Separation and quantification of Tartrazine (E102) and Brilliant Blue FCF (E133) in green colored foods and beverages

Serena Fiorito [a], Francesco Epifano [a,*], Lucia Palumbo [a], Chiara Collevecchio [a], Roberto Spogli [b], Salvatore Genovese [a]

[a] Dipartimento di Farmacia, Università "G. d'Annunzio" Chieti - Pescara, Via dei Vestini 31, 66100 Chieti Scalo (CH), Italy
[b] Prolabin & Tefarm Srl, Via dell'Acciaio 9, 06134 Perugia, Italy

ARTICLE INFO

ABSTRACT

In the present study we investigated the capacities of a panel of 25 solid sorbents represented by layered structures, inorganic oxides and hydroxides, and phyllosilicates, to effectively remove in high yield Tartrazine (E102) and Brilliant Blue FCF (E133) from aqueous solutions, and more notable, green colored food matrices. Quantification of the title compounds have been achieved by HPLC-DAD analyses. Contents of E102 and E133 in real samples were in the range 1.3–36.5 µg/mL and 1.0–20.1 µg/mL, respectively. After a treatment of 1 min., in most cases a complete bleaching of solutions and deep coloring of the solid phase was recorded. The most effective solids to this aim were seen to be aluminium based ayered double hydroxides. In the case of magnesium oxide for E102, and magnesium aluminium D. benzensulfonate SDS 01 H8L and Florisil for E133, a selective adsorption (>99.9 %) of only one dye was observed. The adsorption recorded was strictly dependent on the loading of the sorbent. Related values were 300 mg for the separation of E102 by magnesium oxide from all the five food matrices under investigation, and in the range 200 mg–300 mg for magnesium aluminium D. benzensulfonate SDS 01 H8L and Florisil in the case of E133. The application of Langmuir and Freundlich models suggested that the adsorption may take place in the inner layers of the solids with a favourable thermodynamique outcome. Findings described herein offer the concrete possibility of quantifications of individual dyes in matrices containing more than one food colorant.

1. Introduction

Synthetic dyes are organic compounds used for imparting color to textile, paper, leather, plastic, food, beverages, cosmetic and pharmaceutical formulations, and many other goods. In the food sector, such chemicals have witnessed the widest use. Food color additives in fact have the undoubted advantage over naturally occurring pigments (e.g. chlorophyll, carotenoids, xanthophylls, flavonoids, etc.) (Sigurdson, Tang, & Giusti, 2017) in being much more stable to photo- and air oxidation, to changes in pH, to microbial contamination, and to relatively high temperatures. Furthermore, synthetic dyes are commercially available at lower costs, provide uniformity of color to products, and are characterized by a good tinting capacity (Silva, Reboredo, & Lidon, 2022; Sharma, McKone, & Markow, 2011). The more and more growing use of food color additives is essentially justified by marketing reasons, in that the more products are attractive, the more will be purchased and consumed by end users. To this concern, dyes are mainly employed in

confectioneries, jams, sweets, candies, jellies, drinks, syrups, ice-creams, and similar products to reduce color loss during processing, restore and make more brilliant natural colors, and enable an easy identification on sight of commercial foodstuffs by consumers (Amchova, Kotolova, & Ruda-Kucerova, 2015). From a chemical point of view, food dyes are classified into five main groups: a) azo dyes, b) triphenylmethane dyes, c) quinoline dyes, d) indigo dyes, and e) xanthenes dyes (Lipskikh et al., 2020). Tartrazine (TZ) (trisodium 1-(4-sulfonatophenyl)-4-(4-sulfona-tophenylazo)-5-pyrazolone-3-carboxylate), also known as E102, C.I. 19140, Yellow 5, Yellow 5 Lake, Acid Yellow 23, or Food Yellow 4, and Brilliant Blue FCF (BB) (disodium 2-[[4-[ethyl-[(3-sulfonatophenyl) methyl]amino]phenyl]-[4-[ethyl-[(3-sulfonatophenyl)methyl]azaniu-mylidene]cyclohexa-2,5-dien-1-ylidene]methyl]benzenesulfonate), also known as E133, Blue No. 1, or acid blue 9 are among the main representatives and widely employed dyes and belong to the azo and triphenylmethane dyes groups, respectively. These additives are used separately to provide brilliant lemon yellow and blue colors

respectively, or in mixture to produce various green shades. However, it is nowadays notorious that overuse of both TZ and BB in foodstuff represents a serious risk for human health, especially for children (Merinas-Amo, Martinez-Jurado, Jurado-Güeto, Alonso-Moraga, & Merinas-Amo, 2019). Allergic asthma, anaphylaxis reactions, disfunctions of the immune system (Vojdani & Vojdani, 2015), sleeping disorders (Rowe & Rowe, 1995), hyperactivity in infants (Stevens, Burgess, Stochelski, & Kuczek, 2014), teratogenesis (Hashem, Abd-Elhakim, Abo-El-Sooud, & Eleiwa, 2019), kidney (El Rabey et al., 2019) and liver (Saxena & Sharma, 2015) diseases, DNA damage (Mpountoukas et al., 2010), and carcinogenesis (Kobylewski & Jacobson, 2012) may represent the most serious risks associated to a prolonged dietary intake. Therefore, the use of both TZ and BB as food additives is strictly under the control of local laws and regulations, although there are no uniform rules in the world. In Europe, the advice given by the European Food Safety Authority (EFSA) in 2009 for TZ (EFSA Panel on Food Additives and Nutrients Sourced Added to Food, 2009) and 2010 for BB (EFSA Panel on Food Additives and Nutrients Sourced Added to Food, 2010), led the EU Commission to set limit concentrations for acceptable daily intake (ADI) to 7.5 mg/ Kg body weight (b.w.) and 12.5 mg/Kg b.w. for TZ and BB, respectively. Such values have been also adopted by the World Health Organization (WHO). Consequently, monitoring the presence of TZ and BB and the development of easy to handle, rapid, sensitive, efficient, and alternative techniques for their identification and quantification in foodstuffs is a field of research of considerable importance and growing interest. In green-colored food matrices, TZ and BB coexist in different percentages, so the development of analytical methodologies for their simultaneous assessment is always necessary. Despite numerous processes for the determination of TZ (Rovina, Siddiquee, & Shaarani, 2017) and BB (Guo, Wu, Du, & Fu, 2013) have been reported in the literature, much less investigations have been focused to the simultaneous quantitative analyses of their mixture. The few reports include the voltammetric detection at the surface of a multiwalled carbon nanotube paste (Ghoreishi, Behpour, & Golestaneh, 2011), ionic liquid-modified expanded graphite paste and electrodes (Wang, Chen, Zhang, Wang, & Chen, 2015), carbon black-polyethylene composite electrodes (Lipskikh et al., 2020), UV–Vis spectrophotometry without any treatment (Antakli, Nejem, & Katran, 2015) or coupled to solid phase adsorption on Amberlite XAD-7 resin (Bisgin, 2020), adsorption on chitosans (Zhang et al., 2020), and finally high performance thin layer chromatography (Nandanwadkar & Mastiholimath, 2020). Electrochemical methodologies well performed in general especially for what concerned reproducibility, stability, and reusability, even if sophisticated instrumentations are needed. These may be not so promptly at disposition and easy to use, in particular for their cost and maintenance. On the other hand, adsorption-based techniques have undoubted advantages for the simple experimental design, ease of use, regeneration of sorbents, low detection limit, low cost, high preconcentration factor, extraction yields, precision, and robustness (Ścigalski & Kosobucki, 2020). Due to interferences from the matrices and to the low concentrations of dyes, a separation and a pre-concentration step would be highly desirable to improve the effectiveness and significance of the analytical process. In this study, a selective extraction method in the heterogeneous phase of TZ and BB from artificial mixtures and green colored food and beverage samples using a panel of differently structured and functionalized solid materials is reported. To the best of our knowledge, the possibility of separating and handling these two dyes in matrices by a selective adsorption is reported herein for the first time.

