% of the samples did not have any artificial color, 32.85% of the total samples contained authorized synthetic dyes and 7.15% comprised unauthorized synthetic dyes. Therefore, 40% of the samples were considered non-consumable ones. In a similar study conducted by Sultan Dalal *et al.* (2007) on the frequency of unauthorized dyes in cherry and barberry juices in Tehran, it was found that 89% of the total samples contained artificial colors. Of the total number of samples containing artificial dye, 18.5% were unauthorized synthetic dyes and 70.5% possessed authorized artificial dyes (20).

Thus, the use of artificial colors in samples of cherry and barberry juices in Tehran has been more than our research samples. Our findings displayed that the most commonly used synthetic dyes in juices and traditional beverages were the simultaneous combination of carmoisine and sunset yellow, and among the unauthorized oral dyes, tartrazine was the most widely used. Authorized synthetic dyes such as Alorard and Ponce 4R and Indigotine were not used in any product. The highest frequency of artificial dyes was noted in pomegranate juice. No additives were found in cantaloupe and carrot juice. In a sample of mango juice, an unauthorized oral dye, tartrazine, was noticed.

In a study conducted by Mohammadi *et al.* (2014) on fruit products and traditional juices in Tehran, 51% of the total samples contained artificial colors. The highest permissible food color was related to carmoisine red and the highest color combination was related to the combination of two colors, carmoisine and sunset yellow (21). The similarity of this study with the present study is that the type of color used in juices was the same, and some producers of traditional juices and beverages used authorized and unauthorized artificial colors without a license.

Moreover, based on the results of this study on slush products, all samples contained artificial colors. Despite the permissible use of synthetic dyes in slush products (22), some of the slushed beverages used the unauthorized tartrazine dye. Alipour Hafshjani *et al.* (2015) investigated the prevalence of food

contamination with artificial colors. The results indicated that 33.8% of the total samples contained synthetic dyes and tartrazine with 67.8% was the most synthetic dye used in this study (23). The results of both studies showed that despite the proximity of the use of synthetic dyes, the use of the illegal dye tartrazine was lower in our study.

According to previous research, tartrazine synthetic dye can have harmful effects on human health, including causing allergic reactions such as urticaria, asthma, eczema, and itching in adults, and restlessness, bad mood, and hyperactivity in children. It can also cause purple spots, migraines, and inflammation of the nose and skin (21-23). One of the reasons for using this illegal dye may be the lack of awareness of the sellers about the type of colors used in the raw materials. Sometimes the raw materials are imported, which may contain the unauthorized synthetic dye tartrazine, which is allowed in some other countries. Numerous studies have been conducted in different cities on the colors used in various food products, all of which confirm the use of permitted and unauthorized synthetic colors in these products (24-30).

According to the results obtained from the analysis of artificial colors in traditional juices and drinks, it was found that due to attractiveness or profitability, a percentage of traditional juices and drinks in Shiraz, Iran contained artificial colors. The use of artificial colors in traditional beverages and juices showed that this is due to the poor health and safety knowledge of food retailers, the low price of artificial food colors, easy access, and ultimately the low level of supervision by health officials and the increase in the number of stores.

On the other hand, the use of artificial colors in these products can cause carcinogenicity, neurological disorders, genetic disorders, attention deficit hyperactivity disorder in children (ADHA), anaphylactic reactions, urticaria, allergies, weakened immune system, and other serious injuries (2, 8). Therefore, it should be noted that the use of these dyes has adverse effects on children's health, especially in long run in the human body, and can be a warning sign for human health. Due to the absence of the technical manager, lack of knowledge and information about the production instructions, and without having health licenses, some people in small workshops and unsuitable places produce unnatural juices, with a natural title and deceive the customer. This issue not only causes problems for society in terms of health but also in terms of justice.

Conclusion

As the use of these artificial dyes may be due to the lack of awareness of food manufacturers and suppliers, increasing awareness in various ways, including face-to-face and distance education can be effective in preventing this violation. Therefore, continuous monitoring and control should be done with more accuracy and intensity.

Acknowledgment

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Conflict of Interest

None declared.

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