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Evaluation of natural and synthetic dyes in saffron barbecued chicken collected from meat shops and restaurants in Babol using thin-layer chromatography

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ABSTRACT

Synthetic colors have advantages in comparison to natural colors, but they cause cancer, attention-deficit hyperactivity disorder, immune system suppression and vitamin deficiency. In the present study, authorized and unauthorized synthetic colors in saffron barbecued chicken from meat shops and restaurants in Babol city have been identified by thin-layer chromatography. Fifty samples of saffron barbecued chicken from meat shops and restaurants in Babol city have been collected from July to September 2019. Out of 50 samples, 20 had natural color, while 27 and 3 samples contained authorized and unauthorized synthetic colors respectively. According to the Institute of Standards and Industrial Research of Iran, the application of synthetic colors is banned in saffron barbecued chicken. Therefore, 40% were consumable and 60% were inconsumable, out of which 54% contained quinoline yellow and 6% had tartrazine. About 58.06% and 63.15% of samples from meat shops and restaurants were inconsumable, respectively. There were no significant differences regarding the frequency of the evaluated colors between samples from meat shops and restaurants ($p>0.05$). Based on the results, it is essential that the presence of synthetic colors is traced constantly and more strictly in food products and that the perpetrators receive more serious punishment.

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1. Introduction

Food additives are substances that are intentionally added to food during production, processing, packaging, transportation and storage to increase shelf life, improve organoleptic properties and nutritional value (1).

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Food colors are added to color the discolored foods or to color foods that have lost their color during heat treatment or exposure to light, air, and moisture (2). These materials are also used to create color uniformity in all products of a food product and to enhance the color of food to motivate the consumer.

In addition, food color usually indicates food quality and safety. Food colors are divided in two groups of natural and synthetic colors (3,4).

Natural dyes are obtained from minerals, animals and plants such as anthocyanins, betanine, beta-carotene and chlorophyll (5,6).

Saffron is one of the most important natural colors that its anti-cancer properties have also been proven (1,7). Saffron is the flower of the Crocus sativus plant which has a yellow-orange color and a pleasant smell (8,9).

Its coloring properties are attributed mainly to water-soluble carotenoids derived from crocins (10). Dyeing rice and chicken meat using saffron has been common in Iran but its high price has caused some profiteers to use artificial colors instead of saffron. Saffron is the only permitted color for use in barbecued chicken (11).

Synthetic dyes are water soluble and acidic (1). These dyes can be from azo, quinoline, xanthene, triaryl methane and indigo groups and are produced from coal tar during chemical processes (12). Synthetic colors are made in the form of granules, powders, gels, pastes and liquids. These dyes are used in beverages, confectionery, ice cream, jellies, meat and dairy products (4,7).

Artificial colors have advantages over natural colors. For example, these colors are cheaper, easier to use, faster to coloring, more resistant to heat, light, pH and oxygen, and their effects last longer. But artificial colors cause cancer, attention deficit hyperactivity disorder, behavioral - neurodevelopmental disorders, impulsive behavior, skin problems, headache, nausea, weakness, obesity, asthma, diabetes, immunosuppression, vitamin deficiency, allergic reactions (13), pulmonary and gastrointestinal obstruction, genome toxicity in human lymphocytes (5), abortion, decreased IQ in children, anaphylactic reactions (14), inhibition of dopamine uptake by nerve terminals and sleep disorders (12).

Quinoline yellow (E104, FD & C Yellow 10) is an artificial yellow dye that is allowed to be used in the food according to Iranian Standard No. 740 (15,16). Quinoline yellow can cause asthma, skin rashes and lower blood pressure in humans (17).

Tartrazine (E102, FD & C Yellow 5) is an azo-orange dye that is soluble in water (18). Some of the adverse effects of this dye include urticaria, hyperactivity, skin rashes, aspirin intolerance, adverse effects on behavioral and cognitive behavior, toxicity to the reproductive system, DNA damage in gastrointestinal cells, increased number of eosinophils and lymphocytes in mice (19). The use of tartrazine in food products has been banned by the Iranian Standard Organization (16).

Some of the analytical methods for color detection in food are available such as high performance liquid chromatography (HPLC), electrochemical sensor, thin-layer chromatography (TLC), spectrophotometry, capillary electrophoresis and liquid chromatography-tandem mass spectrometry (LC-MS) (6). Thin-layer chromatography is one of the best methods for the qualitative analysis of food colors. TLC is the best choice for the qualitative analysis of food dyes as it gives reasonable results in a very short time span and requires less complex apparatus and methodology as compared to other chromatographic techniques. TLC is a cheap and accurate method and a small amount of soluble material is used. It can also evaluate several samples simultaneously (5).

Several studies have been conducted on food colors in various food products in Iran. But there is little information about artificial colors in saffron barbecued chicken in northern Iran. The present study identifies synthetic colors in saffron barbecued chicken from meat shops and restaurants in Babol using thin-layer chromatography.