2. Materials and methods

2.1. Chemicals, equipment, and matrices

CH₃CN, H₂O, MeOH, and EtOH (all HPLC grade), NH₄OAc (purity ≥ 99.99 % trace metals basis), Tartrazine (analytical standard, purity ≥ 97.0 % HPLC), and Brilliant BlueFCF (analytical standard, purity ≥ 97.0 % HPLC) were acquired from Honeywell Research Chemicals (Charlotte, North Carolina, USA) and Merck Millipore (Darmstadt, Germany). Sorbents listed as entries A-N and S-W in Table 1 are products commercialized and kindly supplied by Prolabin & Tefarm Srl (Perugia, Italy). Solids indicated by entries O-R and X-Z have been obtained from Merck Sigma-Aldrich (Darmstadt, Germany).

The same HPLC apparatus Agilent 1100, as already reported (Fiorito et al., 2022), was employed to accomplish analyses and data processingon artificial mixtures and food and beverages matrices containing TZ and BB (Fiorito et al., 2021). The other main experimental steps have been performed using weighing balance mod. Sartorius BSA224-CW (Sartorius Lab Instruments GmbH & Co. KG, Goettingen, Germany), a vortex mixer Neuation mod. iSwix VT (Neuation Technologies Pvt. Ltd, Gujarat, India), a centrifuge Eppendorf mod. 5430R (Eppendorf, Hamburg, Germany), and a pH meter Cole Parmer mod. P200 (Cole Parmer Srl, Cernusco sul Naviglio, Milan, Italy).

Six different green colored food and beverages matrices were used: 1 liqueur (high alcoholicgrade), green apple flavoured candies, dried pomelo [*Citrus maxima* (Burm.) Merr., Rutaceae] peels, "icing green" food colouring agent, 1 soft drink (low alcoholic grade), and pistachio (*Pistacia vera* L., Anacardiaceae) cream. All samples were purchased from a local market and stored at room temperature before use.

2.2. HPLC-DAD analytical conditions and parameters

HPLC analytical conditions (e.g. column, mobile phase composition, flow rate, injection volume) were the same as recently reported (Fiorito et al., 2021), with a total run time of 18.0 min. Wavelengths for the qualitative and quantitative analysis were set at 427 nm for TZ and 630 nm for BB. Validation in terms of precision, accuracy, linearity, limits of detection (LOD) and limits of quantification (LOQ) was accomplished

Table 1
List of inorganic and inorganic/organic solidsstudied herein for their effectivenessin removing E102 and E133from aqueous solutions and food and beverages matrices.

	Entry
Layered double hydroxides	
Zinc aluminium oleate	A
Zinc aluminium nitrate	B
Zinc aluminium chloride	C
Magnesium aluminium nitrate	D
Magnesium aluminium azelate	E
Magnesium aluminium hydroxide chloride	F
Magnesium aluminium hydroxide acetate	G
Magnesium aluminium hydroxide carbonate	H
Magnesium aluminium acetate	I
Zinc hydroxy chloride	J
Lamellar solids	
Zirconium phosphate (type B)	K
Zirconium phosphate (type B) + octadecylamine	L
Oxides/Hydroxides	
Alumina	M
Silica	N
Magnesium oxide	O
Magnesium hydroxide	P
Phyllosilicates	
Bentonite	Q
Talc	R
Mica L	S
Mica F	T
Mica SFG 20	U
Magnesium aluminium D. Benzensulfonate SDS 01 H8L	V
Zinc aluminium D. Benzensulfonate SDS 02 H8L	W
Florisil	X
Montmorillonite	Y

following the provisions given by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (Ntrallou, Gika, & Tsochatzis, 2020). Intra-day and inter-day precisions have been accomplished following the same route as recently reported (Fiorito et al., 2021). Precision was determined at the following concentration values for quality control (QC): QC$_{low}$ = 0.5 µg/mL, QC$_{medium}$ = 10.0 µg/mL, and QC$_{High}$ = 40.0 µg/mL. Accuracy was assessed by spiking the filtrate obtained after application of MgO (entry M) to the TZ aqueous solution and after application of Florisil (entry X) to the one containing BBwith the just mentioned concentrations (low, medium, and high) of the respective pure chemical standard solutions. Calibration curves were constructed by injecting standard mixture solutions at the following ten concentration values:0.1 µg/mL, 0.5 µg/mL, 1.0 µg/mL, 2.5 µg/mL, 5.0 µg/mL, 10.0 µg/mL, 15.0 µg/mL, 20.0 µg/mL, 30.0 µg/mL, and 40.0 µg/mL. LODs and LOQs were calculated following the same route as recently reported (Fiorito et al., 2021). Values of the above listed and other main analytical parameters are reported in Table 2.

2.3. Sample preparation and extraction

TZ and BBpure chemical standards wereboth dissolved into HPLC-grade H$_2$O to a final concentration of 20.0 µg/mL. The one so obtained represented the blank solution. This latter was also employed for the treatment for solid phase adsorption experiments purposes. In particular, the initial treatment consisted in adding 100 mg of each of the 25 solid supports listed in Table 1 added to the blank solution (5 mL) to assess their performance and their selectivity in the adsorption of individual TZ and/or BB. The resulting heterogeneous mixtures were vigorously magnetically stirred at room temperature for 5 min. As the final step each suspension was poured into an Eppendorf tube and centrifuged (5000g, room temperature, 5 min.). The resulting supernatant was decanted and submitted to HPLC analyses. The amount of TZ and BB retained on each solid was determined by subtracting the quantity present in the parent blank solution and the one in the discarded supernatant.

