Simultaneous determination of saffron and synthetic dyes in ready-to-cook Iranian barbecued chicken by HPLC

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ABSTRACT

Saffron, the dry stigmas of the plant *Crocus sativus* L., is widely used as a natural food additive for coloring and flavoring of foods. Because of high price, the coloring of food with synthetic food dyes are widely used by food industries and restaurants instead of saffron. This study was carried out to develop a method for extraction and simultaneous determination of saffron, Tartrazine (E102); Quinoline Yellow (E104) and Sunset Yellow (E110) in ready-to-cook Iranian barbecued chicken. During the year 2018, a total of 160 ready-to-cook barbecued chicken samples, including 136 samples from restaurants and 24 samples from food industries were collected from the central part of Iran. The results showed that only 41 samples (25.62%) colored with Saffron and 119 samples (74.38%) colored with other food dyes. The detection rates of synthetic dyes in the samples were 10.0% for Tartrazine, 25.62% for Sunset Yellow, and 18.75% for Quinoline Yellow. The occurrence of Tartrazine was significantly higher \((P < .05)\) in samples of restaurants than those from food industries, while the occurrence of Sunset Yellow and Quinoline Yellow was significantly higher \((P < .05)\) in samples obtained from food industries compared to those obtained from restaurants. In conclusion, the presence of illegal synthetic dyes in ready-to-cook barbecued chicken can be a potential hazard for the health of consumers. Further investigations should be carried out to determine illegal synthetic dyes in other foodstuffs.

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Introduction

Food color additives are any dye, pigment or substance that imparts color when added to foods. Natural and synthetic colors are two major categories of food colors.\(^1,2\) Natural food colorants (e.g. curcumin, saffron, and beta carotene) obtained from vegetable or animal origins by derivation procedures. Synthetic food colorants (e.g. Tartrazine, Sunset Yellow, and Quinoline Yellow) are manufactured by chemical reaction and are commonly used as food additives in food industries.\(^3,4\)

Saffron is one of the most expensive food additives obtained from the dry stigmas of the plant *Crocus sativus*. Saffron is known for the yellow color of its aqueous or alcoholic extracts. This spice is widely used as a natural food additive for coloring and flavoring of foods and beverages, and its coloring properties are attributed mainly to water-soluble carotenoids derived from crocetin metabolites named crocins.\(^5–7\)

In Iran, barbecued chicken as a traditional meat product is one of the most popular and delicious ready-to-cook foods in which saffron is used for coloring and flavoring.\(^8\) Unfortunately, since saffron is an expensive natural food additive, the coloring of barbecued chicken with synthetic food dyes are widely used by food industries and restaurants instead of saffron.\(^9,10\) Some of these substances and their metabolites create potential risk to human health and may even be carcinogenic, particularly if consumed in large amounts. Tartrazine (E102), Quinoline Yellow (E104), and
Sunset Yellow (E110) are three synthetic food dyes that can make yellow color like saffron. The toxicity of these dyes for human (e.g. carcinogenicity and genotoxicity), have reported in several studies.\textsuperscript{[11–13]}

European Union controlled the use of synthetic dyes in foods rigorously by legislation.\textsuperscript{[14]} The Institute of Standards and Industrial Research of Iran have banned the use of Tartrazine in food products and set the national legal limit for Quinoline Yellow and Sunset Yellow in some food products.\textsuperscript{[15]} According to Iranian veterinary organization, saffron is the only food color additive in ready-to-cook barbecued chicken; and the use of other natural or synthetic colorants is banned in this type of food.\textsuperscript{[16]}

Several analytical methods including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC–MS) have been developed to determine dyes in saffron solutions.\textsuperscript{[17–19]} However, no analytical method has been developed to determine saffron extracted from tissues. Therefore, this study aimed to develop a method for extraction and determination of saffron and three synthetic yellow dyes from ready-to-cook Iranian barbecued chicken. Moreover, the occurrence and levels of the mentioned colorants were determined in ready-to-cook barbecued chicken samples obtained from the central part of Iran.

