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Analytical Methods

Determination of synthetic dyes in selected foodstuffs by high performance liquid chromatography with UV-DAD detection

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Abstract

A relatively fast method was developed and applied to the determination of selected synthetic food dyes (Sunset Yellow, Tartrazine, Amaranth, Brilliant Blue and Red-40) in three different kinds of foodstuffs: solid juice powders, solid jelly powders and soft drinks. High performance liquid chromatography with UV-DAD detection was employed. The developed chromatographic method employed an ODS Zorbax column (250 mm; 4.6 mm; 5 μm). Two different solvent systems were employed depending on the expected dyes in the studied samples. Sample preparation consisted of dissolving and filtering the samples and showed high throughput. Adequate detection and quantification limits together with high recoveries (better than 98.8%) were obtained. All studied samples showed dye levels in conformity with Brazilian legislation. Indeed some products showed poor quality and/or production controls due to the variability between lots. This fact was more critical for Tartrazine and Sunset Yellow in solid juice powders and there is concern that these substances can exceed legislated values. It was also observed that different producers use different dyes and/or composition in similar products.

Keywords: Food dyes; HPLC analysis; Regulation

1. Introduction

Food additives are commonly used in processed foodstuffs to improve appearance, flavor, taste, color, texture, nutritive value and conservation (Hathcock & Rader, 2003). Since the visual aspect is an important factor for the selection of products by final consumers, synthetic food dyes stand out as one of the essential additive class for food industry in the conquest of markets (Ashfaq & Masud, 2002; Clydesdale, 1993).

When compared to natural dyes, synthetic dyes show several advantages such as high stability to light, oxygen and pH, color uniformity, low microbiological contamination, relatively lower production costs, etc. (Hathcock & Rader, 2003). The use of food dyes is at least controversial because they are only of esthetical role. Moreover many of them have been related to health problems mainly in children that are considered a very vulnerable group (Clydesdale, 1993; Polônio, 2002). Furthermore in some cases the use of food dyes is also indicative of foodstuff adulteration such as in their addition to fruit juices (Kiseleva, Pimenova, & Eller, 2003).

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Many food dyes are controlled or even forbidden in many places of the world. The Brazilian Agency ANVISA (1999a,b, 2002) imposed limits of concentrations for several food dyes in different foodstuffs in Brazil following international regulations. Only Tartrazine (E-102), Sunset Yellow (E-110), Amaranth (E-123), Ponceau 4R (E-124), Red 40 (E-129), Eritrosine (E-127), Indigotine (E-132) and Brilliant Blue (E-133) are allowed in food (ANVISA, 2002).

However according to the Brazilian legislation, although it is obligatory to list the added dyes in product labels the specification of their actual concentrations are not required. This situation points to the need of reliable methods of high throughput for the determination of these substances allowing foodstuff control.

Spectrometric and chromatographic approaches have been employed for food dye determination but other techniques such as capillary electrophoresis have also been used (Dossi et al., 2007; Garcia-Falcon & Simal-Gandara, 2005, & references therein). High performance liquid chromatography (HPLC) with UV–VIS, UV–VIS-DAD and/or mass spectrometry detectors have been employed for dye determination (Kiseleva et al., 2003; Ma, Luo, Chen, Su, & Yao, 2006; Kirschbaurn, Krause, Pfalzgraf, & Brockner, 2003; Garcia-Falcon & Simal-Gandara, 2005; Prado & Godoy, 2002, 2003a, 2003b). A method that allows the simultaneous determination of selected dyes, sweeteners and preservatives was also recently published (Dossi, Toniolo, Susmel, Pizzariello, & Bontempelli, 2006).

A fast chromatographic method using two different solvent systems and the same column was developed and employed in the analysis of dyes in selected foodstuffs. The method required a minimal sample preparation step that consisted of filtering the samples following dissolution of the solid samples in water (juice powders) or hot water (jelly powders), or after degassing (soft drinks). Although the limits imposed by the Brazilian legislation (Table 1) were considered during method development, it is sufficiently robust and reliable to allow determination of the studied dyes below Brazilian legislation limits.

