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Determination of Synthetic Colors in Some Locally Available Foods of Kashan City, Iran

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Authors
Sharafati Chaleshtori R.* *PhD,*
Golsorkhi F.[1] *BSc*

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*Biochemistry & Nutrition in Metabolic Diseases Research Center, Kashan University of Medical Sciences, Kashan, Iran
[1]Food & Hygiene Control Laboratory, Deputy of Food & Drug, Kashan University of Medical Sciences, Kashan, Iran

Correspondence
Address: Qotb-e-Ravandi Avenue, Pezeshk Boulevard, Kashan University of Medical Sciences, Kashan, Iran. Postal Code: 8715988141
Phone: +983155540021
Fax: +983155541112
sharafati.reza@gmail.com

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ABSTRACT

Aims The synthetic colors are preferred by the food industry because of the variety of shades, intensity, uniformity, excellent solubility and stability. They are used in a variety of food products such as dairy products, beverages, baked products, confections and pet foods. Food colors may have toxic effects on the human body. This study aimed to examine the amount of synthetic colors in some ready to use foods in Kashan, Iran.
Instrument & Methods In this cross sectional study, conducted in Kashan City, Iran, in March to October 2015, a total of 52 samples of meat products, 33 samples of sweets, 43 samples of drinks and 21 samples of miscellaneous foods were collected. The coloring agents were extracted of samples and purified using the hydrochloric acid extraction method. Thin layer chromatography was used to analyze the samples.
Findings 72 samples (48.30%) contained no coloring and 77 samples (51.7%) contained artificial colors. The most coloring agents were in sweets (72.7%), drinks (51.2%) and meat products samples (48.10%). The quinoline yellow, tartrazine and sunset yellow were the most common coloring used in the various foods.
Conclusion About 52% of examined foods contained artificial colors that have been banned by the national Iranian standards organization.

Keywords Coloring Agents; Chromatography, Thin Layer; Food

CITATION LINKS

[1] Determination of 13 synthetic food colorants in water-soluble foods by reversed-phase high-performance liquid chromatography coupled with ... [2] Determination of synthetic food dyes in commercial soft drinks by TLC and ... [3] A review of the genotoxicity of food, drug and cosmetic colours and other azo, triphenylmethane and xanthene ... [4] Genetic damage induced by a food coloring dye (sunset yellow) on meristematic cells of Brassica campestris L. J Environ Public ... [5] Simultaneous determination of red and yellow artificial food colourants and carotenoid pigments in food ... [6] Surveillance on artificial colors in different confectionary products by chromatography in ... [7] Evaluation of food color consumption and determining color type by thin layer ... [8] US FDA "Redbook II" immunotoxicity testing guidelines and research in immunotoxicity evaluations of food chemicals and new food ... [9] Surveillance on artifical colours in different ready to eat ... [10] Anaphylaxis following ingestion of ... [11] Food anaphylaxis following ingestion of carmine [12] Synthetic colourings of some snack foods consumed by primary school children aged 8-9 years in ... [13] Estimates of dietary exposure of children to artificial food colours in ... [14] Assessment of synthetic dyes in food stuffs produced in confectioneries and restaurants in ... [15] Quantitative analysis, in vitro assessment of bioavailability and antioxidant activity of ... [16] Determination of 40 synthetic food colors in drinks and candies by high-performance liquid chromatography using a short column with photodiode array ... [17] Determination of synthetic dyes in selected foodstuffs by high performance liquid chromatography with UV-DAD ... [18] Determination of food dyes in soft drinks containing natural pigments by liquid chromatography with minimal ... [19] Qualitative identification of permitted and non-permitted color additives in ... [20] Trace analysis of Ponceau 4R in soft drinks using differential pulse stripping voltammetry at SWCNTs composite electrodes based on ... [21] Determination of magnesium in foods by single-sweep ... [22] Simultaneous spectrophotometric determination of synthetic dyes in food samples after cloud point extraction using multiple response ... [23] A simple and compact fluorescence detection system for capillary electrophoresis and ... [24] Permitted food additives - Food colors list and ... [25] Identification and determination of seven synthetic dyes in ... [26] Permitted food addetive-synthetic food ... [27] Study of concentration of ... [28] Determination of eight synthetic dyes in ... [29] Synthetic colours in some ... [30] Ottawa, Ontario: Canada Health ...