2. Materials and Methods

2.1. Solutions and materials

Standard quinoline yellow and tartrazine colors were purchased from Sigma (Sigma-Aldrich Co., St. Louis, MO, USA). Glacial acetic acid, ammonium hydroxide and butanol were purchased from Merck (Darmstadt, Germany).

2.2. Sample Collection

A total of 50 samples of saffron barbecued chicken were collected randomly from meat shops and restaurants in Babol from June to September in 2019. The formula for sample size determination in cross-sectional studies has been used. Prevalence was considered 74%, confidence interval equals to 95% and precision was 0.1 (10). Samples were divided into 3 groups based on the identified color: Natural dye (saffron), permitted synthetic dye (quinoline yellow) and unauthorized synthetic dye (tartrazine). According to the national standard of the country, only samples that have natural colors can be consumed and samples containing permitted and unauthorized synthetic dyes are unusable (11).

2.3.Color Extraction

Extraction of food colors was done according to the standard method of Iran (20). Five grams of crushed saffron chicken samples with 80 mL of water were put in a 100 mL glass beaker. One mL of acetic acid was added to the solution and mixed well. Then white wool was put in the beaker and heated for 1 h. Due to the acidic condition, synthetic colors and wool proteins have negative and positive charges, respectively, and the dyes are absorbed by the wool (4). The white wool is then washed with cold water to remove food particles. White wool was heated with 30 mL of distilled water and 1 mL of ammonium hydroxide in a beaker. After heating the solution, the artificial food dyes are separated from the wool and introduced into the solution. Heating is continued until all the solution has evaporated and only the synthetic dye remains. If the solution is colorless at this stage, no further steps are required and the sample is reported without artificial color.

2.4.Thin Layer Chromatography Analysis

Silica gel plates (25 × 25 cm) were activated by heating in an oven at 90 to 100°C for 8 to 10 min to remove moisture. Activated silica gel acts as a stationary phase. A few drops of water were added to the sample to dissolve the dried dye. The plate was stained by the samples from a distance of 3 cm at the end and intervals of 1.5 to 2 cm. Colors should not be more than 0.5 mm in diameter. Standard quinoline yellow and tartrazine dyes were also stained on the plate for comparison and identification. The color stains were dried by a hairdryer. TLC tank was thoroughly washed and 100 mL of moving phase (butanol, acetic acid and distilled

water (10: 5: 6 v/v/v)) was added to the tank. The TLC tank was then covered with a lid and the silica gel plate was placed vertically in the TLC tank. The moving phase on the plate with the capillary property moves upwards and the sample spots move in the same direction (20). When the solution moves 15 cm, the plate is removed from the tank and is examined. The colors are identified by comparison of samples movement with standard color movements.

2.5. Statistical analysis

Data analysis was performed using SPSS software version 22. Chi square test was used to determine the frequency difference between natural and artificial colors among saffron barbecued chicken samples obtained from meat shops and restaurants. In all analysis, a significance level of less than 5% was considered.

3. Results

The frequency of artificial colors in saffron barbecued chickens distributed in Babel is shown in Figure 1. Among the 50 samples of saffron barbecued chicken, 20 samples had natural colors, while 27 samples contained permitted synthetic colors and 3 samples contained unauthorized synthetic colors. According to the national standard of the country, the use of any kind of artificial color in the production of saffron barbecued chicken is not allowed. Therefore, 40% of the samples can be consumed, while 60% were unusable. Among the non-consumable samples, 54% had quinoline yellow and 6% had tartrazine.

According to table 2, there is no significant difference in the frequency of natural color, permitted artificial color and unauthorized artificial color among saffron barbecued chicken samples from super meat and restaurants ($p>0.05$). Thirty-one barbecued chicken samples were taken from meat shops of which 41.93% were consumable and 58.06% were inconsumable. Nineteen barbecued chicken samples were taken from restaurants of which 36.84% were consumable and 63.15% were inconsumable.

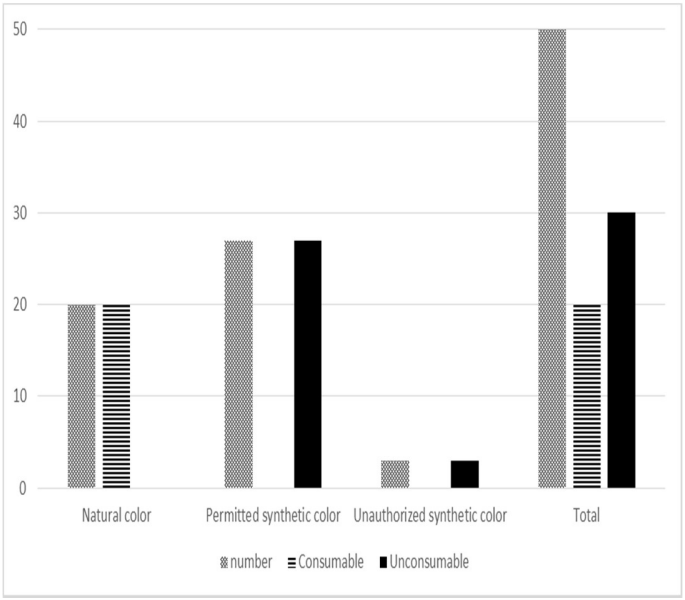

Figure 1. Distribution of food colors in saffron barbecued chickens offered in Babol

