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Original article

Dietary intake of artificial food color additives containing food products by school-going children

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ABSTRACT

Nutritional risk in children is associated with food safety. This is the first study to identify the food type consumed by 6–17-year-old school-going children in Saudi Arabia. Eight permitted artificial food color additives, including Tartrazine (E102), Sunset Yellow (E110), Carmoisine (E122), Allura Red (E129), Indigo Carmine (E132), Brilliant Blue (E133), Fast Green (E143), and Black PN (E151), and two non-permitted ones, Erythrosine (E127) and Red 2G (E128), were determined using 24-h dietary recall questionnaires. Artificial color additives in 839 food products were divided into nine categories, including biscuits, cakes, chocolates, chips, ice cream, juices and drinks, candy, jelly, and chewing gum, are determined using high performance liquid chromatography and diode array detector. The results indicated a high intake of juices and drinks, ice cream, and cakes, but low consumption of chewing gum among school-going children. Among the permitted artificial food color additives, Brilliant Blue (E133) (54.1%) and Tartrazine (E102) (42.3%) were the most commonly used. Sunset Yellow (E110) in one chocolate sample, Tartrazine (E102) and Sunset Yellow (E110) in one and two juice and drink samples, respectively, and Brilliant Blue (E133) in two candy samples exceeded the permitted level. Therefore, further investigations are needed to provide insights into the possible adverse health effects of high intake of these additives in artificial food coloring on the test population are warranted.

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1. Introduction

Food color is any substance incorporated to food or beverage to alter its color. Adding color to food and drink has been a normal practice over many centuries. Color was generally added to serve as a visual cue for quality, induce recognition of flavor, and meet consumer expectations. Today, color is still added during the manufacture of products such as biscuits, pastries, cakes, processed meats, cheese, margarine, confectionery, ice cream, cordials, and soft drinks. The addition of color to food and beverage also serves many technological purposes, such as offsetting color loss caused by processing conditions, including temperature, time, and heat,

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enhancing color that is already present in food, ensuring batch to batch uniformity, which preserves food identity, and protecting flavors and vitamins that may be sensitive to sunlight during storage (Ghorpade et al., 1995). Before the 1950s, addition of color to food was a simple process. Manufacturers colored candy and food using natural color compounds of plant and vegetable origin, such as chlorophyll (green), beets (pale red), and extracts of other plants and spices (yellow and orange) Shamina et al. (2007). The chemical industry boomed after world war two (WWII) due to enhanced collaboration within the food industry. This led to incorporation of artificial petroleum-based ingredients in artificial food colors, which became widely popular over natural colors due to their manufacturing benefits, such as reduced costs and prolonged shelf life (Martins et al., 2016).

The safety of a chemical compound is determined primarily by testing the amount required to kill 50% of the experimental animal population. The data are then extrapolated to assess the risk in humans. Regrettably, no toxicology studies were performed when artificial food color additives were first used. Toxicology studies are important to evaluate the potential adverse effects of a chemical compound on human health (Boekelheide et al., 2010).

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In general food additives and in particular color additives have increasingly accessed in recent years for their safety (FAO/WHO, 1978). Artificial food color additives, also known as food dyes contains multiple chemicals. Most of these chemicals are produced from highly toxic sources, and can cause numerous human diseases, disorders and mutations.

Today, due to increased public awareness, there are concerns about the effects of long-term use of artificial food color additives, for example, in most of the countries Amaranth (E123) is banned except Japan (Das and Mukherjee, 2004). Numerous studies have investigated the carcinogenic changes associated with the consumption of food color additives and flavors. However, few studies have evaluated the behavioral effects in children due to consumption of foods containing artificial color additives (Arnold et al., 2012).

Recently (McCann et al., 2007) reported that a mixture of artificial color additives and the preservative sodium benzoate in food products increased hyperactivity in children aged 3, 8, and 9 years. In an earlier report, it was found that 3-year-old children showed adverse behavior, as measured by parental ratings, after consumption of a mixture of four artificial food color additives and sodium benzoate (Bateman et al., 2004). The FDA Redbook, also known as "Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food," was reported to have been revised, as directed by the US Congress, stating that all new and existing food colors should undergo routine neurotoxicological screening for postmarked surveillance. It has been postulated that some generally recognized as safe compounds should also undergo neurotoxicity testing. Some colorants, such as Tartrazine, have been reported to cause allergies, asthma (Nevado et al., 1997) and childhood hyperactivity (Rowe and Rowe, 1994). From July 20, 2010, the UK Food Standards Agency and European Food Safety Authority required all products containing Tartrazine, Quinoline Yellow, Sunset Yellow, Carmoisine, Ponceau 4 R, and Allura Red to carry the label "may have an adverse effect on activity and attention in children."