Moving to food and beverages matrices, slightly different protocols were applied. For the liqueur (high alcoholicgrade) the stock solution was prepared by diluting 2 mL of the same with HPLC-grade H$_2$O (3 mL). The resulting mixture was then used for further experiments and HPLC analyses. Green food colouring agent (2.5 mg), pistachio cream (5.0 g), and pieces of dried pomelo peels (4.0 g) were weighted and extracted with HPLC-grade H$_2$O (5 mL). The resulting suspensions were allowed to react under magnetic stirring for 24 h at room temperature and filtered to obtain the final solutions used for the subsequent experiments and HPLC analyses. Candies (1.4 g) were finely triturated and the so obtained powder dissolved in HPLC-grade H$_2$O (5 mL) until a homogenous green color of the liquid phase was observed. This was then used for experiments and HPLC analyses. Finally, for the soft drink (3 mL) a pretreatment with 1 N NaOH (290 µL) to adjust the pH value to 7 was necessary prior accomplishing further experiments and HPLC analyses (e.g. citric acid was among the main ingredients, thus altering the structure of the chromophore of both TZ and BB by protonation of sulfonate residues).

All the above-mentioned aqueous solutions were then submitted to treatments with solids (200 mg for the preliminary screening of the respected efficiency) listed in Table 1 and the adsorbed amounts of TZ and BB assessed in the same way as outlined above.

2.4. Isotherm adsorption experiments

Isotherm adsorption experiments were performed using the protocol recently reported by Zhang and coworkersin 2020. Thus, the Langmuir and Freundlich isotherm equations were used to model the experimental data. Both models are described by the following equations.

(a)

$$\frac{C_e}{q_e} = \frac{1}{(K_L \times q_m)} + \frac{C_e}{q_m}$$

(b)

$$\log q_e = \log K_F + \frac{1}{n}\log C_e$$

where C_e is the equilibrium concentration of TT and BB (µg/mL), q_e is the amount of dyes adsorbed (µg/mg), q_m is the maximum amount of dyes per unit weight of sorbents(µg/mg), K_L is the Langmuir adsorption equilibrium constant (mL/µg); $K_F$isthe Freundlich constant (µg/mg (mL/µg)$^{1/n}$), while $\frac{1}{n}$ is the dimensionless parameter representing the measurement of adsorption intensity.

Sample materials for the elaboration of diagrams were MgO (entry M) for TZ and Florisil (entry X) for BB and the liqueur (high alcoholicgrade) and the green food colouring agentfor food samples. Adsorption studies were carried out by adding different amount of each sorbent, namely 10, 25, 50, 100, and 200 mg for MgO, and 25, 50, 100, 200, 300, and 400 mg for Florisil to an aqueous solution deriving from dilution of these two matrices performed as described above.

All the assays reported in this manuscript were accomplished in triplicate. Statistical analyses were elaborated following the same general procedure as already reported in the literature (Epifano et al., 2020).

3. Results and discussion

The panel of 25 solid materialsemployed to accomplish the removal in the heterogenous phase of TZ and BB from the above-mentioned matrices is reported in Table 1. Different classes of sorbents were

Table 2
HPLC-DAD method validation parameters for Tartrazine and Brilliant Blue.

	TZ	BB
Retention time (min.)	9.92	13.56
Slope(S)	14,059	12,733
Intercept	−804	−274
r^2	0.9991	0.9992
SD$_s$ (slope)	997	821
SD$_i$ (intercept)	57	66
SD$_r$ (residuals)	0.241	0.198
LOD (µg/mL)	0.23	0.21
[3.3 × SD$_s$/S]		
LOQ (µg/mL)	0.71	0.64
[10 × SD$_s$/S]		
Precision		
Intra-day	**% RSD (n = 3)**	
QC$_{low}$	100.2/0.995	100.3/0.997
QC$_{medium}$	100.1/0.994	100.2/1.003
QC$_{high}$	100.3/1.004	100.2/0.998
Inter-day	**% RSD(n = 3)**	
QC$_{low}$	100.1/1.008	99.7/1.001
QC$_{medium}$	99.7/0.992	99.8/1.002
QC$_{high}$	100.2/1.003	99.7/0.996
Accuracy		
Intra-day	**% Recovery (n = 3)**	
QC$_{low}$	101.1/1.005	100.7/1.003
QC$_{medium}$	101.4/0.999	101.1/1.002
QC$_{high}$	100.8/0.999	100.6/0.997
Inter-day	**% Recovery (n = 3)**	
QC$_{low}$	100.1/0.997	100.1/0.997
QC$_{medium}$	101.4/0.998	100.8/0.999
QC$_{high}$	104.1/0.991	102.0/1.006
Capacity factor	1.1 ± 0.03	1.2 ± 0.04
Resolution	1.2 ± 0.03	1.1 ± 0.05
Peak symmetry	1.03 ± 0.01	1.02 ± 0.02
Tailing	0.99 ± 0.01	0.98 ± 0.02

used: layered double hydroxides (LDHs), zirconium-based lamellar solids, inorganic oxides and hydroxides, and phyllosilicates. Before moving to food and beverage matrices, we screened the efficiency of all the listed solids on artificial mixtures containing both TZ and BB. To this aim, a stock solution containing both dyes was prepared by dissolving them into HPLC-grade H₂O. The same green coloured solution was used as the blank for the subsequent HPLC analyses and quantitative determination. Adsorption experiments were carried out by adding each solid to the blank solution, as indicated in the Material and Methods section. At a glance, the outcome of the reactions, for some sorbents, was dramatically clear even with the naked eye: some sorbents were colored in green while some others in deep blue or yellow. This indicated that some solids exhibited a selectivity towards TZ and BB (vide infra). Subsequently, each suspension was placed into an Eppendorf tube, centrifuged and the supernatant decanted, filtered under vacuum, and submitted to HPLC analyses. The amount of TZ and BB adsorbed was calculated as the difference ofthe amount of both dyes in the parent blank solution and the one determined in the supernatant. Values of the quantifications are reported in Table 3. Final recorded data are the results of three independent HPLC runs. For all assays t-test values [95 % confidence level ($\nu = 2$)] are lower than the theoretical ones (data not shown).