Table 1. The frequency of natural and artificial colors in saffron barbecued chicken obtained from meat shops and restaurants in Babol

	Natural color	Permitted color	Unauthorized artificial color	Consumable (%)	Inconsumable (%)
Meat shops (31 sample)					
Number of positive	13	17	1	13	18
samples	41.93	54.83	3.22	41.93	58.06
Percentage of positives					
Restaurants (19 sample)					
Number of positive	7	10	2	7	12
samples	36.84	52.63	10.52	36.84	63.15
Percentage of positives					
P-value	0.721	0.879	0.291	0.721	0.721

4.Discussion

A study on barbecued chicken in the central part of Iran showed that 25.62% had saffron and 74.38% had other food dyes. Quinoline yellow was detected in 17.65% of restaurants and 25% of food industries samples. Tartrazine was detected in 11.03% of restaurant barbecued chicken samples, which is in accordance with the results of the present study (10).

In another study, a higher percentage of permitted and unauthorized colors (18.18 and 81.81%) was reported in barbecued chickens in Nazarabad (21). Fifty two percent of saffron solutions, saffron rice and saffron barbecued chicken in Tehran had at least one artificial color. The most commonly used dyes were tartrazine, quinoline yellow, and sunset yellow. About 13.4% of saffron barbecued chickens had at least one artificial color (9).

While in the present study, 60% of the barbecued chickens distributed in Babol had artificial colors.

About 7.1% of raw and cooked barbecued chickens in Shahrekord had artificial colors and 59.5% of saffron solutions had artificial colors. Tartrazine was detected in 7.1% of barbecued chickens and 48.6% of saffron solutions. Low price, stability and color similarity to saffron are the reasons for using tartrazine (22).

In a study conducted on saffron solutions in Arak, it was shown that 86.66% of the samples had artificial colors that were banned by the Standard Institute. About 57.1% and 44.28% of cookies, ice cream and saffron solutions contained tartrazine and quinoline, respectively (23).

Confetti and rock candy in Shahrekord had unauthorized and authorized colors at the rate of 6.52% and 34.73%, respectively. About 41.25% of the samples were unusable. Quinoline yellow and tartrazine were detected in 32% and 1.7% of the samples. The reasons for using this amount of artificial colors are the disadvantages of natural colors and the lack of facilities to measure small amounts of artificial colors (1).

Most beverage samples were identified as having authorized color brands, while unauthorized food colors were found in local food samples, which adversely affected consumer health (4). Tartrazine is used in beverages such as orange juice and other foods, medicines and especially in foods that contain saffron. 0.15 mg tartrazine causes an acute asthma attack that occurs due to Immunoglobulin E and prostaglandin synthesis (1). Anaphylactic purpura and false allergy have been reported due to the use of tartrazine in food (12). Tartrazine at a dose of 2000 mg/kg causes DNA damage in mice clone after 24 h of administration (24). About 5.8% of foods and beverages in Sri Lanka had unauthorized artificial colors and 85% had permitted artificial colors. Tartrazine was detected in 55.83% of the samples. Only 9.2% of the samples did not have artificial colors and therefore were consumable. While in the present study, 40% of barbecued chicken was consumable (12).

Avazpour et al. (2013) investigated the use of food coloring at confectionary products in Ilam, Iran. 91 samples (50.55%) contained permitted synthetic colors and 42 (23.33%) contained natural colors. Quinoline yellow was detected in 20% of the products. Manufacturers have used quinoline yellow and sunset yellow to replace turmeric, orange juice, pineapple juice and saffron (2). About 26% of confectionery products in Turkey contained tartrazine. The reason for the difference between the results of this study and the present study can be attributed to the permissibility of tartrazine dye in Turkey (25). Similar to the results of the current study, researchers showed that only 39% of confectionery products in India did not contain artificial colors in 2003 (26).

5.Conclusion

The results of the present study showed that the frequency of permitted artificial colors in saffron barbecued chicken in Babol was high which is considered a fraud. Therefore, the presence of artificial colors in food should be examined more strictly and continuously, and stricter penalties should be set for fraudulent production units. Moreover, incentives should be set for production units that comply with regulation and increase the awareness of producers and consumers about unauthorized colors and food that contain colors above the permitted limits. According to the national standard No. 740, the permissible daily intake for the permitted synthetic quinoline yellow dye is defined as a maximum of 5 mg/kg body weight (16). The use of permitted colors in all types of processed barbecued chickens is prohibited, and violators usually use large amounts of such colors to color the final product, regardless of the allowed daily amount. It is suggested to evaluate the artificial colors present in the supply of barbecued chicken quantitatively in future studies in order to prevent possible harm to consumers. In this study, the frequency of unauthorized tartrazine dye was low, which can be attributed to the fact that

consumers and manufacturers are aware of the health problems caused by unauthorized dyes or the precise monitoring carried out by health organizations.

Conflict of interest

The authors have no conflict of interest to declare.

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