Introduction

Synthetic food colorants are a very important class of food additives. They are widely used for maintenance and improvement of color appearance and to compensate for the loss of natural colors of foods that destroyed during processing and storage, and to provide the desired colored appearance. However, some of these substances create a potential risk to human health, especially in children if they excessively are consumed [1]. The effects of synthetic colors are due to azo (N=N) functional groups and aromatic ring structures [2]. Some studies have reported about genotoxicity of food, drug and cosmetic azo dyes and other dyes [2-5].

There is little information about toxicity of food colors on human [6]. Synthetic colors may cause to various health problems such as asthma, hyperactivity of children, negative effects on children's IQ, anaphylactic reactions, urticaria, abortion, carcinogenicity, idiosyncrasy, hypertension, weakening of the immune system, decrease the WBC and lymphocyte count, sleeping disorders and vitamin B6 deficiency [7, 8]. They also effect on mitochondrial respiration, hepatic and renal function and T3 and T4 production [8]. Ponceau 4R and sunsets yellow have also been implicated in adverse reactions in patients with chronic urticarial [9]. However, the health hazards due to consumption of natural food colors have been reported by FAO/WHO in 1994. Anaphylactic reactions after consuming Annatto as a natural color were shown by certain individuals [9]. Also, anaphylactic reactions after consumption of carmine that extracted from the cochineal insects were reported in women at a dose of 1mg/kg body wt. [10, 11]. Lok *et al.* demonstrate that dietary exposure to synthetic colors for an average primary school student was considerably lower than the threshold for acceptable daily intake (ADI) for their ages, except for sunset yellow. They have shown that the average daily intake of sunset yellow was 51% over the ADI threshold in 9-year old boys. The higher intakes of sunset yellow were mainly due to the high consumption of soft drinks and desserts such as jellies, which have high concentrations of this synthetic color [12]. In addition, Hussain *et al.*, indicate that intake of tartrazine, sunset yellow, carmoisine and allura red were exceeded their ADIs in 5-14 year old children in the State of Kuwait [13]. In Iran, Rezaei *et al.*, have shown the sunset yellow (60%), tartrazine (57.1%), quinoline yellow (44.28%), azorubine (28.57%), ponceau 4R (8.57%) and allura red (2.8%) in confectioneries and restaurants products in Arak [14].

In recent years, natural food colors such as curcumin, anthocyanins and β-carotene have been increasingly used for the consumer preference. The lutein, β-carotene and lycopene control the yellow, orange and red colors in many fruits and vegetables. They have been confirmed to possess some health benefit functions, such as supplying vitamin A to the body, reducing the damage of the eye retina from exposure to near-ultraviolet light, preventing the risk of prostate cancer, etc. [5, 15]. However, they are relatively unstable and the costs are higher than synthetic food colors [16]. Synthetic dyes have several advantages such as relatively lower production costs, color uniformity, high stability to light, oxygen and pH, low microbiological contamination, etc. [17].

The synthetic colors added to a variety of foods such as drinks, sweets and etc., have exceed the authorized levels. For example, the maximum level of sunset yellow, tartrazine and brilliant blue dyes should not be more than 10mg/100ml (individually) in non-alcoholic beverages, and 5mg/100ml to Amaranth [2]. Monitoring of the levels of dyes in high consumption products becomes therefore of paramount importance [18]. There are many analytical methods for determination food colors, such as thin layer chromatography (TLC) [19], voltammetry [20], polarography [21], spectrophotometry [22], capillary electrophoresis (CE) [23], and high-performance liquid chromatography (HPLC) [16].

Many food colors are controlled or even forbidden in many countries of the world. Following international regulations, the Institute of Standards and Industrial Research of Iran (ISIRI) imposed limits of concentrations for several food colors in variety foodstuffs in Iran [24]. Only quinoline yellow (E-104), sunset yellow (E-110), azorubine (carmoisine) (E-122), ponceau 4R (E-124), allura red (E-129), indigotine (E-132) and brilliant blue (E-133) are allowed in food [24]. Figure 1, shows the acceptable daily intake

(ADI) values synthetic dyes for humans approved by Iranian National Standardization Organization and also demonstrates countries prohibiting these dyes [24]. Accordingly, Green S, orange RN, yellow 2G and red 2G are banned from use in foods in the United States. As of January 1986, amaranth is not permitted in foods in the United States. However, amaranth is permitted in foods in Canada [22, 25].

