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Original Article

Simultaneous determination of some common food dyes in commercial products by digital image analysis

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ABSTRACT

A simple and relatively fast image-analysis method using digital images, obtained with a flatbed scanner, has been described. The method was used for the simultaneous determination of four common food dyes, namely, carmoisine, brilliant blue, sunset yellow, and quinoline yellow, in binary mixtures in commercial products without a need for any prior separation steps. The results obtained were validated against a standard high-performance liquid chromatography method and a good agreement was obtained. The parameters affecting the experimental results were optimized. Under the optimal conditions, the method provided acceptable linear ranges (20–250 mg/L) with correlation coefficients higher than 0.998, suitable precision (relative standard deviation $\leq$ 4.5%), and limits of detection between 4.82 and 8.05 mg/L.

1. Introduction

Food additives are commonly used in processed foodstuff to improve appearance, flavor, taste, color, texture, nutritive value, and preservation. Color is the first quality attribute of food evaluated by consumers, and is therefore an important component of food quality relevant to market acceptance. Although increasing evidence in recent years indicates that the abuse of dyes may cause adverse effects, many kinds of dyes are still widely used due to their low price, high effectiveness, and excellent stability. Therefore, determination of food dyes is important to quality control and control the amount of use permitted [1]. Numerous methods such as sensitive spectrophotometry [2], differential spectrophotometry [3], derivative spectrophotometry [4], spectrophotometry using chemometrics [5,6], colorimetry using a homemade double-beam photocolorimeter [7], reflectometry using a homemade reflectometer [8], electrochemical methods [9,10], capillary electrophoresis [11], ion-pair high-performance liquid chromatography (HPLC) [12], electrokinetic chromatography [13], supercritical fluid extraction–capillary liquid chromatography (LC) [14], thin-layer chromatography [15], HPLC–mass spectrophotometry (MS) [16], solid-phase

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extraction–HPLC [17], HPLC–diode-array detector detection [18,19], LC–MS [20], LC–hybrid linear analysis presented by Goicoechea and Olivieri [21], and gas chromatography–MS [22] have been developed for determination or resolving mixtures of colorants in foodstuff.

Image analysis is the extraction of meaningful information from images, especially from digital images by digital-image-processing techniques. Several techniques have been used for obtaining digital images for different purposes. These techniques include some types of spectroscopy and obtaining images using digital color cameras and scanners [23,24].

The aim of this work is to propose a simple and cheap image-analysis method using flatbed scanners for simultaneous determination of some food colors [quinoline yellow (QY), sunset yellow (SY), carmoisine (CA), and brilliant blue (BL)] in commercial food products. The flatbed scanners in comparison with other imaging devices are relatively inexpensive with the ability to digitize images into a stored array of pixels within a computer. Therefore, the proposed method proved to be simple, relatively fast, and economical for the analysis of the target analytes.

2. Methods

2.1. Chemicals

Dyes of purity higher than 95% were purchased from Grodab Chemie GmbH (Hamburg, Germany). The dyes used were QY, SY, CA, and BL. All other chemicals were obtained from Merck (Germany) and were of the analytical reagent grade. Standard solutions (1000 mg/L) of dyes were prepared using pure water.

2.2. Apparatus and software

The experiments were carried out using a flatbed scanner (LiDE20; Canon, China). All HPLC measurements of colors were carried out using a liquid chromatograph (Waters, Milford, MA, USA), which was equipped with the following: Waters 515 pump, Waters 717plus autosampler, and Waters 2487 dual-wavelength absorbance detector (double channel). A filter paper (ALBET ashless), a thin-chromatography Whatman paper (1 Chr Whatman paper, Thickness, 0.18 mm, UK), and a thick Whatman paper (No. 43; thickness, 0.23 mm, UK) were used as spotting surfaces. Photoshop CS5 (Adobe Systems) and MATLAB 7.0 (MathWorks, Natick, MA, USA) were used for investigating the color intensities from the saved images and calculations, respectively.