Table 1
Maximum allowable concentrations in the studied foodstuffs according to the Brazilian legislation

Dyes	Solid juice powders[b] (mg/100ml[a])	Solid jelly powders[c] (mg/100g[a])	Soft drinks[b] (mg/100ml)
Sunset yellow	10	10	10
Amaranth	5	10	5
Brilliant blue	10	15	10
Tartrazine	10	15	10
Red 40	10	15	10

[a] In the ready to consume product.
[b] ANVISA, Regulation 389, 5th August, 1999.
[c] ANVISA, Regulation 388, 5th August, 1999.

Studied products and brands were chosen considering data of consumption reported in a specific questionnaire applied to child parents in the ambulatory of a local child hospital. The analyzed dyes: Tartrazine (E-102), Sunset Yellow (E-110), Amaranth (E-123), Red 40 (E-129) and Brilliant Blue (E-133) where also selected according to the dyes listed in product labels.

2. Materials and methods

2.1. Chemicals and reagents

Solid dye standards from both AccuStandard (CT, USA) or Aldrich Chemical Co. (WI, USA) were employed (Table 1). Methanol (HPLC grade) was purchased from TediaBrazil (RJ, Brazil). All other reagents (ammonium acetate, EDTA, sodium acetate and acetic acid) were of analytical grade and were purchased from Vetec (RJ, Brazil).

Ultra-pure water was prepared through a Simplicity System (Millipore, EUA) following distillation.

2.2. Standard solutions

Standard stock solutions of all dyes containing 100 mg/L were prepared by weighing sufficient amount of the correspondent solids followed by dilution to 100 mL with ultra-pure water. Working standards of individual dyes were prepared by dilution of aliquots of the stock solutions.

2.3. Samples

Samples consisted of solid jelly powder (pineapple, grape, strawberry and raspberry flavors), solid juice powder for drinks (orange, mango, cashew, pineapple, passion fruit and strawberry flavors) and soft drinks (orange and grape flavors). For each kind of sample, 4 different brands were studied except in the cases of grape jelly powder, strawberry and cashew solid juice powder and grape soft drink. Three different product lots were evaluated in the case of solid juice powder samples and two different lots evaluated for the other studied samples.

Samples were of commercial products usually sold in the local market. They were bought in supermarkets of Rio de Janeiro and Niterói cities, Brazil. Different dealers where labeled as A, B, C and D independently of the kind of foodstuff.

2.4. Sample preparation procedures

Solid samples were previously homogenized in their own packages before sampling. Samples between 2 and 5 g were precisely weighted. Solid juice powder samples were directly dissolved in ultra-pure water at room temperature. Solid jelly powder samples were dissolved in hot ultra-pure water (~60 °C). In both cases they were diluted up to 50 mL with ultra-pure water and filtered through 0.45 μm

filters. Soft drink samples were previously degassed in ultrasonic bath, filtered through 0.45 μm filters and directly analyzed.

2.5. Chromatographic Analysis

Samples were analyzed by high performance liquid chromatography (HPLC). The HPLC system consisted of a binary pump, a degasser, an automated injector, a column oven and an UV-DAD detector (all Agilent 1100 Series, USA). The system was controlled by an Agilent ChemStation.

Chromatographic conditions (mobile phase composition and flow-rate) were evaluated and optimized in ODS column (Zorbax ODS, 250 mm; 4.6 mm; 5 μm) using a guard column of the same characteristics (Zorbax ODS, 30 mm; 4.6 mm; 10 μm).

Two different mobile phase systems were employed to accomplish a quick separation of the analyzed dyes. System I consisted of methanol (solution A) and aqueous ammonium acetate (0,08 mol/L; solution B). System II consisted of methanol (solution A) and an aqueous solution containing EDTA (5×10^{-3} mol/L) and sodium acetate (3×10^{-2} mol/L) with a final pH adjusted to 3.5 with addition of diluted acetic acid (solution B). Prior to use the aqueous solutions and methanol were degassed in ultrasonic bath. Aqueous solutions were further filtered through 0.45 μm membranes.