The lack of studies about the presence of synthetic colors in foodstuffs produced in Kashan city (Iran), this study aimed to examine the amount of synthetic colors in some ready to eat foods in Kashan, 2015.

Instrument & Methods

Sampling

This is a cross sectional study, conducted in Kashan, Iran. The city was divided into three areas, and the samples were collected equally from each area in March to October of 2015. Total of 149 food samples including drinks (n=43), meat products (n= 52), sweets (n=33) and miscellaneous foods (n=21) were taken randomly from different super markets in the city.

Chemicals and reagents

Samples of ten synthetic colors (sunset yellow (E-110), quinoline yellow (E-104), ponceau 4R (E-124), allura red (E-129), azorubine (E-122), tartarazine (E-102), brilliant blue (E-133), amaranth (E-123), red 2G (E-128) and orange RN) were purchased from Merck; Germany.

- Chromatography tank (27 × 7 × 27) cm and lid as well as glass capillary tubes.
- Thin Layer Chromatography (TLC) plate-silica gel (20×20cm; Merck; Germany) as stationary phase.
- Acid acetic, n-butanol (Merck; Germany) and distilled water as mobile phase.

Color extraction

For extraction of dyes in confectionary, meat and milk products (foods contained starch), about 10-15g of the sample in Erlen-mayer flask were mixed with 100ml of 2% ammonium in ethanol 70%. After 24 hours, supernatant liquid was picked and the precipitate was removed. Then, the solution was poured into a beaker and its water evaporated on a Bain-Marie bath (100°C) about 1 hour. Refer to similar study 20ml sample was used directly for liquid foods as drinks [14]. Afterwards, 80ml distilled water and 1ml concentrate hydrochloric acid (37%HCl) were added. Pure white wool was added to the acidified color extract and placed on a Bain-Marie bath (100°C) about 1 hour. The colored wool was removed and washed well with cold tap water. Then, the washed wool was transferred to a beaker containing l% (v:v) ammonia solution and placed on a Bain-Marie bath (100°C) about 30min for extraction the colors. The wool was removed and the solution obtained was dried on a Bain-Marie bath (100°C) [14, 26].

Thin layer chromatography analysis (TLC)

Acid acetic, n-butanol and distilled water 5:10:6 (v:v:v) as mobile phase were poured into the chromatography tank to a depth of about 2cm. Then, the TLC tank was covered by a lid. Afterwards, the silica gel was activated in an incubator with the temperature of 90-100°C for 8-10min and allowed to be cooled. The TLC plate was marked using a soft lead pencil so that the distances baseline was 3cm from the bottom of the plate. Then, small amount of the separated color solution was labeled on the plate by a clean capillary tube. At least 1.5cm was left between spots. The stained plate was placed in the TLC tank and put the lid on the tank. When the solvent front approaches almost 4cm to the upper end of the plate, the plate was taken out of the tank and let dry completely under a hood [26].

Figure 1) Acceptable daily intake (ADI) values synthetic dyes for humans, approved by Iranian National Standardization organization (ISIRI) and countries prohibiting these dyes

Synthetic color	Color	Maximum ADI (mg/kg of bw)	Use prohibition
Quinoline Yellow	Yellow	5	Canada, USA
Sunset Yellow	Yellow	4	Norway
Azorubine (Carmoisine)	Red	4	USA, Canada, Japan, Norway, Sweden
Ponceau 4R	Red	4	USA, Norway
Allura Red	Red	7	Denmark, Belgium, France, Switzerland
Indigotine	Blue	5	Denmark, Belgium, France, Norway, Switzerland, Sweden
Brilliant Blue	Blue	12/5	Belgium, France, Germany, Switzerland, Sweden, Austria, Norway

To determine the colors, the retention factors (Rf) was calculated using: distance travelled from the baseline by the food coloring/distance travelled from the baseline by the solvent. The position of each spot was taken from its center. Then, Rf obtained samples compared with Rf standard colors [26]. Data were analyzed using SPSS 16.

Findings

72 samples (48.30%) showed no synthetic colors and 77 samples (51.7%) contained artificial colors that have been banned by the national Iranian standards organization. Sweets (72.7%) and drinks (51.2%) samples contained the most prohibited colors. (Figure 2).