Materials and methods

Standard and reagents

Tartrazine (E-102), Quinoline Yellow (E-104), Sunset Yellow FCF (E-110) and crocin standards were purchased from Sigma (Sigma-Aldrich Co, St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade), ammonium hydroxide and ammonium acetate were acquired from Merck (Darmstadt, Germany). Deionized water was prepared by using the Milli-Q water system (Millipore, Bedford, MA, USA). Stock standard solution of each colorant (100 mg/l) was prepared in deionized water and stored at 4°C in the dark.

Sample collection

During year 2018, a total of 160 barbecued chicken samples, including 136 samples from restaurants and 24 samples from food industries were obtained randomly from the central part of Iran. The samples were transported to the laboratory inside an icebox and stored at –20°C until analyses.

Sample extraction and clean-up

Saffron and synthetic dyes from barbecued chicken were extracted with a mixture of methanol and 0.02 M ammonium acetate (i.e. 1:1, v/v) with a few drops of 10% ammonium hydroxide. A portion of the chicken sample (5.0 g) was accurately weighed and dissolved in 10 ml of the extracting solution in a centrifuge tube and thoroughly vortexed for 1 min. The mixture was sonicated for 10 min and centrifuged for 5 min at 4000 rpm at room temperature (20°C). The supernatant was transferred to another tube and the extraction procedures were repeated one more time. The combined extract was made up to 30.0 ml with an extracting solution and filtered through a folded filter paper. The extract was filtered through a 0.45 μm PVDF syringe filter before HPLC analysis.

High-performance liquid chromatography analysis

HPLC analyses were carried out with an Agilent 1260 Infinity HPLC system (Agilent Corporation, USA) equipped with a vacuum degasser, a quaternary pump, an analytical guard column (ZORBAX Eclipse XDB-C18, 5 μm particle size, 250 mm 4.6 mm i.d.; Agilent Corporation, USA), and
a G1315D diode-array detector (DAD) was used for chromatographic analysis. The DAD was set at an absorbance wavelength of 250 and 440 nm for simultaneous detection of synthetic dyes and crocins, respectively. The instrument control and data processing were conducted by using ChemStation software. The mobile phase consisted of ammonium acetate solution (0.1 M, pH 6.7) as solvent A and methanol as solvent B using a gradient elution primary with 10% of solvent B, increased linearly to 80% in 40 min, and held at 80% for 5 min. The flow rate was 1.0 mL/min and a total run time was 45 min. The injection volume of the sample or standard solutions was 20 µl.

**Methods validation**

Validation of the method for determination of saffron and synthetic dyes was based on international guidelines described by Yoshioka and Ichihashi (2008).\(^{[20]}\) Limits of detection (LOD) and limits of quantification (LOQ) were calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively, for evaluation the sensitivity of the method. A six-point calibration curve was constructed using different concentrations (1–20 mg/l) of each colorant to check the linearity. Inter-day repeatability of the method was determined by analyzing the spiked-barbecued chicken samples on six different days and Intra-day precision was assessed by analyzing six spiked samples during 1 day.

**Statistical analyses**

Statistical analyses were performed using chi-square test of the SPSS software version 20 for windows (SPSS Inc., Chicago, IL, USA) to evaluate the differences of distribution between the locations. The differences were considered significant at \( P < .05 \).

**Results and discussion**

The HPLC chromatograms for the mixture of color standards and barbecued chicken samples contained analyzed colors are presented in Figure 1. According to the validation procedure, the accuracy of the method was evaluated as recovery, which is determined by analyzing of blank samples spiked with known amounts of colorants. The mean recoveries were in the range of 86.8–90.2% for saffron, 87.8–91.0% for Tartrazine, 84.6–90.1% for Quinoline Yellow, and 88.5–89.3% for Sunset Yellow. The LODs were 0.11 mg/kg for saffron, 0.15 mg/kg for Tartrazine, 0.10 mg/kg for Sunset Yellow, and 0.13 mg/kg for Quinoline yellow. The inter-day RSD for analyzed dyes in spiked samples ranged from 3.7% to 7.2%, and the intra-day RSD ranged from 4.2% to 9.9% (Table 1). Both inter-day and intra-day RSDs and recovery values were acceptable.