Isocratic conditions were employed for both solvent systems and the mobile phase was composed of 45% of solution A and 55% of solution B. A constant flow rate of 1.0 mL/min was used.

2.6. Dye identification and quantification

Optimum absorption wavelengths for each dye were previously evaluated using standard solutions. For quantitative analysis the following wavelengths were used: Brilliant Blue (632 nm), Amaranth (524 nm), Red 40 (508 nm), Sunset Yellow (484 nm) and Tartrazine (454 nm).

The characteristics of the DAD-UV detector and of the ChemStation allowed the simultaneous detection and posterior treatment of up to 5 wavelengths. Dyes were identified by comparison with retention times of true compounds and by their absorption spectra. The method allowed the separation and detection of the 5 dyes in less than 6 min leading to a high throughput of sample evaluation.

Quantification of the studied dyes was performed by external standard calibration. Six level analytical curves (1.00, 5.00, 10.00, 20.0, 50.0 and 100.0 mg/L) were used and each calibration point represented the mean of 3 injections of each standard. Detection limit (DL) and quantification limit (QL) were determined by considering, respectively 3 and 10 times the signal to noise ratios estimated by the regression lines (ACS, 1980). For the calculation of the DL and QL in solid samples a typical mass of 5.00 g was considered.

Recovery evaluations were performed by spiking known amounts of the studied dyes to the solid sample before processing and comparing the obtained results with these of the same sample without spiking. Recoveries were estimated by difference of concentrations and expressed as percentages.

Final treatment of data and statistical analysis (Dixon test, Student *t*-test and *F*-test) were performed by datasheets prepared in Microsoft Excel®.

3. Results and discussion

Since all the five studied dyes are not usually simultaneously present in foodstuffs, a different strategy and approach that involved two different isocratic HPLC conditions for the separation of groups of dyes is presented here. This approach represents an alternative to methods of analysis of several dyes including those that usually do not occur simultaneously in real samples. This method can be useful in quality control and it shows also a high throughput due to the use isocratic conditions that reduce the stabilization time necessary between consecutive determinations.

Fig. 1(a–d) shows typical chromatograms of the selected dyes in some of the studied samples in the two mobile phase systems. All dyes could be separated and detected within 5 min. Good chromatographic resolution can be observed for Tartrazine and Sunset Yellow with methanol/ammonium acetate as mobile phase in the wavelength of 484 nm. The chromatogram of Tartrazine and Red-40 with methanol/aqueous EDTA 5×10^{-3} mol/L and sodium acetate 3×10^{-2} mol/L at pH = 3.5 in the wavelength of 508 nm shows also a good resolution of that pair.

Qualitative and quantitative analysis were allowed with selective wavelength detection in the DAD detector. Five dyes: Tartrazine (E-102), Sunset Yellow (E-110), Amaranth (E-123), Red 40 (E-129) and Brilliant Blue (E-133) where determined according to the listed dyes in the studied product labels.

As for linearity, the calibration functions were calculated by least-square regression. Three replicates for each concentration were analyzed allowing the evaluation of detection limits (DL), quantification limits (QL) and correlation coefficients of the studied dyes as shown in Table 2.

Detection limits of dyes were ranged from 0.01 (Red 40) to 0.15 (Brilliant blue) mg/100 mL and QL were between 0.03 (Red 40) and 0.50 (Brilliant blue) mg/100 mL. These values correspond to the same nominal values as expressed by mg/100 g when 5 g of sample and a final volume of 50 mL are considered. Quantification limits were far below the Brazilian legislated values (Table 1) satisfying this legislation for the determination and control of these dyes in foodstuffs and soft drinks.