2.3. Preparation of the real sample solutions

The proposed method was applied for determination of QY, SY, CA, and BL dyes present as individual components or in binary mixtures within commercial products. The paper chromatography method was used for identification of food dyes in commercial products. In this method, the chromatogram is developed by applying aqueous samples and standard dye solutions to a normal-phase paper. The solutions were developed in the mobile phase of sodium citrate (2.4% w/v):pure ethanol (1:1). Retention factor values, that is, the ratio of distance traveled by the dye to the distance traveled by the solvent, were estimated for the samples and the standard solutions. Various solid jelly powders (Farmand; Iran) and traditional chocolate (Morvarid, Minoo, Iran) samples were purchased from local supermarkets (Tabriz, Iran). An unnamed shoddy orange soft-drink sample was supplied by the Quality Control Laboratory of the Food and Drug Department of Tabriz University of Medical Sciences. Approximately 6 g of color-coated chocolates (shells) were transferred into a flask. The colored shells were then dissolved in distilled water, centrifuged, and diluted with an equal volume of 3M CH_3COOH solution. Approximately 200 mg (per 10 mL of sample) of white commercial wool yarn was washed with detergent, rinsed with distilled water, dried, and then added to the aforementioned diluted sample, and the mixture was heated (60 minutes at 60°C or 10 minutes at 90°C). The colored yarn was then taken out, and washed with plenty of distilled water. The dyes were recovered by mixing the yarns with 10 mL of NH_3 (2M) and heating for 10 minutes at 90°C.

2.4. Analytical procedures

Different concentrations of dye samples were prepared by appropriate dilutions of stock solutions (1000 mg/L) with deionized water (Ghazi Company, Tabriz, Iran). To prepare the color spots of the dyes, the prepared solutions (20 μL) were spotted on Whatman No. 43 paper, left for a while (40 minutes), and placed on the glass plate of the flatbed scanner. The spots on the paper were separated by Microsoft Paint (XP) software. The selected images were saved in the bitmap format composed in the three channels of the RGB model using Photoshop. The magnitude of each component (red, green, and blue) was determined, and the color value was calculated by subtracting the suitable RGB channel intensities of samples from those of the white sheet. This procedure was performed using MATLAB.

2.5. High-performance liquid chromatography

The aforementioned method was validated by running a parallel HPLC test for the commercial food products. Measurements were made at 460 nm for CA, SY, and QY and at 640 nm for BL using NaH_2PO_4/Na_2HPO_4 buffer solution (pH = 6.0); acetonitrile (65:35 v/v) was used as the mobile phase with a flow rate of 0.9 mL/minute at room temperature. The retention times were 2.91, 3.06, and 3.51 minutes at the first channel (460 nm) for QY, SY, and CA, and 3.47 minutes at the second channel (640 nm) for BL. No efficient interaction between BL and CA was observed as they had similar retention times.

3. Results and discussion

3.1. The RGB color model and selection of the suitable RGB component

A digital image is intrinsically a multivariate system, which is a collection of data stored in pixels, each usually highly correlated to its neighbors [25]. The numerical information of

each pixel can be decomposed into three channels that correspond to red, green, and blue, which are added in various ways to reproduce a broad array of colors. This is known as the *RGB model* [26]. The abbreviation RGB is the initials of the three primary colors, red, green, and blue. Thus, a color in the RGB model is described by indicating how much of each component (red, green, or blue) is included (each component can vary from zero to a defined maximum value). Fig. 1 shows the linear RGB values corresponding to each wavelength. As shown in the figure, there are R, G, and B values on this plot for every wavelength.

The scanner optically scans the images and converts them to digital images (each point is expressed as an RGB triplet, the components of which vary from 0 to 255). These three parameters are the color intensities of the red (R), green (G), and blue (B) channels [27].

The absorption spectra of the dyes (at pH 6 and 11; recorded and represented in Fig. 2) indicate that the dyes were active in the ultraviolet and visible regions. Within the 400–700-nm range, the spectra of QY, CA, and SY are highly overlapped, whereas within the same range, a lower spectral overlapping was observed between BL and other dyes. Furthermore, a negligible spectral shift was observed, when the solution was made alkaline. Such a high overlapping (particularly between QY, SY, and CA) limits the analysis of these dyes in food products by conventional methods, whereas the proposed method was successfully applied for determining some dyes in binary mixtures. Considering the wavelengths of the maximum absorbance of the dyes (Fig. 2) and RGB values for each wavelength (Fig. 1), the suitable RGB component was selected for determination of any food color as an individual component or as a binary mixture (Table 1).