Many countries have their own specific regulations governing the type, purity, use, and amount of artificial color additives permitted in the food industry (Husain et al. 2006). Dietary modeling is an important tool in establishing chemicals from food in the diet and is an important part of the risk assessment process. To estimate dietary exposure to chemicals in food, concentration and consumption data are used, the results are then compared with established health standards. Internationally, dietary modeling has been used in the risk assessment process for many years by food regulatory agencies to establish unacceptable risks to public health. The quality of food concentration and consumption data for dietary modeling determines accuracy of the dietary exposure estimate (Kettler et al., 2015).

Food and Drug Administration (FDA) of the United States has permitted the nine artificial color additives to use in food (USFDA, 2017). In order to contain the improper use, number of countries has developed regulations to limit the quality, purity, use and quantity of permitted artificial color additives in food. Food and Agriculture Organization (FAO) and World Health Organization (WHO) joint expert committee on food additives (JECFA) highly advice to the governments frequently monitor the overall intake of each food additives, in particular, if there use is exploited and/or if the overall intake exceeds the permissible levels. The permissible levels of each artificial food color additives are determined by the national level dietary studies. Saudi Arabia, regrettably, studies have not been conducted till date on the level or total intake of artificial food color additives in food products consumed by school-going children. The aim of this study was to identify and quantitatively determine the amount of artificial color additives in food items sold locally and consumed by school-going children. Additionally, this is the first study conducted in Saudi Arabia on the pattern of consumption of food products containing artificial color additives by school-going children.

2. Materials and methods

2.1. Target group

Saudi and non-Saudi school-going children were the target population enrolled in public, private aged 6–17 year old primary and secondary schools, because they are considered to be the major consumers among the various population groups of Saudi Arabia, for the drinks and food having artificial food color additives.

2.2. Sample size and design

The data was obtained from the statistical department of the Ministry of Education of Saudi Arabia. The overall enrolled student population of Riyadh schools for the academic year 2014–2015, are segregated into several categories called strata. This covers nationality (Saudi and non-Saudi) and other characteristics of the population such as sex (male and female), educational level (primary and secondary), governorate (north, south, east, west, and central), school sector (public, private, and international), and age group (between 6 and 17 years) (Fig. 1). Each stratum was considered a different population, and an individual sample was then randomly taken from each stratum.

2.3. Twenty four-hour dietary recall

Before carrying out the survey, we procured 839 food products, which were generally consumed by school-going children, from different supermarkets in Riyadh, Saudi Arabia.

Such food products were divided into nine groups: chocolates, chips, biscuits, ice cream, cakes, juices and drinks, candy, jelly, and chewing gum. Fig. 2 shows the percentage of food samples used during interview.

The study was performed by the dietary study team, which consisted of members from the food science and nutrition department. Members of the team were extensively trained for the interview of the children on the 24-h dietary recall and collect and verify the data. The survey team interviewed children independently at schools and told them to identify the types and amount of food products they consumed during breakfast, lunch, dinner, and in between meals in the last 24 h using photographs of the food products.

The set of questions was designed to collect the information needed to assess the intake of food products by school-going children aged 6–17 years. The questionnaire was divided into five sections, information about school, student, parent, child height and weight, and food consumed during the last 24 h. Field survey data for 24-h dietary recall has been statistically analyzed using the Statistical Analysis System, Version 9.1.3, (SAS, 2011).

2.4. Food sample analysis.

Perishable food products are stored at 4 °C, dry food products (e.g. chips and biscuits) were stored at room temperature, whereas ice cream samples are stored at -20 °C up to testing is done. All food products were extracted and analyzed in triplicate.