In Iran, TLC method is a common method to determine various dyes in foodstuffs. However, this method is not suitable to determine saffron adulteration because of the overlapping of crocin and adulterant spots. However, the HPLC method can be an efficient method to detect saffron adulteration due to the separation of crocins from other synthetic dyes.

The occurrence and levels food colorants in barbecued chicken samples obtained from the central part of Iran are shown in Table 2. Synthetic dyes were detected in 68 samples (50.0%) obtain from restaurants and 19 samples (79.17%) obtain from food industries. The most prevalent colors were Sunset Yellow (25.62%) and Quinoline Yellow (18.75%), at mean values of 20.09 ± 1.522 and 19.08 ± 1.858 mg/kg for restaurants and 19.08 ± 1.858 and 32.81 ± 6.852 mg/kg for food industries, respectively. In the samples obtained from restaurants, 24 samples (17.65%) colored with Quinoline Yellow, 29 samples (21.32%) with Sunset Yellow, and 15 samples (11.03%) with Tartrazine. Considering the samples obtained from food industries, 6 samples (25.0%) colored with Quinoline Yellow, 12 samples (50.0%) with Sunset Yellow, and 1 sample (4.17%) with Tartrazine. The saffron was detected in 40 samples (29.41%) obtained from restaurants and 1 sample (4.17%) obtained from food industries. The occurrence of Tartrazine was significantly higher \(( P < .05 )\) in samples of restaurants than those from food industries, while the occurrence of Sunset Yellow
Figure 1. Chromatographic response for (a) saffron (crocins) and (b) mixed synthetic dyes standard solution (10 mg/l). (c, d, e, f) HPLC chromatogram of barbecued chicken samples. 1-crocin 1, 2-crocin 2, 3-Tartrazine, 4-Quinoline Yellow 1, 5-Sunset Yellow, 6-Quinoline yellow 2.
and Quinoline Yellow was significantly higher \( (P < .05) \) in samples obtained from food industries compared to those obtained from restaurants (Table 2).

The addition of illegal synthetic dyes to foodstuffs can be a potential hazard for the health of consumers. In this regard, few surveys have been conducted in Iran to assess the occurrence of illegal synthetic dyes in various foodstuffs.\(^{21-23}\) Sayadi et al. found that 43.8% of food samples obtained from restaurants located in Fasa city of Iran contained synthetic dyes such as Tartrazine and Sunset Yellow.\(^{24}\) In another study performed in Kashan city of Iran, artificial colors were detected in sweets (72.7%), drinks (51.2%), and meat products (48.1%); and Quinoline Yellow, Tartrazine, and Sunset Yellow were the most common colors.\(^{25}\) Moradi-Khatoonabadi et al. determined synthetic dyes from saffron chicken, saffron solutions, and saffron rice samples obtain from restaurants and found that 52% of samples were positive for at least one synthetic dye; and the prevalent dyes were Tartrazine (44%), Quinoline Yellow (9.1%), and Sunset Yellow (8.4%).\(^{26}\) In a survey conducted in Arak city of Iran, synthetic illegal dyes such as Sunset Yellow, Tartrazine, Quinoline Yellow, Azorubine, and Allura Red were detected in 56 out of 70 samples (80%) of cookies, ice cream, and saffron aqueous solutions obtained from confectionaries and restaurants.\(^{27}\) In Hong Kong, the occurrence of synthetic colors was determined in snacks and found that Tartrazine (27.5%) and Sunset Yellow (24.0%) were the most commonly used synthetic colorants.\(^{28}\) Tripathi et al. detected non-permitted synthetic dyes in 31% of various foodstuffs in India; and these dyes were more prevalent in foodstuffs from rural markets than those from urban markets.\(^{29}\)