Data reported in Table 3 allows to appreciate how nearly the half (12 out of 25) of the materials tested were able to adsorb one or both dyes in percentages > 85 %.9 out of 25 provided a complete adsorption on solids. However, the most interesting data are those recorded for MgO (/entry O), MgAl D. Benzensulfonate SDS 01 H8L (entry V), and Florisil (entry X) for which a selective and complete adsorption of TZ (in the case of MgO) and BB (in the case of Mg Al D. Benzensulfonate SDS 01 H8L, and Florisil) was achieved. For other solids, namely Zn Al nitrate (entry B), Zn Al chloride (entry C), Mg Al nitrate (entry D), Mg Al azelate (entry E), Mg Al hydroxide chloride (entry F), and Zr phosphate (type B) + octadecylamine (entry L) a complete bleaching of the green coloured solution was observed. Those outlined in the previous section of the present manuscript represented the best experimental route in terms of sorbent loading, experimental times, temperature, and adsorption yields. The following variations of some chemical-physical parameters have been made in the case of this preliminary screening of adsorption

efficiency: increases of the quantity of sorbent materials up to 500 mg of times to 30 min., and of temperatures to 75 °C. All these resulted in no appreciable improvements of dye removal, also in the case of the most effective sorbents. There was no need to adjust the pH of mixtures undergoing treatment with solid sorbents to further optimize the experimental conditions and recovery yields. This can be explained by the fact that clays has a well documented high capacities of ion exchange and buffering in their interlayer spaces (Takahashi et al., 1987). As a consequence, both TZ and BB can find in this microenvironment the best conditions for their large adsorption, without the need to modify pH of the medium to adjust their ovella charge and polarization. Meanwhile, also the addition to working solutions of TZ and BB of potentially interfering species found as ingredients in food samples like glucose, fructose, saccharose, potassium sorbate, sodium benzoate, ascorbic acid, and sodium chloride provided no effects on recoveries of both TZ and BB. These values remain unaffected and comprised in the range 99.8 %−100.2 %.

These findings were extremely encouraging to move on with investigations. In fact, it could be hypothesized that the same trend of selective extraction of TZ or BB could be observed also in the case of green coloured food and vegetable matrices. To this aim the following commercially available products were selected: one liqueur (high alcoholic grade), green apple flavoured candies, dried pomelo peels, "icing green" food colouring agent, one soft drink (low alcoholic grade), and pistachio cream. For all, the presence, but not the quantity, of only E102 and E133 was declared on the label. The first acquisition was the content of individual dyes in the respective matrices, prepared in a form suitable for HPLC analyses as described in the Material and Methods sections (e. g. aqueous solutions). For the above listed food and drink matrices we decided to test only the most effective sorbents in terms of extractive yields and, more importantly, selectivity. Thus, MgO (entry O), Mg Al D. Benzensulphonate SDS 01 H8L (entry V), and Florisil (entry X), were used at different amounts ranging from 25 to 400 mg, depending on the complexity of the matrices (e.g. consistency of the mixtures and physical state). Such solids were so added to solutions derived from food and drink matrices directly into an Eppendorf tube, vortexed and centrifuged. The resulting supernatant was finally analyzed by HPLC to evaluate the adsorption effectiveness and capacity. Data were recorded as percentages (%) and reported in Table 4. Results from experiments accomplished with pistachio cream are not reported as the consistency of the mixture (very thick emulsion) did not allow an effective mixing.

Data outlined in Table 4 revealed that, also in the case of food and beverages derived matrices, the pattern of selective adsorption of TZ and BB was very similar to that recorded with artificial mixtures. For every sorbent a dose dependent increase of adsorption capacities was observed. At the highest amount applied a quantitative extractive yield was recorded and, in some instances, (e.g. Mg Al D. Benzensulphonate SDS 01 H8L, 25 mg for the liquer high alcoholic grade but practically also for the green food coloring agent and the soft drink with low alcoholic grade, and Florisil 200 mg for all food and beverages entries) even for the lowest loading of the sorbent. In only one case for a 100% extraction of a dye the percentages of the other exceed 4%, while in all the others, values were in the range 1.3%−2.0%.

A relevant point emerging from the data reported in Table 4 is that both dyes recorded a higher percentage of adsorption, especially for lower sorbent loadings, when part of matrices having alcohol as an ingredient. In this case the addition of H₂O as a sample preparation treatment before accomplishing the solid phase adsorption step, resulted in a hydroalcoholic mixture, that may favour a higher retention on tested solids of both TZ and BB. A similar pattern was also recorded in our previous experiences with natural pigments like anthraquinones (Epifano et al., 2020).

The picture of the putative intimate interaction between synthetic dyes like TZ and BB with the solid sorbents listed herein has been already provided (Fiorito et al., 2022). Briefly, the large adsorption recorded with possible location of the two analytes in the interlayer

Table 3
Quantitative determination of TZ and BBadsorbed onto sorbents (A-Z) from the respective artificial aqueous solution at the concentration of 20 μg/ml by HPLC-DAD. Values are expressed as μg/ml, percentage, and as mean ± SD (n = 3).

Sample entry	TZ		BB	
	μg/mL	%	μg/mL	%
A	7.0 ± 0.04	36.4 ± 0.02	19.7 ± 0.02	92.9 ± 0.01
B	20.0 ± 0.01	100 ± 0.01	20.0 ± 0.02	100 ± 0.01
C	20.0 ± 0.02	100 ± 0.01	20.0 ± 0.01	100 ± 0.01
D	20.0 ± 0.02	100 ± 0.01	20.0 ± 0.01	100 ± 0.01
E	20.0 ± 0.01	100 ± 0.01	20.0 ± 0.01	100 ± 0.01
F	20.0 ± 0.01	100 ± 0.01	20.0 ± 0.01	100 ± 0.01
G	14.5 ± 0.08	75.2 ± 0.04	6.4 ± 0.06	30.2 ± 0.04
H	6.4 ± 0.03	32.9 ± 0.01	19.7 ± 0.03	94.7 ± 0.02
I	12.6 ± 0.07	65.2 ± 0.04	8.3 ± 0.06	39.1 ± 0.03
L	20.0 ± 0.01	100 ± 0.01	20.0 ± 0.01	100 ± 0.01
M	9.9 ± 0.09	51.4 ± 0.05	10.9 ± 0.06	51.4 ± 0.04
N	6.4 ± 0.05	33.0 ± 0.03	17.5 ± 0.09	82.5 ± 0.05
O	20.0 ± 0.02	100 ± 0.01	2.4 ± 0.05	11.7 ± 0.02
P	12.8 ± 0.06	66.1 ± 0.03	8.4 ± 0.05	39.7 ± 0.03
Q	2.8 ± 0.01	22.8 ± 0.01	10.9 ± 0.06	51.5 ± 0.04
R	8.5 ± 0.04	43.9 ± 0.02	19.7 ± 0.05	94.7 ± 0.03
S	12.2 ± 0.08	62.8 ± 0.05	17.6 ± 0.07	83.2 ± 0.04
T	8.5 ± 0.07	43.8 ± 0.04	18.2 ± 0.07	86.0 ± 0.04
U	8.7 ± 0.07	45.0 ± 0.03	17.1 ± 0.05	80.6 ± 0.02
V	2.1 ± 0.03	10.5 ± 0.03	20.0 ± 0.01	100 ± 0.01
W	6.6 ± 0.02	34.0 ± 0.04	14.8 ± 0.08	69.9 ± 0.05
X	1.6 ± 0.02	8.0 ± 0.01	20.0 ± 0.02	100 ± 0.01
Y	6.4 ± 0.04	33.0 ± 0.02	8.4 ± 0.05	39.7 ± 0.03

Table 4

Adsorption capacity of TZ and BB contained in food and drink matrices for MgO (entry O), Mg Al D. Benzensulphonate SDS 01 H8L (entry V), and Florisil (entry X). Values are expressed as µg/mL (percentages in parenthesis), andas mean ± SD (n = 3).