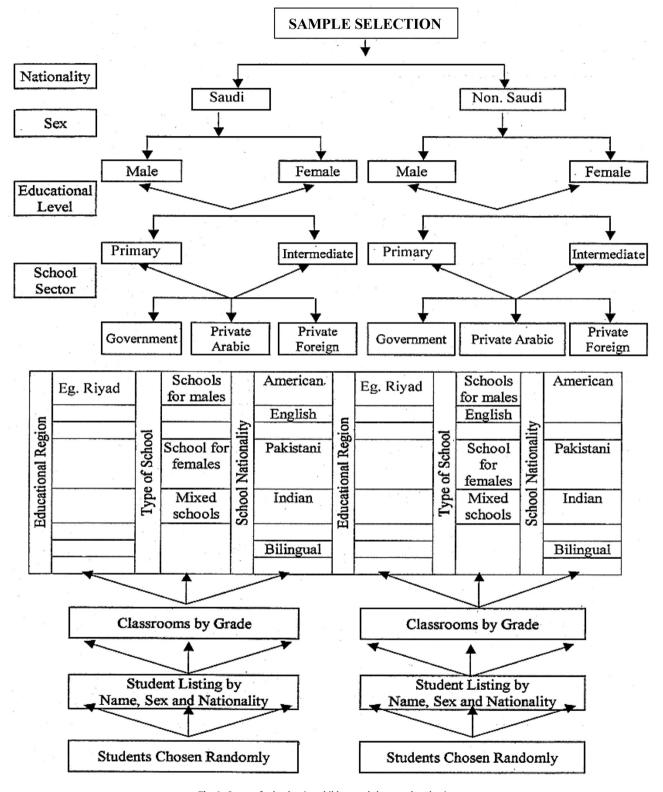


Fig. 1. Strata of school-going children and the sample selection.

2.5. Sample preparation (extraction)

Artificial food color additives were extracted according to the method described by Ha et al. (2013) with some modifications. Briefly, solid samples, such as biscuits, cakes, chips, candy, and jelly (10 g), were crushed, homogenized, diluted with water 2–10-times

of volume, color was extracted with heating on a hot plate, chewing gum solid fractions were removed by centrifugation. The sample was dissolved in 50-80% (v/v) ethanol. To prepare high-fat samples, the crushed and homogenized sample (5-10 g) was defatted with petroleum ether prior to color extraction. Carbonated drink samples were sonicated for 5 min to remove gas.

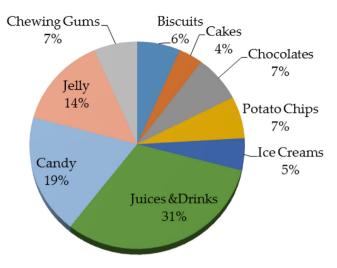


Fig. 2. Food category group's percentage distribution of samples.

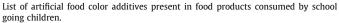
Volume reduction was adjusted with distilled water, then additional (10 mL) distilled water was added.

The ice cream samples were prepared according to the method described by Del Giovine and Piccioli Bocca (2003). Ice cream (10 g) was weighed in a beaker, and ammonical ethanol (30 mL) (EtOH + 5% conc. NH4OH mixture) was added. The sample was allowed to rest in a refrigerator to ease separation of the thickeners. The sample was then filtered, and the colored liquid was collected in a 100 mL volumetric flask. The extraction was repeated twice with ammonical ethanol (15 mL), and the liquid was collected in the calibrated volumetric flask each time. Then the required volume was obtained by adding water. The resultant solutions were filtered through a 0.45 μ m membrane prior to high performance liquid chromatography (HPLC) injection.

2.6. Analysis of artificial food color additives using HPLC

Samples are analyzed for artificial food color additives using Agilent Technologies, RP-HPLC, reversed phase high performance liquid chromatograph with the following details: column, Zorbax Sb-C18 ($250 \times 4.6 \text{ mm}$, 5 µm), detector 1260 DAD-VL. Ammonium acetate 10 nM solution mobile phase A and acetonitrile mobile phase B with flowrate of 1 mL/min with optimized gradient programme (A:B) initially 95:5, at 30 min 50:50 at 30.1 min 95:5. The absorbance was monitored at 426 nm for Tartrazine (E102), 482 nm for Sunset Yellow (E110), 514 nm for Carmoisine (E122), 530 nm for Erythrosine (E127), 509 nm for Allura Red (E128) and Red 2G (E128), 613 nm for Indigo Carmine (E132) and Black PN

Table 2



Artificial food color additive	International numbering system	Mol. Structure
Tartrazine	E-102	
Sunset Yellow	E-110	
Carmoisine	E-122	$\bigcup_{O=0\\O=0\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\O\\$
Erythrosine	E-127	
Red 2G	E-128	
Allura Red	E-129	
Indigo Carmine	E-132	NaO-S-NaO-S-ONA
Brilliant Blue	E-133	
Fast Green FCF	E-143	
Black PN	E-151	

(E151), and 628 nm for Brilliant Blue (E133) and Fast Green FCF (E143). Peaks were identified and quantified using the retention time of standards absorption spectra.