Correlation coefficients were always larger than 0.999 and closer to the unity showing a good relationship

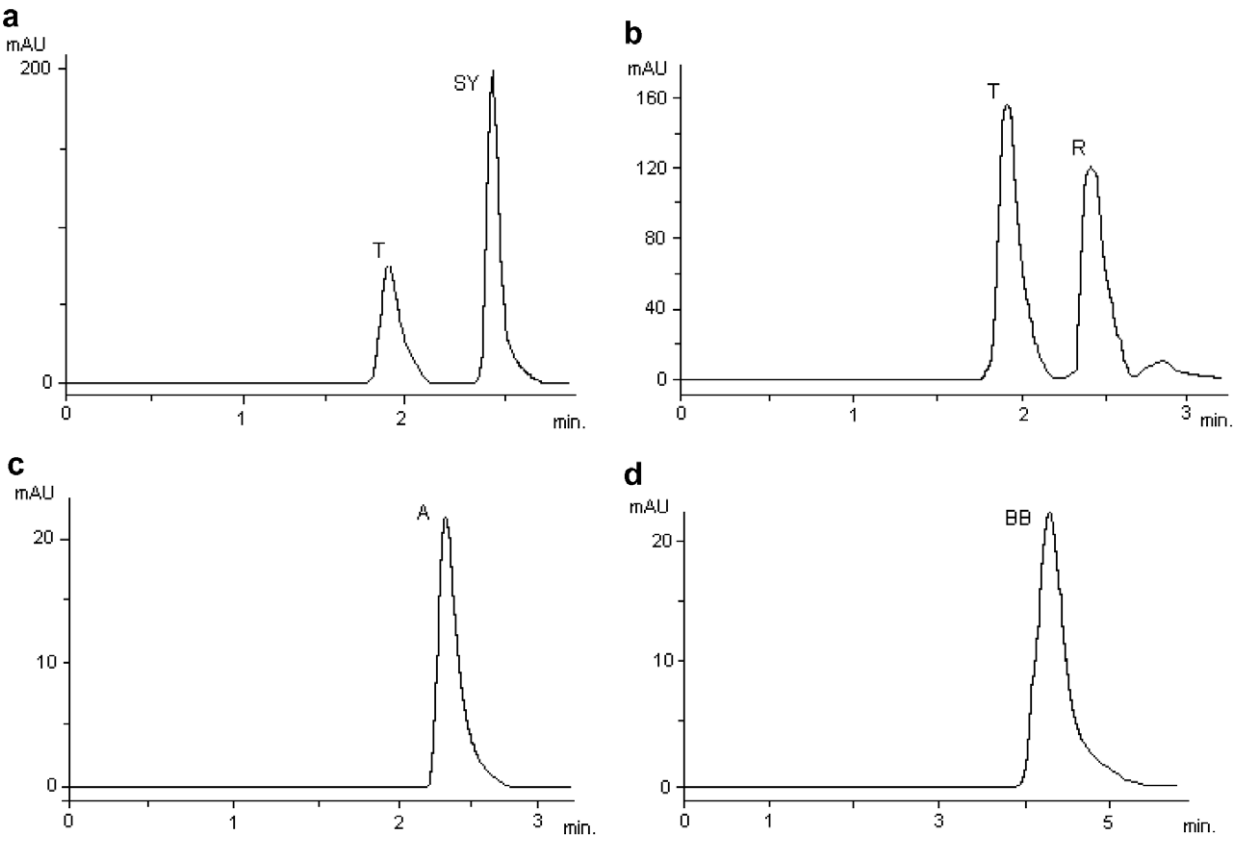

Fig. 1. (a) Chromatogram of Tartrazine (T) and Sunset Yellow (SY) in passion fruit juice powder ($\lambda = 484$nm); mobile phase: methanol/ammonium acetate (0.08 mol/L) (45:55); (b): Chromatogram of Tartrazine (T) and Red-40 (R) in strawberry juice powder ($\lambda = 508$ nm); mobile phase: methanol/aqueous EDTA 5×10^{-3} mol/L and sodium acetate 3×10^{-2} mol/L at pH = 3.5 (45:55); (c): Chromatogram of Amaranth (A) in jelly powder ($\lambda = 524$ nm); mobile phase: methanol/aqueous EDTA 5×10^{-3} mol/L and sodium acetate 3×10^{-2} mol/L at pH = 3.5 (45:55); (d) Chromatogram of Brilliant Blue (BB) in grape soft drink ($\lambda = 632$ nm); mobile phase: methanol/ammonium acetate (0.08 mol/L) (45:55).