3.2. *Optimization of parameters*

3.2.1. *Selection of suitable surface for dyes spotting*

To study the effect of spotted surface on the analytical results, black surfaces (paper and fabric) were selected at the first step, because the RGB values are zero for black backgrounds. However, the color spots were not detectable on the scanned images with black backgrounds. Thus, white surfaces (paper and fabric) were investigated next. Because the RGB factors on the various white fabrics were not constant at 255 in different points of each surface, paper surfaces consisting of filter paper, paperboard, and two types of Whatman paper (1 Chr and No. 43) were tested for further studies. Because of the weak absorbance strength of the paperboard, the best spotted surface was determined by comparison of repeatability of analytical signals with the other three surfaces. Based on the data presented in Table 2, the Whatman paper no. 43 (i.e., the thick one) was selected as the optimized spotted surface.

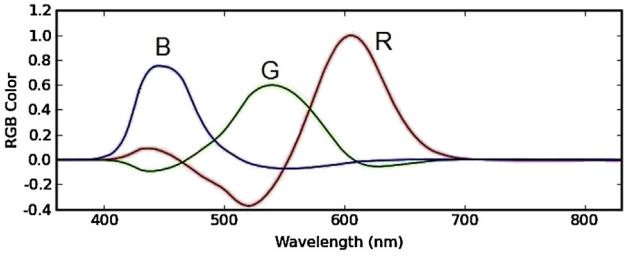

Fig. 1 – RGB values for the pure spectral lines.

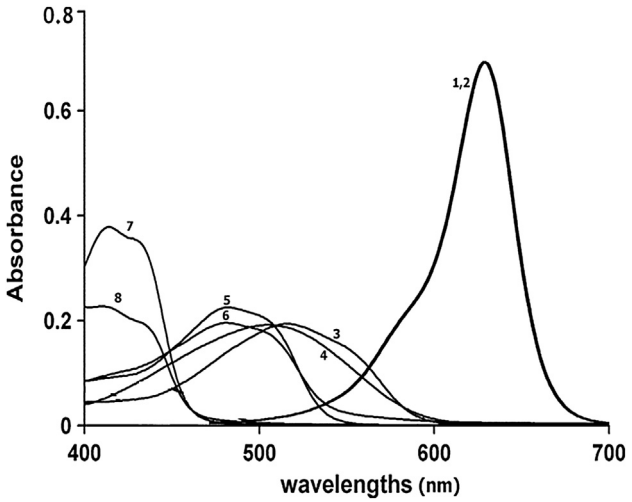

Fig. 2 – Absorption spectra of brilliant blue (1, 2), carmoisine (3, 4), sunset yellow (5, 6), and quinoline yellow (7, 8) at pH 6 and 11, respectively. Concentrations of all dyes are 10 g/L.

3.2.2. *Optimization of spotted sample volume*

The optimum spotted sample volume was selected based on its effect on repeatability of results and determination efficiency. The influence of the spotted sample volume was studied in the 5–50 μL range. The results obtained showed that by increasing the volume of sample from 5 to 20 μL, analytical signals increase and then remain constant until 50 μL. With increases in volume, the repeatability also improved. Based on these results, 20 μL was selected as the suitable volume of spotted sample for scanning.

Table 1 – Suitable components of RGB for determination of food dyes as individual components and in binary mixtures.

Analyte	Food color with	Suitable component of RGB
Brilliant blue	—	R
Brilliant blue	Sunset yellow	R
Brilliant blue	Carmoisine	R
Brilliant blue	Quinoline yellow	R
Carmoisine	—	G
Carmoisine	Brilliant blue	G
Carmoisine	Quinoline yellow	G
Carmoisine	Sunset yellow	G
Sunset yellow	—	B
Sunset yellow	Brilliant blue	B
Sunset yellow	Carmoisine	B
Quinoline yellow	—	B
Quinoline yellow	Brilliant blue	B
Quinoline yellow	Carmoisine	B

Table 2 – Statistical data for selection of spotted surface.