Table 1

Intake of food containing artificial color additives by school-going children in Saudi Arabia (g/day).

Age years)	6–7		8-9		10-11		12-13		14-15		16-17	
Food category	Male	Female										
Biscuits	40.80	50.33	48.05	38.73	58.30	35.05	50.52	41.10	40.23	45.78	35.59	38.46
Cakes	67.80	44.72	51.60	47.73	52.79	55.31	54.26	56.62	63.72	56.77	69.08	62.67
Chocolates	44.50	42.51	52.60	35.37	46.24	40.15	45.14	44.10	45.31	46.16	40.05	47.13
Potato chips	32.90	38.68	33.71	43.27	37.48	55.88	37.02	59.15	43.66	52.53	34.23	62.37
Ice cream	0.00	215.61	93.00	184.33	238.26	188.46	218.26	214.35	141.61	222.81	141.50	113.80
Juices and drinks	366.00	298.41	322.86	283.05	303.99	303.93	353.21	314.13	355.44	299.73	441.56	313.82
Candy	0.00	22.73	68.32	18.62	41.12	35.21	27.99	35.76	23.13	13.27	64.00	15.54
Jelly	0.00	31.14	19.33	38.36	57.04	58.91	31.54	57.98	49.25	73.69	16.27	0.00
Chewing Gum	0.00	8.13	6.71	4.48	8.48	4.26	4.93	3.74	4.24	4.52	4.55	4.88

	Tartrazine	Tartrazine Sunset Yellow Carmo isine Erythro sine	Carmo isine	Erythro sine	Red 2G	Allura Red	Allura Red Indigo Carmine Brilliant Blue Fast Green Black PN	Brilliant Blue	Fast Green	Black PN	Permitted	Permitted Permitted GMP	GMP	GMP
Food category											India	Australia USA	USA	UK
Α	I	217-217	I	I	I	I	I	I	I	I	100	290	I	2-265
В	0.17-0.19	2.39-4.98	I	I	I	2.8-2.8	I	I	I	I	100	290	10-200	1-151
C	0.6 - 26.3	0.32-224.6	2.28-114	I	4.56 - 109.4		I	0.56-5.12	I	I	100	70	5 - 200	1-20
D	0.32-19.7	0.02-46.5	0.18-12.3	1.1 - 1.1	1.24 - 49.7	2.58-76.3	59.3-59.3	0.24-193.2	0.03 - 0.57	I	100	290	10 - 400	2-265
Е	0.1 - 5.8	0.52-14.3	5.14 - 5.14	0.2-0.2	0.25-26.5	0.74-42.8	I	0.74-33.2	0.99 - 11.9	175.3-175.3	200	290	10 - 400	13-136
F	I	8.19-8.19	10.46 - 15.9	I	I	I	I	0.03-0.57	I	I	100	290	I	I

A = Chocolates; B = Ice cream; C = Juices and drinks; D = Candy; E = Jelly; F = Gums

Artificial food color additives concentration ranges in different food product vs good manufacturing practices (GMPs) and permitted levels in some countries (mg/kg)

3. Results

3.1. Target sample size

A sample size of 5000 children, aged 6–17 years (2509 male and 2491 female), from primary and secondary schools in Riyadh, Saudi Arabia, representative of the target population was statistically determined and no more than 5% error estimated at 95% of the confidence level was ensured. Details of each governorate sample size were as follows: (boys) north 550, south 537, east 495, west 496, and central 431, and (girls) north 360, south 319, east 735, west 566, and central 511.

3.2. Pattern of consumption of food products

Among the nine food categories, juices and drinks, ice cream, and cakes were highly consumed by school-going male and female children, as observed in Table 1. Based on the food frequency questionnaire, chewing gum was not consumed as much. Male school-going children of all age groups consumed solid foods and liquids containing artificial color additives daily in the following ranges: biscuits 36–58 g, cakes 52–69 g, chocolates 40–53 g, potato chips 33–44 g, ice cream 93–238 g, juices and drinks 304–442 mL, candy 23–68 g, jelly 16–57 g, and chewing gum 4–9 g. However, the female school-going children consumed food and beverages with artificial color additives daily in the following ranges: biscuits 35–50 g, cakes 45–63 g, chocolates 35–47 g, potato chips 39–62 g, ice cream 114–223 g, juices and drinks 283–314 mL, candy 13–36 g, jelly 31–74 g, and chewing gum 4–8 g.