	TZ					BB				
Entry	1	2	3	4	5	1	2	3	4	5
Blank O	18.1 ± 0.05	14.4 ± 0.04	13.3 ± 0.04	36.5 ± 0.07	1.2 ± 0.02	19.3 ± 0.04	20.1 ± 0.03	1.1 ± 0.01	17.9 ± 0.02	1.0 ± 0.01
50 mg	9.7 ± 0.04 (53.6 ± 0.05)	7.0 ± 0.03 (48.8 ± 0.04)	7.7 ± 0.02 (57.9 ± 0.06)	17.5 ± 0.06 (48.1 ± 0.07)	0.9 ± 0.02 (79.4 ± 0.04)	NA	NA	NA	NA	NA
100 mg	15.6 ± 0.06 (87.0 ± 0.06)	9.3 ± 0.02 (64.7 ± 0.05)	9.2 ± 0.03 (69.2 ± 0.04)	31.3 ± 0.08 (85.8 ± 0.05)	1.0 ± 0.02 (90.1 ± 0.02)	NA	NA	NA	NA	NA
200 mg	17.6 ± 0.04 (97.2 ± 0.03)	15.6 ± 0.06 (88.1 ± 0.02)	11.2 ± 0.04 (84.3 ± 0.06)	34.5 ± 0.07 (94.5 ± 0.02)	1.2 ± 0.01 (100.0 ± 0.01)	0.3 ± 0.01 (1.9 ± 0.01)	0.3 ± 0.02 (1.7 ± 0.02)	NA	0.4 ± 0.01 (2.1 ± 0.03)	NA
300 mg	18.1 ± 0.03 (100.0 ± 0.02)	14.4 ± 0.04 (100.0 ± 0.02)	13.3 ± 0.03 (100.0 ± 0.03)	36.5 ± 0.01 (100.0 ± 0.01)	1.2 ± 0.01 (100.0 ± 0.02)	0.7 ± 0.03 (3.6 ± 0.02)	0.8 ± 0.02 (4.2 ± 0.04)	NA (3.3 ± 0.02)	0.9 ± 0.03 (5.1 ± 0.02)	NA
V										
25 mg	0.4 ± 0.02 (2.4 ± 0.01)	NA	NA	NA	NA	19.3 ± 0.02 (100.0 ± 0.02)	14.0 ± 0.03 (69.8 ± 0.02)	0.9 ± 0.01 (79.5 ± 0.02)	17.2 ± 0.02 (96.3 ± 0.04)	0.9 ± 0.04 (94.2 ± 0.04)
50 mg	0.5 ± 0.03 (2.6 ± 0.02)	NA	NA	NA	NA	19.3 ± 0.03 (100.0 ± 0.03)	15.8 ± 0.06 (78.9 ± 0.03)	0.9 ± 0.02 (83.0 ± 0.03)	17.9 ± 0.02 (100.0 ± 0.02)	1.0 ± 0.02 (100.0 ± 0.01)
100 mg	0.5 ± 0.01 (2.7 ± 0.01)	0.2 ± 0.01 (1.3 ± 0.02)	NA	1.0 ± 0.02 (2.8 ± 0.03)	NA	19.3 ± 0.05 (100.0 ± 0.01)	19.2 ± 0.07 (95.5 ± 0.04)	1.1 ± 0.01 (100.0 ± 0.01)	17.9 ± 0.02 (100.0 ± 0.02)	1.0 ± 0.01 (100.0 ± 0.03)
200 mg	0.5 ± 0.01 (2.8 ± 0.02)	0.3 ± 0.02 (1.9 ± 0.01)	NA	1.6 ± 0.04 (4.5 ± 0.04)	NA	19.3 ± 0.01 (100.0 ± 0.01)	20.1 ± 0.03 (100.0 ± 0.02)	1.1 ± 0.04 (100.0 ± 0.03)	17.9 ± 0.01 (100.0 ± 0.01)	1.0 ± 0.01 (100.0 ± 0.02)
X										
200 mg	0.4 ± 0.01 (2.1 ± 0.02)	NA	NA	0.4 ± 0.02 (1.1 ± 0.02)	NA	18.9 ± 0.07 (97.9 ± 0.05)	19.1 ± 0.02 (95.2 ± 0.04)	1.0 ± 0.01 (98.7 ± 0.03)	16.7 ± 0.06 (93.6 ± 0.04)	1.0 ± 0.02 (100.0 ± 0.01)
300 mg	0.4 ± 0.03 (2.2 ± 0.01)	NA	NA	0.4 ± 0.03 (1.3 ± 0.03)	NA	19.3 ± 0.01 (100.0 ± 0.01)	20.1 ± 0.02 (100.0 ± 0.01)	1.1 ± 0.01 (100.0 ± 0.03)	17.9 ± 0.04 (100.0 ± 0.03)	1.0 ± 0.02 (100.0 ± 0.02)
400 mg	0.4 ± 0.02 (2.4 ± 0.04)	0.3 ± 0.01 (2.3 ± 0.04)	NA	0.5 ± 0.02 (2.1 ± 0.02)	NA	19.3 ± 0.03 (100.0 ± 0.02)	20.1 ± 0.02 (100.0 ± 0.02)	1.1 ± 0.01 (100.0 ± 0.01)	17.9 ± 0.04 (100.0 ± 0.02)	1.0 ± 0.01 (100.0 ± 0.01)

NA = not present or below LOQ; **1** = liqueur (high alcoholicgrade), **2** = green apple flavoured candies, **3** = dried pomelo peels, **4** = green food colouring agent, **5** = soft drink (low alcoholic grade),

spaces of the solid materials investigated herein could be due to the wide versatility and binding capacity (e.g. ionic bonds, hydrogen bonds, acid-base interactions, Van der Waals interactions, etc.) present in the chemical structure of these same.

To get further insights into the adsorption process, we calculated the adsorption isotherms using MgO (entry O) and Florisil (entry X) as references for solid materials and the liqueur (high alcoholic grade) and the green food colouring agent for food and beverages matrices. The Langmuir and Freundlich equations were used to model the experimental data. Isotherm constants and parameters are reported in Table 5 while the related curves are shown in Fig. 1.

Comparing the r^2 values, the Langmuir model better describes the adsorption of both TZ and BB onto both MgO and Florisil. This evidence suggests that the adsorption may take place in the monomolecular layers of solid materials. The $1/n$ values obtained from the Freundlich model were all <1, which indicated that retention in the solid phase of both TZ and BB was thermodynamically favorable.

Up to now, most of the methods proposed in the literature for the determination of TZ and BB in green colored food matrices refer to simultaneous quantifications. To the best of our knowledge, only six methodologies have been reported in the literature for the quantification of TZ and BB. A survey and comparison between the method reported herein and the ones already reported in the literature have been reported

Table 5

Isotherm constants for Tartrazine (TT)/MgO and Brilliant Blue (BB)/Florisil.