Table 2
Analytical figures of merit of the developed method

Dyes	Detection limit (DL) (mg/L)[b]	Quantification limit QL (mg/L)[a]	R	Percentages of dye recoveries (mean ± standard deviation)		
				Solid juice powder	Solid jelly powder	Soft drinks
Tartrazine	0.04	0.14	0.9998	103.1 ± 3.77	102.8 ± 3.55	101.0 ± 1.21
Sunset yellow	0.05	0.15	1.000	100.4 ± 3.37	98.9 ± 2.59	103.8 ± 1.55
Amaranth	0.10	0.32	1.000	101.0 ± 3.05	101.3 ± 3.84	99.8 ± 3.52
Red 40	0.01	0.03	1.000	98.8 ± 0.03	–[a]	–[a]
Brilliant blue	0.15	0.50	0.9997	–[a]	103.8 ± 1.00	100.9 ± 0.41

[a] No such a kind of sample containing the indicated dye was studied.
[b] The same values of DL and QL expressed as mg/100 g are obtained when considering 5 g of solid samples diluted to 50 mL.

between peak area and concentrations in the studied range 1.00–100.0 mg/L (Table 2).

Recoveries were also evaluated by three independent determinations of the studied samples. For this purpose, samples were analyzed before and after spiking known amounts of the different dyes. Recoveries between 98.8 and 103% (Table 2) were found showing that there are few matrix effects in the determinations and indicating that the simplified method of sample preparation is adequate for the analysis of the studied samples. Standard deviations closer to 3% were always found in recovery evaluations. However considering that two determinations were necessary for recovery evaluation an increase of data dispersion is to be expected. Indeed recoveries were considered to be sufficiently high and precise for a quantitative analysis.

Tables 3–5 show the concentrations (mg/100 g) of the studied dyes in the foodstuffs evaluated in this study. As expected, most of the studied foodstuffs showed two or three dyes in their composition due to the desired colors of final products. An overall precision better than 4% was found in the analysis of the studied foodstuffs.

No qualitative differences were found in the composition of foodstuffs of different lots of each brand. Strawberry powder juice represents an exception since among the

Table 3
Dye concentrations (mg/100g) (mean ± standard deviation) obtained for four independent determinations in different lots of solid juice powder