3.3. Level of artificial color additives in food products commonly consumed by school-going children

Content of artificial color additives Table 2 in food products commonly consumed by 6–17-year-old school-going children in Saudi Arabia were analyzed and the range of detected concentrations are presented in Table 3. Eight permitted food color additives, including Sunset Yellow (E110), Tartrazine (E102), Carmoisine (E122), Brilliant Blue (E-133), Indigo Carmine (E132), Allura Red (E129), Fast Green (E143), and Black PN (E151), and two non-permitted additives, Erythrosine (E127) and Red 2G (E128), were analyzed in 839 food products that were consumed commonly by 6–17 year-old school-going male and female children in Saudi Arabia.

Among these food samples, 141 food items (6.9%) we did not found any artificial color additives, whereas non-permitted artificial color additives found in 43 food products, such as Red 2G

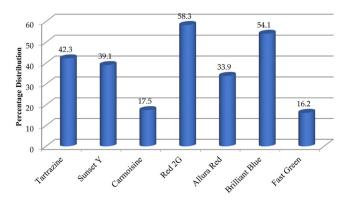


Fig. 3. Percentage distribution of artificial food color additives in the studied categories.

(E128) and Erythrosine (E127). These were identified in juices and drinks, candy, and jelly, between the range 0.3–109.4 mg/kg for Red 2G (E128) and 0.2–1.1 mg/kg for Erythrosine (E127). The per-

centage distribution of the food items, in the various analyzed food categories, tested for permitted artificial color additives are shown in (Fig. 3). It was found that the most commonly used artificial food

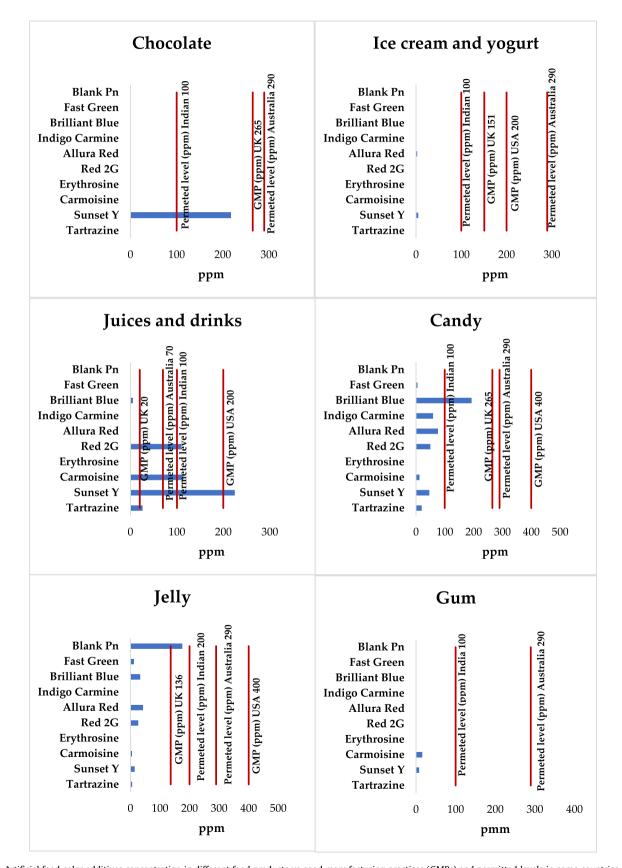


Fig. 4. Artificial food color additives concentration in different food products vs good manufacturing practices (GMPs) and permitted levels in some countries (mg/kg).

color additives were Brilliant Blue (E133) (54.1%) and Tartrazine (E102) (42.3%), followed by Sunset Yellow (E110) (39.1%), Allura Red (E129) (33.9%), Carmoisine (17.5%), and Fast Green FCF (16.2%).