Relative standard deviation of signals ($C = 50$ mg/L, $n = 6$)	Analyte	Kind of paper
9.3	Brilliant blue	Filter paper
8.9	Carmoisine	
7.7	Quinoline yellow	
9.1	Sunset yellow	
14.2	Brilliant blue	Whatman paper
11.8	Carmoisine	(1 Chr)
15.2	Quinoline yellow	
14.3	Sunset yellow	
2.6	Brilliant blue	Whatman paper
2.1	Carmoisine	(No. 43)
2.8	Quinoline yellow	
1.9	Sunset yellow	

Table 3 – Quantitative features of the method for the selected dyes.

Analyte	LR (mg/L)	R^2	LOD (mg/L)	LOQ (mg/L)
Brilliant blue	20–250	0.9996	4.82	16.06
Carmoisine	30–180	0.9982	7.23	24.11
Quinoline yellow	30–250	0.9991	8.05	26.84
Sunset yellow	30–250	0.9989	7.70	25.68

LOD = limit of detection (S/N = 3); LOQ = limit of quantification (S/N = 10); LR = linear range; R^2 = square of correlation coefficient.

3.2.3. *Optimization of drying time of spots*

In this study, *drying time* was defined as the time interval between spotting the analyte on paper and scanning it. To obtain constant signal for each spot, the time of its drying was studied between 0 and 70 minutes. The results obtained show that by increasing drying time, analytical signals fluctuate until the 40th minute, after which they remain constant. Therefore, the optimal drying time was selected as 40 minutes.

3.2.4. *Effect of scanner dots per inch*

Dots per inch (DPI) is a measure of spatial printing or video dot density, in particular the number of individual dots that can be placed in a line within the span of 2.54 cm (1 in.). By increasing the DPI of scanner, the quality of digital image and the time required for scanning can be increased. The effect of DPI was studied in the range of 75–300, and it was found that resolution of images had no effect on the obtained responses and on their reproducibility. Therefore, the resolution of scanner was adjusted at 75 DPI (the minimum value in scanners) in this study.

Table 5 – Tolerable concentration ratios for determination of 30 mg/L of each dye with the proposed method (relative error ± 5%). The analytes and interfering dyes are shown in horizontal and vertical columns, respectively.

	BL	CA	QY	SY
BL	—	50	60	60
CA	30	—	60	50
QY	60	60	—	—
SY	30	50	—	—

BL = brilliant blue; CA = carmoisine; QY = quinoline yellow; SY = sunset yellow.

3.2.5. *Effect of surrounding light*

To investigate the effect of light on the obtained signals, two papers were spotted under the same conditions. For drying these spots, one of the papers was exposed to surrounding light and the other one was placed in a darkroom. It was concluded that the drying condition does not affect the analytical signals. Thus, other experiments were carried out under normal laboratory conditions (i.e., no need for a darkroom).

3.3. *Quantitative features of the method*

The analytical performance of the proposed method was validated by considering the dynamic linear range, square of correlation coefficient, limit of detection (LOD), and limit of quantification (LOQ) for the analytes (summarized in Table 3). In general, the LOD is taken as the lowest concentration of an analyte in a sample that can be detected, but not necessarily

Table 4 – Analytical performances of some similar methods reported in the literature for determination of food dyes.

Parameter	Proposed method	Altınöz and Toptan [4].	Al-Degs and El-Sheikh [5].	Al-Degs [21].	Alves et al [29].	Huang et al [30].	Berzas et al [31].
Method	Image analysis	Spec	Spec	Spec and HPLC	HPLC	CE	Spec
Data processing	NN	Derivative spectrophotometry	PARAFAC and BLLS/RBL	HLA	NN	NN	PLS/PCR
Dynamic range (mg/L)	20–250	1.0–52	1.0–180	0.5–16	1–100	1–500	0.8–20
Number of dyes studied	4	2	3	3	5	8	3
RSD (%)	0.79–4.46	1.50–3.73	<5	2.2–3.2	<3.87	2.04–4.96	0.4–1.15
LOD (mg/L)	4.82–8.05	0.002–0.05	0.22–0.54	0.04–0.3	0.01–0.1	<0.5	0.003–0.06
LOQ (mg/L)	16.06–26.84	NA	NA	NA	0.03–0.5	NA	NA