Flavor	Dyes[b]	Lot	Brands[c]			
			A	B	C	D
Orange	E-102	1	1.43 ± 0.04	1.73 ± 0.07	1.70 ± 0.08[d]	1.12 ± 0.02[d]
		2	1.45 ± 0.03	1.69 ± 0.07	1.09 ± 0.03[d]	0.89 ± 0.01[d]
		3	1.46 ± 0.10	1.66 ± 0.05	1.56 ± 0.02[d]	0.66 ± 0.01[d]
	E-110	1	1.97 ± 0.09	1.64 ± 0.09	2.57 ± 0.11[d]	1.64 ± 0.01[d]
		2	2.05 ± 0.06	1.66 ± 0.14	1.36 ± 0.01[d]	1.24 ± 0.00[d]
		3	2.00 ± 0.04	1.58 ± 0.05	1.99 ± 0.01[d]	2.13 ± 0.05[d]
Passion Fruit	E-102	1	1.14 ± 0.05	1.99 ± 0.03	1.15 ± 0.04[d]	1.09 ± 0.01
		2	1.16 ± 0.01	1.99 ± 0.03	1.31 ± 0.04[d]	1.18 ± 0.01
		3	1.19 ± 0.05	1.97 ± 0.01	0.97 ± 0.01[d]	1.38 ± 0.02[d]
	E-110	1	0.80 ± 0.05	1.45 ± 0.03	0.90 ± 0.02[d]	0.61 ± 0.01[d]
		2	0.84 ± 0.03	1.48 ± 0.08	0.79 ± 0.04[d]	0.57 ± 0.01[d]
		3	0.80 ± 0.07	1.42 ± 0.02	0.98 ± 0.00[d]	0.84 ± 0.05[d]
Mango	E-102	1	2.30 ± 0.10	1.36 ± 0.10	1.27 ± 0.04	3.37 ± 0.03[d]
		2	2.23 ± 0.05	1.41 ± 0.11	1.91 ± 0.08[d]	5.62 ± 0.17[d]
		3	2.22 ± 0.03	1.34 ± 0.12	1.52 ± 0.04	4.93 ± 0.07[d]
	E-110	1	2.82 ± 0.10	1.50 ± 0.04	2.26 ± 0.04[d]	3.69 ± 0.04[d]
		2	2.75 ± 0.05	1.52 ± 0.05	2.02 ± 0.11	9.81 ± 0.26[d]
		3	2.77 ± 0.17	1.49 ± 0.03	2.15 ± 0.09	3.99 ± 0.13[d]
Strawberry	E-102	1	–[a]	0.41 ± 0.01	0.33 ± 0.01	–
		2	–	0.42 ± 0.01	0.33 ± 0.02	–
		3	–	0.43 ± 0.03	0.34 ± 0.01	–
	E-123	1	–	2.38 ± 0.04	–	–
		2	–	2.34 ± 0.16	–	–
		3	–	2.39 ± 0.14	–	–
	E-129	1	3.04 ± 0.05	–	5.10 ± 0.13	–
		2	3.02 ± 0.06	–	5.18 ± 0.04	–
		3	3.05 ± 0.11	–	5.38 ± 0.10	–
Cashew	E-102	1	0.24 ± 0.01	0.15 ± 0.01	0.14 ± 0.00	–
		2	0.24 ± 0.01	0.16 ± 0.00	0.13 ± 0.01	–
		3	0.24 ± 0.02	0.16 ± 0.01	0.14 ± 0.01	–
	E-110	1	0.12 ± 0.00	0.05 ± 0.00	0.03 ± 0.00	–
		2	0.12 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	–
		3	0.12 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	–
Pineapple	E-102	1	0.74 ± 0.01	0.15 ± 0.00	0.29 ± 0.01	0.46 ± 0.01
		2	0.70 ± 0.05	0.15 ± 0.00	0.28 ± 0.02	0.45 ± 0.02
		3	0.70 ± 0.05	0.15 ± 0.01	0.28 ± 0.02	0.46 ± 0.02
	E-110	1	0.16 ± 0.00	0.11 ± 0.00	0.12 ± 0.00	0.25 ± 0.01
		2	0.16 ± 0.00	0.12 ± 0.01	0.12 ± 0.01	0.27 ± 0.02
		3	0.16 ± 0.00	0.12 ± 0.01	0.12 ± 0.01	0.26 ± 0.01

[a] No sample containing the indicated dye was studied in this category.
[b] E-102 (Tartrazine), E-110 (Sunset Yellow), E123 (Amaranth), E129 (Red-40).
[c] Mean ± standard deviation ($n = 4$).
[d] Indicates a significant difference of lots ($P = 0.05$).

studied samples the synthetic dyes employed to reach this color varied. This way only one dye (Red 40) was found in brand A while two dyes were found in brand B (Tartrazine and Amaranth) and in brand C (Red 40 and Tartrazine).

When different lots of solid juice powder of the same brands were compared it was observed that dye concentrations in different lots of brands A and B were very similar showing no significant statistical differences. However some lots of brands C and D showed statistically significant differences of Tartrazine and Amaranth concentrations when orange, mango and passion fruit juice powder were compared by Student *t*-tests ($P = 0.05$). For example, the concentration of Tartrazine in lot 1 of orange juice powder of brand C (1.70 ± 0.08 mg/100 g) was about 70% larger than in lot 2 (1.09 ± 0.03 mg/100 g). Similar results were found when comparing Tartrazine in lot 1 (1.27 ± 0.04 mg/100 g) and in lot 2 (1.91 ± 0.08 mg/100 g) of mango juice powder of brand C.