Tartarazine (n=11), quinoline yellow (n=11) and mixt of sunset yellow with quinoline yellow (n=12) were the most frequently consumed colors in various foods. Also, 10 samples contained other synthetic colors that were not identified. Amaranth was not detected in samples. But brilliant blue with amaranth (n=1 out of 43) and azorubine with sunset yellow and amaranth (n=1 out of 43) were found in drinks (Figure 3).

Figure 2) Frequency (the numbers in parentheses are percentage) of synthetic dyes in samples of drinks, meat products, sweets and miscellaneous in examined foods

Type of sample	Artificial colors	No Artificial colors
Drinks (n=43)	22 (51.2)	21 (48.8)
Meat products (n=52)	25 (48.1)	27 (51.9)
Sweets (n=33)	24 (72.7)	9 (27.3)
Miscellaneous foods (n=21)	6 (28.6)	15 (71.4)
Total (n=149)	77 (51.7)	72 (48.3)

Figure 3) Frequency of occurrence of various synthetic colors identified in examined foods

Various synthetic colors	Drinks (n=43)	Meat (n=52)	Sweets (n=33)	Miscellaneous foods (n=21)	Total (n=149)
Azorubine	1	1	1	0	3
Sunset Yellow	0	0	3	1	4
Brilliant Blue	1	0	0	0	1
Tartarazine	3	3	3	2	11
Quinoline Yellow	3	5	2	1	11
Ponceau	1	0	0	0	1
Red 2G	0	0	1	0	1
Allura Red	0	1	0	0	1
Orang RN	0	1	0	0	1
Sunset Yellow+Quinoline Yellow	1	10	1	0	12
Sunset Yellow+Tartarazine	3	3	2	2	10
Brilliant Blue+Quinoline Yellow	0	0	3	0	3
Quinoline Yellow+Red 2G	0	0	1	0	1
Brilliant Blue+ Amaranth	1	0	0	0	1
Sunset Yellow+Quinoline Yellow+Azorubine	0	0	2	0	2
Sunset Yellow+Quinoline Yellow+ Red 2G	0	0	2	0	2
Sunset Yellow+Tartarazine+Azorubine	0	0	1	0	1
Azorubine+Sunset Yellow+Amaranth	1	0	0	0	1
Other synthetic color	7	1	2	0	10

Discussion

This study aimed to examine the amount of synthetic colors in some foods products in Kashan, Iran. The results indicated that more than half of the samples (51.7%) could not be used, due to application of artificial colors.

In similar study, Arast *et al*. report that 48% of different confectionary products were contained artificial colors [6]. Also, Soltan Dallal *et al.* have declared among 336 juice samples produced in Tehran, 237 samples were contained authorized artificial coloring and 62 samples included unauthorized colorings [27]. Farzianpour *et al.* show 48.47% of synthetic colors in poolak, pastry and rock candy [7]. Moreover, Ashfaq & Masud have demonstrated that 47.56% of the samples (sweet meats and confectioneries) were contained non-permitted food colors [9]. Our results showed that 72.7% sweets and 51.2% drinks were contained artificial colors.

Using green liquid chromatography, Hajimahmoodi *et al.* have determined eight synthetic dyes in four different foods (cookies, colored rice, saffron and fruit juice). Their results show that only 7.5% of cookies, 30% of colored rice, 8% of saffron and 12% of juice samples were in compliance with Iranian National Standards. Tartrazine was reported as the most prevalent food colors in their analyzed samples [28]. The results have been supported by Lim's study as well [29].

It should be noted that the samples contained artificial colors were not approved for consumption, since the Ministry of Health has forbidden using any type of artificial color in facilities such as sweets and drinks [30].

The limitations of this study were small sample size, not identify the recipient of the food groups and also, cross-reactions between the additives and the substances which contaminate foodstuff naturally, such as those occur between salicylates and tartrazine.

It seems, provide the manufacturers and restaurant owners with food safety training can reduce the use of artificial color and guarantee the health of society. It is necessary to extent the quality control of ready to eat foods, restaurant and confectionery products by detecting the synthetic colors added. Finally, for future studies, the authors suggest to consider the dietary of different age's groups which expose to synthetic colors.

Conclusion

51.7% of examined foods contained artificial colors that have been banned by the national Iranian standards organization.

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Conflicts of Interests: Authors have no conflict of interests.

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