BLLS/RBL = bilinear least squares/residual bilinearization; CE = capillary electrophoresis; HLA = hybrid linear analysis; HPLC = high-performance liquid chromatography; LOD = limit of detection; LOQ = limit of quantification; NA = not available; NN = not needed; PARAFAC = parallel factor analysis; PLS/PCR = partial least squares/principal component regression; RSD = relative standard deviation; Spec = spectrophotometry.

Table 6 – Results obtained for determination of dyes in food samples with the proposed method and HPLC (mg/L).

	Image analysis				HPLC			
	BL	CA	SY	QY	BL	CA	SY	QY
Chocolate—green	ND	—	—	34 ± 3	4 ± 1	—	—	34 ± 2
Chocolate—yellow	—	—	—	36 ± 3	—	—	—	37 ± 3
Chocolate—red	—	50 ± 3	—	—	—	47 ± 2	—	—
Chocolate—orange	—	—	35 ± 3	—	—	—	33 ± 3	—
Soft drink—orange	—	ND	57 ± 2	—	—	4 ± 1	56 ± 3	—
Jelly powder—banana	—	—	ND	44 ± 2	—	—	5 ± 1	46 ± 2
Jelly powder—blue raspberry	40 ± 1	—	—	—	41 ± 3	—	—	—
Jelly powder—watermelon	—	48 ± 1	30 ± 2	—	—	47 ± 3	32 ± 2	—

BL = brilliant blue; CA = carmoisine; HPLC = high-performance liquid chromatography; ND = not detected; QY = quinoline yellow; SY = sunset yellow.

quantified, under the stated conditions of the test. The LOQ is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of the test [28].

Despite some limitations, the proposed model offers some advantages over other similar methods, such as simplicity (Table 4). The results indicated that wide linear ranges and good linearity ($R^2 > 0.99$) were achievable for the selected food colors. Acceptable detection and quantification limits in the range of 4.82–8.05 and 16.06–26.84 mg/L, respectively, were obtained by image analysis. Repeatability of the method was assessed by its application on the six similar standard solutions at three different concentrations (40, 90, and 180 mg/L). The relative standard deviations were obtained in the 0.79–4.46% range.

3.4. *Study of the interference*

The interference of a food dye over other dyes was investigated by analyzing solutions containing two dyes. The results obtained with tolerable maximum error of 5% in the reflectance reading are presented in Table 5. As it can be seen, the interference of a dye over another increases with increasing the molar absorptivity of the interfering dye and spectral overlapping of the dyes. Because of the similarity of the RGB factors and the signals obtained for QY and SY in the software used, the examination of the interference of these dyes on each other was not possible.

3.5. *Determination of food dyes in real samples*

The BL, CA, SY, and QY dyes in various samples (solid jelly powders, traditional chocolate, and untitled shoddy orange soft drink) were analyzed. The solutions of food samples were prepared as described in the "Preparation of the Real Sample Solutions" section. The dyes in each sample were then identified by paper chromatography. These samples were also subjected to an HPLC method. The results obtained for each of the two methods were then compared (Table 6). As it can be seen, the results obtained from the proposed method was in a good agreement with those obtained for the HPLC. There was also no significant difference between the results obtained by each of the two methods (*t* test).

4. Conclusion

The proposed analytical approach is a cheap analytical method for obtaining digital images and determination of food colors by a scanner. The optimized technique in conjunction with flatbed scanner was considered to be a facile, economical procedure, and nearly fast for analyzing some food colors in commercial samples. The method has been successfully used for the analysis of some dyes (BL, CA, QY, and SY) as model analytes. The method does not require any preliminary sample-preparation step. The methodology showed acceptable linear ranges for the analytes, with correlation coefficients higher than 0.998, and suitable precision. In this digital imaging method, the costs of equipment and software required are low, and the experimental setup and operating conditions are simple.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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