Mango juice powders of brand D showed the largest discrepancies of Sunset Yellow concentrations among the studied juice powder samples and lot 2 showed concentrations that are larger than twice the value found in lot 3. Moreover the concentration of Sunset Yellow in lot 2 was statistically as large as the maximum regulated value of 10 mg/100 g. The levels of Sunset Yellow in the 3 lots

Table 4
Dye concentrations (mg/100g) (mean ± standard deviation) obtained for four independent determinations in different lots of solid jelly powder

Flavor	Dyes[b]	Lot	Brands[c]			
			A	B	C	D
Pineapple	E-102	1	0.83 ± 0.01	3.18 ± 0.05	1.42 ± 0.02	1.99 ± 0.08
		2	0.81 ± 0.03	3.20 ± 0.02	1.43 ± 0.01	2.00 ± 0.10
	E-110	1	0.75 ± 0.01	0.82 ± 0.03	1.04 ± 0.08	0.25 ± 0.02
		2	0.74 ± 0.02	0.80 ± 0.01	1.31 ± 0.02	0.24 ± 0.02
Strawberry	E-110	1	1.37 ± 0.04	1.24 ± 0.03	1.95 ± 0.11	1.65 ± 0.03
		2	1.34 ± 0.04	1.02 ± 0.02	1.89 ± 0.15	1.55 ± 0.10
	E-123	1	2.05 ± 0.03	2.33 ± 0.06	3.41 ± 0.16	1.65 ± 0.03
		2	2.05 ± 0.06	2.39 ± 0.03	3.37 ± 0.20	1.55 ± 0.10
Raspberry	E-110	1	0.65 ± 0.03	1.96 ± 0.17	0.84 ± 0.03	0.89 ± 0.06
		2	0.62 ± 0.03	1.84 ± 0.06	0.82 ± 0.06	0.94 ± 0.05
	E-123	1	1.12 ± 0.01	3.85 ± 0.06	1.84 ± 0.06	2.31 ± 0.03
		2	1.19 ± 0.03	3.79 ± 0.14	1.79 ± 0.10	2.31 ± 0.04
Grape	E-102	1	0.87 ± 0.07	–[a]	–	–
		2	0.88 ± 0.06	–	–	–
	E-123	1	1.68 ± 0.04	3.86 ± 0.03	0.97 ± 0.02	6.19 ± 0.33
		2	1.67 ± 0.04	3.77 ± 0.05	0.98 ± 0.01	6.07 ± 0.14
	E-133	1	0.50 ± 0.04	0.64 ± 0.01	0.31 ± 0.02	0.61 ± 0.03
		2	0.52 ± 0.02	0.54 ± 0.01	0.29 ± 0.02	0.58 ± 0.01

[a] No sample containing the indicated dye was studied in this category.
[b] E-102 (Tartrazine), E-110 (Sunset Yellow), E123 (Amaranth), E129 (Red-40), E-133 (Brilliant Blue).
[c] Mean ± standard deviation ($n = 4$).

Table 5
Dye concentrations (mg/100 mL) (mean ± standard deviation) obtained for four independent determinations in different lots of soft drinks

Flavor	Dyes[b]	Lot	Brands[c]			
			A	B	C	D
Orange	E-110	1	2.10 ± 0.01	2.05 ± 0.01	3.70 ± 0.01	3.48 ± 0.12
		2	2.12 ± 0.04	2.04 ± 0.02	3.67 ± 0.04	3.50 ± 0.04
	E-123	1	–[a]	–	0.10 ± 0.01	0.16 ± 0.00
		2	–	–	0.09 ± 0.00	0.16 ± 0.00
Grape	E-102	1	–	0.14 ± 0.02	–	–
		2	–	0.14 ± 0.01	–	–
	E-110	1	–	0.20 ± 0.01	–	–
		2	–	0.19 ± 0.00	–	–
	E-123	1	2.01 ± 0.03	2.65 ± 0.05	4.61 ± 0.10	4.06 ± 0.19
		2	2.08 ± 0.05	2.70 ± 0.01	4.58 ± 0.03	4.10 ± 0.07
	E-133	1	2.26 ± 0.06	0.59 ± 0.02	0.58 ± 0.02	0.57 ± 0.01
		2	2.24 ± 0.02	0.60 ± 0.01	0.51 ± 0.04	0.51 ± 0.02

[a] No sample containing the indicated dye was studied in this category.
[b] E-102 (Tartrazine), E-110 (Sunset Yellow), E123 (Amaranth), E129 (Red-40), E-133 (Brilliant Blue).
[c] Mean ± standard deviation ($n = 4$).

of passion fruit juice powder of brand D were also shown to be statistically different.