4. Discussion

4.1. Food products consumption pattern

The trend of food intake observed in this study was similar to that reported in a study (French et al., 2003) on national trends of soft drink consumption among 6–17-year-old children in the United States, which showed a 48% increase. Rao et al. (2004) reported intake of beverages in the range of 25–540 mL and 32–840 mL by 1–5-year-old and 6–18-year-old children, respectively, in India. The beverages fruit drinks, squashes, and sherbets are consumed by both age groups.

4.2. Level of artificial color additives in food products commonly consumed by school-going children

Artificial food color additives use in Europe is strictly regulated and harmonized with the formulation of Directive 94/35/EU (Minioti et al., 2007). In Saudi Arabia, the use of artificial colors in food follows the GCC Food color standard 285/1999, which is currently being updated by the Saudi food and drug authority, SFDA.

Many tested samples did not contain food color additives, and this could be due to the below detection low levels of the additives or use of natural colors, as confirmed by the E numbers listed on the label. Rao and Bhat (2003), studied the artificial food colors types and levels in the food products of Hyderabad city and its surrounding areas. Out of the 1000 food-items (700 from urban areas and 300 from rural areas) collected and analyzed, 7% of the foods from urban areas and 5% of the foods from rural areas contained non-permitted color additives. In beverages, such as artificial syrups and sherbets, the concentrations of Tartrazine (E102) and Sunset Yellow (E110) were 9.45 mg/g and 4.57 mg/g, respectively.

In another study conducted by Jonnalagadda et al. (2004), 545 ready-to-eat foods prepared in the non-industrial sector were investigated. The data obtained showed that 73% of the samples exceeded 100 ppm, whereas 27% are within the approved limit set by Indian Prevention of Food Adulteration (PFA) Act (1954) for the permitted artificial colors.

Certain observations, similar to the outcome of the current study, have been reported from India (Rao et al., 2004) and Kuwait (Sawaya et al., 2008). Non-permitted color Red 2G (E128), among all food color additives, was the highest consumed additive (58.3%). The approved artificial food color additives level detected in commonly consumed food products by the school-going children in Saudi Arabia were compared with GMP ranges or maximum levels and/or approved levels of USA, UK, Australia (Pascual, 1985) and India (Rao and Bhat, 2003), (Table 3). In general, the artificial color additives level in all the food categories are within GMP and permitted levels of these countries. Only one sample in the chocolate food category showed maximum concentration of Sunset Yellow (E110) (217 mg/kg) that exceeded the permitted level (100 mg/kg) in India. In the juice and drink food category, only one sample showed a concentration of Tartrazine (E102) (26 mg/kg) that exceeded GMP range (1-20 mg/kg) in UK (Fig. 4). Two samples in the juice and drink food category showed Sunset Yellow (E110) level that exceeded the permitted level. One sample contained 224.6 mg/kg of Sunset Yellow, exceeding the GMP range in UK (1-20 mg/kg) and USA (10-200 mg/kg) and the permitted level in India (100 mg/kg). The second sample showed a concentration of 53.9 mg/kg, exceeding the GMP range in UK (Fig. 4). Three samples in the juice and drink category showed 114, 100, 68.14 mg/kg of another artificial color additive Carmoisine (E122) in UK, India, and Australia, respectively, exceeding the GMP range in UK (1–20 mg/kg) and permitted levels in India (100 mg/kg) and Australia (70 kg/mg). When concentrations of approved artificial color additives found in candy food category are compared (Fig. 4), two samples showed 193.2 and 103.8 mg/kg of Brilliant Blue (E133), exceeding the permitted level in India (100 mg/kg). The relatively higher levels of added artificial colors in the food products could be due to imports of major food items in Saudi Arabia from industrialized countries, whose standards are, vary from each other's.

5. Conclusions

The current study was conducted for the first time in Saudi Arabia. Data obtained demonstrated a higher intake of juices and drinks, ice cream, and cakes by 6-17-year-old school-going children (Saudi, non-Saudi, male and female). Brilliant Blue (E133) (54.1%) and Tartrazine (E102) (42.3%) were found to be the most commonly used permitted artificial color additives in the various analyzed food items. Many tested samples did not contain food color additives, and this could be due to below detection levels of the additives or use of natural colors. One chocolate sample showed levels that exceeded the permitted level in India. Five samples of juices and drinks showed levels of some artificial food color additives that exceeded the permitted level in India and GMP ranges in UK and USA. Data on the concentrations of artificial color would be important to help understand food consumption pattern in school-going children and select appropriate products to avoid intake of large amount of additives, which can lead to health problems in children.

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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.

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