### Table 1. Validation of saffron and synthetic dyes determination by HPLC.

| Analyte             | LOD \(^a\) (mg/kg) | LOQ \(^b\) (mg/kg) | Linearity \(r^2\) | Spiked level (mg/kg) | Inter-day Recovery \((n = 6)\) | Intra-day Recovery \((n = 6)\) |
|---------------------|---------------------|---------------------|-------------------|----------------------|-------------------------------|-------------------------------|
| Saffron             | 0.11                | 0.34                | 0.998             | 5                    | 86.8 ± 3.7                    | 89.1 ± 4.2                   |
|                     |                     |                     |                   | 10                   | 90.2 ± 4.9                    | 93.0 ± 6.0                   |
| Tartrazine          | 0.15                | 0.46                | 0.988             | 5                    | 91.0 ± 3.9                    | 89.4 ± 5.3                   |
|                     |                     |                     |                   | 10                   | 87.8 ± 6.1                    | 87.0 ± 9.9                   |
| Sunset yellow       | 0.10                | 0.31                | 0.997             | 5                    | 88.5 ± 5.5                    | 93.0 ± 6.8                   |
|                     |                     |                     |                   | 10                   | 89.3 ± 7.2                    | 93.2 ± 8.1                   |
| Quinoline yellow    | 0.13                | 0.39                | 0.999             | 5                    | 84.6 ± 4.0                    | 86.3 ± 4.2                   |
|                     |                     |                     |                   | 10                   | 90.1 ± 6.6                    | 88.7 ± 7.7                   |

\(^a\) Limit of detection.  
\(^b\) Limit of quantitation.

### Table 2. The Colorant level detected in ready-to-cook Iranian barbecued chicken samples.

| Colorant       | Location   | Samples, \(n\) | Positive samples, \(n\) (%) | Concentration in positive samples (mg/kg) | Mean ± SEM | Range \(^a\) |
|----------------|------------|----------------|----------------------------|-------------------------------------------|------------|--------------|
| Saffron        | Restaurant | 136            | 40 (29.41) \(^x\)          | 37.95 ± 3.344 14.75–81.41                 |            |              |
|                | Industry   | 24             | 1 (4.17) \(^y\)           | 18.37                                     |            |              |
|                | Total      | 160            | 41 (25.62) \(^z\)         | 37.24 ± 3.337 9.01–81.41                  |            |              |
| Tartrazine     | Restaurant | 136            | 15 (11.03) \(^x\)         | 12.18 ± 1.678 6.73–40.11                   |            |              |
|                | Industry   | 24             | 1 (4.17) \(^y\)           | 17.26                                     |            |              |
|                | Total      | 160            | 16 (10.00) \(^z\)         | 12.50 ± 1.601 6.73–40.11                   |            |              |
| Sunset Yellow  | Restaurant | 136            | 29 (21.32) \(^x\)         | 20.09 ± 1.522 5.81–34.6                    |            |              |
|                | Industry   | 24             | 12 (50.00) \(^y\)         | 38.44 ± 5.80 15.2–72.29                    |            |              |
|                | Total      | 160            | 41 (25.62) \(^z\)         | 25.46 ± 2.366 5.81–72.29                   |            |              |
| Quinoline Yellow| Restaurant | 136            | 24 (17.65) \(^x\)         | 19.08 ± 1.858 7.12–34.48                   |            |              |
|                | Industry   | 24             | 6 (25.00) \(^y\)          | 32.81 ± 6.852 17.34–62.15                  |            |              |
|                | Total      | 160            | 30 (18.75) \(^z\)         | 21.83 ± 2.202 7.12–62.15                   |            |              |

\(^x\) Minimum and maximum values in positive samples.  
\(^xy\) Occurrence of each color with different superscript letters are significantly different between restaurant and industry \((P < 0.05)\).
Conclusion

Monitoring of synthetic dyes in foods is very important. In this study, a reliable analytical method for the extraction, detection, and quantitation of saffron and three synthetic food dyes in ready-to-cook barbecued chicken was developed. Based on the results, Synthetic dyes were detected in 50.0% of the restaurants and 79.17% of food industries samples. It was shown that different food color additives, like Tartrazine, Sunset Yellow and Quinoline Yellow are widely used in barbecued chicken. Sunset Yellow was the most prevalent (25.62%) synthetic dye used in barbecued chicken followed by Quinoline Yellow (18.75%) and Tartrazine (10.0%). The presence of illegal synthetic dyes in ready-to-cook barbecued chicken can be a potential hazard for the health of consumers. It seems that providing food safety training for manufacturers and restaurant owners can play an important role in reducing the use of synthetic dyes in barbecued chicken and other food products. Further investigations should be carried out to determine illegal synthetic dyes in various food products.

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