	TZ					
Matrix*	Langmuir**			Freundlich**		
	q_m	K_L	r^2	q_m	K_F	r^2
1	0.219	0.56	0.9979	0.172	0.3153	0.9666
2	1.483	0.205	0.9967	0.110	0.2576	0.9564

	BB					
	Langmuir**			Freundlich**		
	q_m	K_L	r^2	q_m	K_F	r^2
1	0.588	0.085	0.9910	0.038	0.2899	0.9882
2	1.57	0.077	0.9832	0.0487	0.1671	0.9792

*1 = liqueur (high alcoholic grade), 2 = green food colouring agent. ** Values are expressed as the following: q_m (µg/mg); K_L (mL/µg); K_F (µg/mg (mL/µg)$^{1/n}$).

in Table 6.

Among the methodologies reported in this Table, the ones that more closely resemble the proceduredescribed herein, are the pioneering studies reported in 2021 and 2022 by Fernández-Andrade and co-workers. These Authors employed rice husk as the sorbent material and
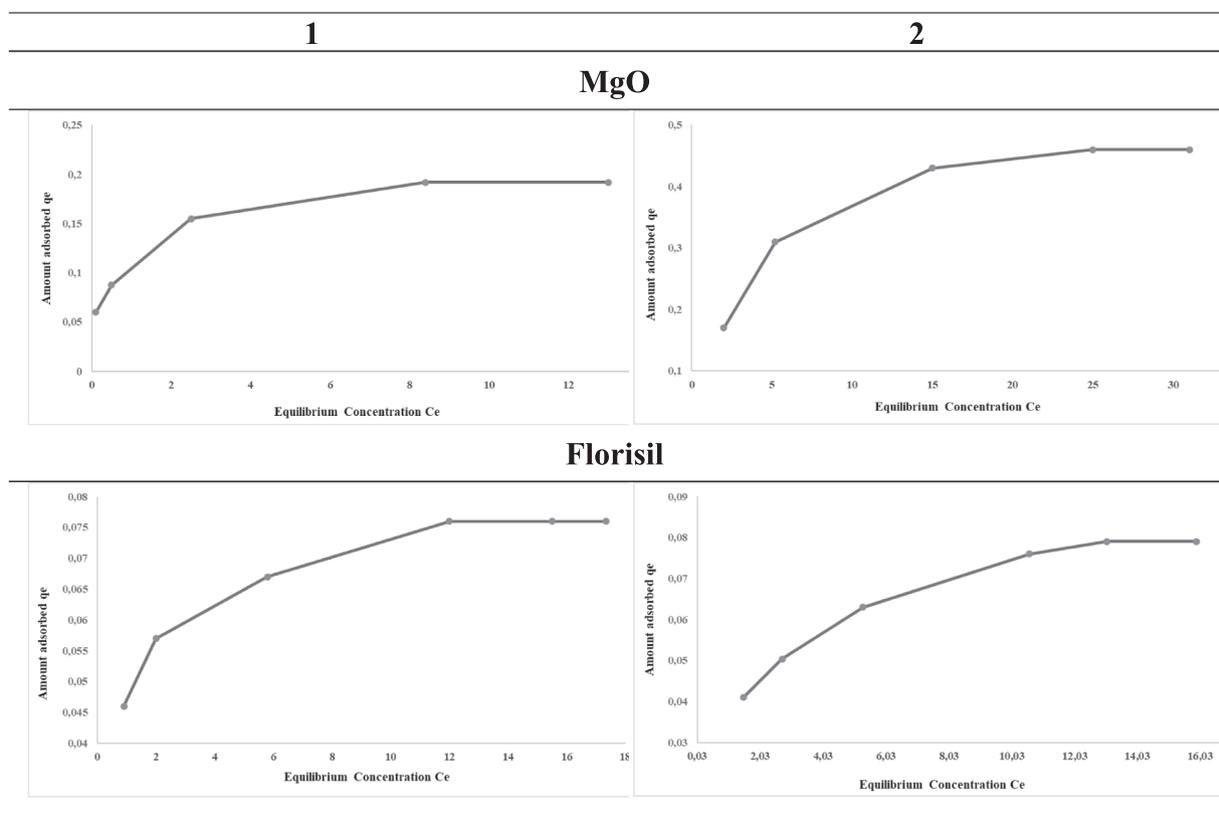
Fig. 1. Equilibrium isotherms for adsorption of TZ and BB components of the liqueur (high alcoholicgrade) (**1**) and the green food colouring agent (**2**) onto MgO and Florisil.

first elaborated a detailed and powerful mathematical model based on the Langmuir and Freundlich adsorption isotherms for mixtures containing TZ and BB at different concentrations. By this way, the Authors could gain insights on phenomena occurring during the adsorption step and study in details the adsorption balance between TZ and BB (Fernández-Andrade, Ávila-Toro, Baquerizo-Crespo, & Rodríguez-Diaz, 2021). Data acquired after a series of preliminary experiments revealed an antagonistic behavior between these two dyes (e.g. the mixture interacted with the sorbent material in such a way to reduce the adsorption capacities of at least one component). In a second series of experiments, using the same materials, these Authors applied a dynamic range (fixed-bed column) and a 3-level response surface design to evaluate the adsorption process. Even if this was found to be dependent on the concentration of TZ and BB, a competitive behavior was still recorded. The sorbent exhibited a higher adsorption capacity towards BB (Fernández-Andrade et al., 2022). The investigation proposed herein can be considered an ideal continuation of studies recently reported by Fernández-Andrade. The same idea of investigating the balance of adsorption of TZ and BB from mixtures containing both was shared, but the methodology was also applied to food and beverages matrices. It was also widened the panel of sorbents and identified some for which a competitive behavior could be claimed (e.g. those materials for which a partial and non-selective adsorption was recorded), while for few others (namely MgO, Mg Al benzensulfonate SDS 01 H8L, and Florisil) a clear selectivity was revealed. Fernández-Andrade and coworkers provided details on the intimate interactions between TZ and BB and the sorbent and accounted for the higher preference of this latter towards BB in terms of the higher number of sulfonate moieties. In the case depicted herein the observed selectivity of adsorption can be also rationalized in terms of the different numbers of sulfonate groups between TZ and BB as well, but also in terms of the differences of 3D structures between Mg Al benzensulfonate SDS 01 H8L and Florisil on one hand, and MgO on the

other. The first are multilayered materials rich in "hard" cations like Mg^{+2} and Al^{+3} located in the interlayer spaces. These same are known to have a high affinity towards sulfonate groups. Thus, as properly hypothesized by Fernández-Andrade and coworkers the selectivity we observed for BB can be due to its higher numbers of such moieties and in the meantime to the absence of other basic sites, according to Lewis, in its structure. Furthermore, BB has a large planar structure that can well fit into the interlayer spaces of both Mg Al benzensulfonate SDS 01 H8L and Florisil by intercalation. On the other hand MgO has a brucite-like structure with interlayer spaces with a minimal distance. Its latice consist of a sequence of alternate Mg^{+2} and O^{-2} ions with which TZ, having a greater variety of basic sites according to Lewis, can effectively simultaneously interact by ionic (on Mg^{+2}) and hydrogen bonds (on O^{-2}).