It is worth of note that brands C and D that showed the largest variations of concentration values represent cheaper products with a higher consumption in lowest income people. Our results may indicate poor production and quality controls or even both for these foodstuffs.

The concentrations of the studied dyes in different lots of jelly powder and soft drink are shown in Tables 4 and 5, respectively. No significant differences were found between lots the studied samples indicating better production and quality control of these products.

Sunset Yellow was the most widely employed dye and it appeared in 78% of the studied foodstuffs. Tartrazine and Amaranth were present in 57% and 44% of the products while Red 40 and Brilliant Blue were used in few products. Tartrazine was frequently used in solid juice powders while the use of Sunset Yellow and Amaranth predominated in jelly powders and soft drinks.

Tartrazine concentrations varied between 0.11 and 5.62 mg/100 g in the studied products depending on the considered flavor, brand and foodstuff. Certain products such as mango juice powders presented the largest concentrations of Tartrazine (mean of 4.64 mg/100 g). Pineapple

juice powder (brand B) also presented a large level of Tartrazine (3.19 mg/100 g). A comparison of different brands of the same flavors shows that the concentrations of Tartrazine varied widely in some cases corresponding to a factor of 5. Flavors corresponding to the palest colors (pineapple and cashew) showed in general the lowest concentrations of Tartrazine (0.03–0.74 mg/100 g).

The concentrations of Sunset Yellow varied widely (0.05–9.31 mg/100 g) depending also on the product, flavor and brand. Moreover different lots of brands C and D showed concentrations that were statistically significant different. Besides, as pointed above mango juice powder of brand D showed a concentration similar to the limit established by Brazilian legislation.

The other dyes were present in fewer products and all of then showed concentrations below the maximum values of the Brazilian legislation. Amaranth concentrations varied between 0.97 and 6.17 mg/100 g and its largest concentrations were found in grape soft drinks with values that correspond to 40–92% of the maximum legislated value.

It is worth of note that dye concentrations were always below the maximum legislated values except for Sunset Yellow in one lot mango juice powder of brand D.

However our results indicate the importance of displaying dye concentrations in the labels of commercial products since that data would allow the final consumer to choose between brands, flavors and products selecting those containing the lowest concentrations quantities and number of dyes. For example pineapple juice leads to a consumption of a lower quantity of Tartrazine than pineapple jelly and brand B contains the lowest concentration of this dye. Moreover considering that the studied foodstuffs are not nutritionally essential or adequate and that in many cases they contain other food additives, it would be possible to choose or opt between products considering the concentrations and number of dyes that each product contains.

This possibility of choice is more relevant in products containing Tartrazine that is suspected of causing several health problems (Beseler, 1999; Ortolani et al., 1999). Furthermore it has to be considered that most of the studied foodstuffs have children as their major final consumers and this way their higher physiological susceptibility in neglected.

4. Conclusions

The proposed method showed to be fast and to have a high throughput that is adequate for the study of a large number of samples. The figures of merit of the method (detection limits, quantification limits, recoveries and linearities) were also adequate for the simultaneous study of samples containing a wide range of concentrations.

The levels of the studied dyes were lower than their maximum values established by the Brazilian legislation. However it was observed that in some brands the concentrations of certain dyes varied widely when different lots of products were compared. It is possible that this lack of production and quality controls may allow the eventual production of foodstuff containing dye levels larger than the legislated values.

Furthermore dye concentrations varied between brands with values that were up to 6 times the lowest levels of products with the same flavor. Data about the concentrations of dyes would be an important instrument to help consumers in selecting more adequate products to avoid the intake of large amounts of additives, which can led to health problems, mainly in children.

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