As stated above, whatever the method used, all the six in so far reported processes for the qualitative and quantitative analyses of TZ and BB from different matrices are all based on a simultaneous determination. The aim of reaching an exact quantification of the title synthetic dyes using the until now reported approaches may be hampered by interferences between these two dyes, as somewhat recorded in previous reports. Thus, what we proposed herein, namely the capacity of some inexpensive and widely accessible solid materials coupled to HPLC/DAD analyses, to provide the selective adsorption of only TZ and BB from green colored food and beverages matrices, have the potential to overcome drawbacks featuring previous investigations. An additional advantage is represented by the easy-to-handle procedure and by the fact that the key step of pre-concentration of TZ or BB is successfully accomplished in "simple" deionized H_2O without the need to adjust pH and/or ionic strength values of extracting solutions to render the process more effective. It is noteworthy also to highlight that regardless of whether the matrix was represented by an artificial solution, or by liquid or solid foodstuffs, the behavior and the effectiveness of the sorbents

Table 6
Comparison of the methodology reported herein and previously reported processes.

Reference	Technique employed	Advantages	Drawbacks
Methodology set-up herein	Solid phase adsorption coupled to HPLC/DAD analyses	a) Easy-to-handle procedure; b) selective separation of the two analytes;c) applicability to a wide range of food matrices; d) use of inexpensive sorbent and totaly recyclable materials; e) good analytical performance; f) no need of pH adjustement.	–
Ghoreishi et al., 2011	Electrochemical analysis (multiwalled carbon nanotube paste electrode)	a) Good analytical performance: b) regeneration of the electrode sensor surface; c) long time stability and reproducibility	Interferences between the two analytes
Wang et al., 2015	Electrochemical analysis (ionic liquid-modified expanded graphite pase electrode)	a) good reproducibility, stability; b) reusability of the electrode	Application limited to soft drinks
Antakli et al., 2015	Spectrophotometry	Good precision and accuracy	Report of rough analytical data
Lipskikh et al., 2020	Electrochemical analysis (carbon black-polyethylene composite electrode)	a) Good efficiency and reproducibility; b) applicable also to solid foodstuff	Higher LODs
Bişgin, 2020	Preconcentration (Amberlite XAD7) + spectrophotometry	a) Applicability to a wide range of food matrices; b) good analytical performance; c) good recoveries.	Pre-concentration yields strictly dependent on pH of the media
Zhang et al., 2020	Preconcentration (Chitosans) + spectrophotometry	a) Application of a multilayer statistical physics modelling to describe the adsorption process	a) Report of rough analytical data, b) only theoretical study
Fernández-Andrade et al. 2021, 2022	Rice husk (dynamic regime + response surface analysis)	a) Good adsorption yields for both dyes; b) use of inexpensive sorbent and totaly recyclable materials; c) easy to handle procedure, d) possibility of application as post treatment columns	a) Competitive adsorption between TZ and BB at equal concentration. Higher affinity for BB by the sorbent, b) application only to artificial mixtures

highlighted in this study is practically identical.

4. Conclusions

In this paper the adsorption of two relevant food dyes by a panel of differently structured solid sorbents was investigated. It was demonstrated for the first time in the literature that the selective removal of a synthetic dye from a mixture of two can occur. This finding may represent an initial, but concrete address to develop novel and alternative methodologies towards the separate rather than simultaneous detection of foodstuffs and beverages coloring agents in complex matrices, thus abolishing and/or greatly diminishing the risk of possible interferences between dye chemicals. The versatility of the solid materials we employed during our investigation, allowing to tailor their intimate chemical and bidimensional and tridimensional structures to a wide array of synthetic dyes, will surely allow to reach the selective separation of several other coloring agents from multicomponent matrices in the next future. Such a kind of investigation is still ongoing in our laboratories.

CRediT authorship contribution statement

Serena Fiorito: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Francesco Epifano:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Lucia Palumbo:** Investigation, Methodology, Software, Validation, Visualization. **Chiara Collevecchio:** Investigation, Methodology, Software, Validation, Visualization. **Roberto Spogli:** Conceptualization, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization. **Salvatore Genovese:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Amchova, P., Kotolova, H., & Ruda-Kucerova, J. (2015). Health safety issues of syntheticfood colorants. *Regulatory Toxicology & Pharmacology, 73*, 914–922. https://doi.org/10.1016/j.yrtph.2015.09.026

Antakli, S., Nejem, L., & Katran, S. (2015). Simultaneous determination of Tartrazine and Brilliant Blue in foodstuffs by spectrophotometric method. *International Journal of Pharmacy & Pharmaceutical Sciences, 7*, 214–218.

Bişgin, A. T. (2020). Simultaneous determination of Tartrazine and Brilliant Blue in diverse sample matrices after solid phase extraction. *Journal of AOAC International, 103*, 1478–1485. https://doi.org/10.1093/jaoacint/qsaa056

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). (2009). Scientific opinion on the re-evaluation Tartrazine (E 102). *EFSA Journal, 7*, 1331.

EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). (2010). Scientific opinion on the re-evaluation of Brilliant Blue FCF (E 133) as a food additive. *EFSA Journal, 8*, 1853.

El Rabey, H. A., Al-Seeni, M. N., Al-Sieni, A. I., Al-Hamed, A. M., Zamzami, M. A., & Almutairi, F. M. (2019). Honey attenuates the toxic effects of the low dose of tartrazine in male rats. *Journal of Food Biochemistry, 43*, e12780.

Epifano, F., Genovese, S., Marchetti, L., Palumbo, L., Bastianini, M., Cardellini, F., … Fiorito, S. (2020). Solid phase adsorption of anthraquinones from plant extracts by lamellar solids. *Journal of Pharmaceutical & Biomedical Analysis, 190*, Article 113515. https://doi.org/10.1016/j.jpba.2020.113515

Fernández-Andrade, A., Ávila-Toro, B., Baquerizo-Crespo, R., & Rodríguez-Díaz, J. M. (2022). Estimation of the bicomponent adsorption behavior of dyes: a modeling approach. In *Communication, Smart Technologies and Innovation for Society: Proceedings of CITIS 2021* (pp. 41–51). Singapore: Springer. https://doi.org/10.1007/978-981-16-4126-8_5.

Fernández-Andrade, K. J., González-Vargas, M. C., Rodríguez-Rico, I. L., Ruiz-Reyes, E., Quiroz-Fernández, L. S., Baquerizo-Crespo, R. J., & Rodríguez-Díaz, J. M. (2022). Evaluation of mass transfer in packed column for competitive adsorption of Tartrazine and brilliant blue FCF: A statistical analysis. *Results in Engineering, 14*, Article 100449. https://doi.org/10.1016/j.rineng.2022.100449

Fiorito, S., Epifano, F., Palumbo, L., Collevecchio, C., Bastianini, M., Cardellini, F., … Genovese, S. (2022). Efficient removal of tartrazine from aqueous solutions by solid sorbents. *Separation & Purification Technology, 290*, Article 120910. https://doi.org/10.1016/j.seppur.2022.120910

Fiorito, S., Epifano, F., Marchetti, L., Palumbo, L., Mascioli, F., Bastianini, M., … Genovese, S. (2021). An improved method for the isolation of amarogentin, the bitter principle of yellow gentian root. *Food Chemistry, 364*, Article 130383. https://doi.org/10.1016/j.foodchem.2021.130383

Ghoreishi, S. M., Behpour, M., & Golestaneh, M. (2011). Simultaneous voltammetric determination of Brilliant Blue and Tartrazine in real samples at the surface of a multiwalled carbon nanotube paste electrode. *Analytical Methods, 3*, 2842–2847. https://doi.org/10.1039/C1AY05327B

Guo, J., Wu, H., Du, L., & Fu, Y. (2013). Determination of Brilliant Blue FCF in food and cosmetic samples by ionic liquid independent disperse liquid–liquid micro-extraction. *Analytical Methods, 5*, 4021–4026. https://doi.org/10.1039/C3AY40362A

Hashem, M. M., Abd-Elhakim, Y. M., Abo-El-Sooud, K., & Eleiwa, M. M. E. (2019). Embriotoxic and teratogeniceffects of Tartrazine in rats. *Toxicological Research, 35*, 75–81. https://doi.org/10.5487/TR.2019.35.1.075

Kobylewski, S., & Jacobson, M. F. (2012). Toxicology of food dyes. *International Journal of Occupational & Environmental Health, 18*, 220–246. https://doi.org/10.1179/1077352512z.00000000034

Lipskikh, O. I., Korotkova, E. I., Barek, J., Vyskocil, V., Saqib, M., & Khristunova, E. P. (2020). Simultaneous voltammetric determination of Brilliant Blue FCF and Tartrazine for food quality control. *Talanta, 218*, Article 121136. https://doi.org/10.1016/j.talanta.2020.121136

Merinas-Amo, R., Martinez-Jurado, R., Jurado-Güeto, S., Alonso-Moraga, A., & Merinas-Amo, T. (2019). Biological effects of food coloring in *in vivo* and *in vitro* model systems. *Foods, 8*, 176. https://doi.org/10.3390/foods8050176

Mpountoukas, P., Pantazaki, A., Kostareli, E., Christodoulou, P., Kareli, D., Poliliou, S., … Lialiaris, T. (2010). Cytogenetic evaluation and DNA interaction studies of the food colorants amaranth, erythrosine and tartrazine. *Food & Chemical Toxicology, 48*, 2934–2944. https://doi.org/10.1016/j.fct.2010.07.030

Nandanwadkar, S. M., & Mastiholimath, V. (2020). A novel USP-HPTLC protocol compliant method for the simultaneous quantification of E-102, E-124, and E-133 azo dyes in consumer goods. *Journal of Planar Chromatography - Modern TLC, 33*, 405–412. https://doi.org/10.1007/s00764-020-00038-9

Ntrallou, K., Gika, H., & Tsochatzis, E. (2020). Analytical and sample preparation techniques for the determination of food colorants in food matrices. *Foods, 9*, 58. https://doi.org/10.3390/foods9010058

Rovina, K., Siddiquee, S., & Shaarani, S. M. (2017). A review of extraction and analytical methods for the determination of Tartrazine (E 102) in foodstuffs. *Critical Review in Analytical Chemistry, 47*, 309–324. https://doi.org/10.1080/10408347.2017.1287558

Rowe, K. S., & Rowe, K. J. (1995). Synthetic food coloring and behaviour: A dose response effect in a double blind, placebo-controlled, repeated-measures study. *Journal of Pediatrics, 125*, 691–698. https://doi.org/10.1542/peds.96.2.380a

Saxena, B., & Sharma, S. (2015). Food color induced hepatotoxicity in Swiss albino rats, *Rattus norvegicus. Toxicology International, 22*, 152–157. https://doi.org/10.4103/0971-6580.172286

Ścigalski, P., & Kosobucki, P. (2020). Recent materials developed for dispersive solid phase extraction. *Molecules, 25*, 4869. https://doi.org/10.3390/molecules25214869

Sharma, V., McKone, H. T., & Markow, P. G. (2011). A global perspective on the history, use, and identification of synthetic food dyes. *Journal of Chemical Education, 88*, 24–28. https://doi.org/10.1021/ed100545v

Sigurdson, G. T., Tang, P., & Giusti, M. M. (2017). Natural colorants:Food colorants from natural sources. *Annual Review in Food Science & Technology, 8*, 261–280. https://doi.org/10.1146/annurev-food-030216-025923

Silva, M. M., Reboredo, F. H., & Lidon, F. C. (2022). Food colour additives: A synoptical overview on their chemical properties, applications in food products, and health side effects. *Foods, 11*, 379. https://doi.org/10.3390/foods11030379

Stevens, L. J., Burgess, J. R., Stochelski, M. A., & Kuczek, T. (2014). Amounts of artificial food colors in commonly consumed beverages and potential behavioral implications for consumption in children. *Clinical Pediatrics, 53*, 133–140. https://doi.org/10.1177/0009922813502849

Takahashi, M., Muroi, M., Inoue, A., Aoki, M., Takizawa, M., Ishigure, K., & Fujita, N. (1987). Porperties of bentonite clay as buffer material in high level waste geological disposal. Part I: Chemical species contained in bentonite. *Nuclear Technology, 76*, 221–228. https://doi.org/10.13182/NT87-A33876

Vojdani, A., & Vojdani, C. (2015). Immune reactivity to food coloring. *Alternative Therapies in Health & Medicine, 21*, 52–62.

Wang, W., Chen, Y., Zhang, J., Wang, X., & Chen, Z. (2015). Electrochemical determination of Brilliant Blue and tartrazine based on an ionic liquid-modified expanded graphite paste electrode. *Journal of AOAC International, 98*, 817–821. https://doi.org/10.5740/jaoacint.14-232

Zhang, L., Sellaoui, L., Franco, D., Dotto, G. L., Bajahzar, A., Belmabrouk, H., … Li, Z. (2020). Adsorption of dyes brilliant blue, sunset yellow and tartrazine from aqueous solution on chitosan: Analytical interpretation via multiplayer statistical physics model. *Chemical Engineering Journal, 382*, Article 122952. https://doi.org/10.1016/j.cej.2019.122952