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Risk Assessment of Colorant Additives and Heavy Metal Content of Jelly Products Targeting Pediatric Populations in Arak Market, Iran

Abbas Alimoradian[1], Behnoosh Ansari Asl[2], Sonia Asadi[3], Mahdi Abdollahi[3], Rahmatollah Moradzadeh[4], Khazra Alimoradian[5], Nafiseh Khansari[2*]

[1] *Department of Pharmacology, School of Medicine, Arak University of Medical Sciences, Arak, Iran.*
[2] *Food and Drug Deputy, Arak University of Medical Sciences, Arak, Iran.*
[3] *Arak University of Medical Sciences, Arak, Iran.*
[4] *Department of Epidemiology, School of Public Health, Arak University of Medical Sciences, Arak, Iran.*
[5] *School of Paramedicine, Arak University of Medical Sciences, Arak, Iran.*

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* **Corresponding author:**
Nafiseh Khansari, Food and Drug Deputy, Arak University of Medical Sciences, Arak, Iran.

E-mail: khansari.n@arakmu.ac.ir

ABSTRACT

Background: The present research aimed at the assessment of the contamination levels of heavy metals and synthetic colorants present in soft jelly and jelly powder products, alongside an evaluation of the associated potential health risks for pediatric populations.
Methods: The study utilized a screening technique grounded in thin-layer chromatography for the identification of synthetic colorants. Following a novel microwave-assisted sample preparation process, the quantification of artificial colorants was executed via high-performance liquid chromatography. Furthermore, the detection of lead, cadmium, and copper was conducted using established methodologies.
Results: The research indicated the presence of synthetic colorants in the analyzed samples, including orange, yellow, red, and blue. Notably, no samples were found to contain a synthetic green colorant. The maximum concentration of synthetic colorants was recorded at 15.46 mg/kg Brilliant Blue in jelly powder, whereas the minimum concentration was identified at 4.6 mg/kg Sunset Yellow in soft jelly. The concentration ranges for heavy metals were delineated as follows: lead (0.0019-1.4950 µg/g), cadmium (0.0001-0.0415 µg/g), and copper (13.0247-21.0031 µg/g). In addition, hazard indices were calculated to be 0.882 for jelly powder and 5.28 for soft jelly.
Conclusion: The results of this study highlighted the critical necessity of ongoing surveillance of synthetic colorant and heavy metal concentrations in food products, especially those aimed at children. The hazard index values exceeding 1 imply a potential health hazard for children consuming these products, thereby underscoring the imperative for further research and the development of potential mitigation strategies.

Keywords: Artificial colors, Heavy metal, Jelly products, Risk assessment

Introduction

The food industry has employed a wide array of coloring agents in its products, with a discernible predominance of synthetic colors attributed to their superior cost-effectiveness and accessibility compared to their natural counterparts [1, 2]. In light of this observation, several nations have sought to regulate the use of coloring agents in food items and have instituted guidelines to mitigate the excessive application of synthetic colors [3, 4].

In accordance with the international standards set forth by various food administration organizations, including the World Health Organization (WHO), Codex Alimentarius, and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), individual countries have formulated their own regulations pertaining to synthetic colorants [3, 5-7]. Consequently, the Iranian National Standards Organization has approved seven synthetic colorants: Quinoline Yellow, Sunset Yellow, Indigotin, Brilliant Blue, Ponceau 4R, Carmoisine, and Allura Red. Nonetheless, this organization has yet to establish specific limits for each colorant concerning various food categories, particularly those predominantly consumed by children [7-12]. The potential health implications for the pediatric population represent a paramount concern that health organizations must address. Children aged 4-7 are particularly inclined to favor visually appealing and soft-textured products. In Iran, there exists no regulatory restriction on the utilization of synthetic colorants in these products, thereby posing a significant risk to children's health and causing serious conditions, such as attention deficit hyperactivity disorder (ADHD) [13]. Various qualitative and quantitative methodologies are implemented for the assessment of colorants in food laboratories, as per the guidelines of Codex and the Official Agricultural Chemists (AOAC) [6, 8, 14-16]. Notably, the high-performance liquid chromatography (HPLC) technique stands out as a practical approach for the determination of food colorants. One of the critical advantages of employing HPLC for the quantitative analysis of colorants is its capacity for simultaneous measurement of multiple colors within a chromatogram, thereby enhancing both efficiency and cost-effectiveness [17-22]. The accumulation of heavy metals through food products in the human body can be deleterious, leading to severe health complications. Therefore, it is imperative to scrutinize the contamination levels of unauthorized artificial colors in food items [23]

Atomic absorption spectroscopy is recognized as the traditional technique for the detection of heavy metals in food samples. On the contrary, the microwave digestion method offers enhanced convenience and accuracy, as it facilitates the thorough digestion of samples under elevated pressure and temperature conditions [23, 24]. In the present study, a novel digestion technique was employed to quantify heavy metals utilizing an atomic absorption spectrometer. Moreover, four widely utilized artificial colorants in jelly powder and soft jelly were simultaneously analyzed using both HPLC and thin-layer chromatography (TLC). In addition, a risk assessment was conducted to evaluate the potential hazards associated with the elevated presence of heavy metals in jelly products targeting the pediatric demographic.

Materials and Methods

Sample collection

A total of 25 samples were selected from five distinct brands, each representing five different colors: red, blue, green, yellow, and orange, specifically for each category of jelly powder and soft jelly.

Reagents and standards

The calibration standards employed for the determination of concentration were obtained from Sigma Aldrich for both natural and synthetic colorants, while heavy metals standards were sourced from Merck (Germany), with an initial concentration established at 1000 mg/liter. Furthermore, other solvents utilized, including nitric acid, hydrogen peroxide (designated for atomic absorption analysis), methanol, and acetonitrile of HPLC grade, were of laboratory-grade quality.

HPLC-UV analysis

In the sample preparation procedure, the samples were initially subjected to digestion (Sineo MDS 10, China), where 10 grams of each sample were dissolved in a methanol/water mixture at a 1:1 ratio. The resultant mixture was subsequently filtered through a 0.45 µm PVDF filter. The filtered samples underwent analysis via HPLC-UV (ultraviolet detector) using the Platin Blue UPLC system (Knauer, Germany). The mobile phase comprised a buffer acetate at 10 M (pH=7) as elution A and acetonitrile as elution B, combined in a 70:30 ratio, delivered into a C18 column (Nucleodur-100-3 C18, 250 × 2 mm, 3 µm packing, Knauer, Germany) at a flow rate of 0.8 mL/min. An injection volume of 10 µL was utilized, with the column temperature maintained at 30°C. The chromatogram analysis was performed utilizing Clarity Chrome software (Knauer, Germany). The area under the curve of the standard solutions was employed for the comparative analysis of color concentrations within the samples.

Thin-layer chromatography analysis

To conduct a qualitative assessment of the jelly powder and soft jelly, 5-10 grams of each sample were dissolved in a mixture of water and glacial citric acid. Following this, the samples were immersed in a water bath. The chromatography tank was prepared with a composition of 60 ml butanol, 30 ml glacial acetic acid, and 36 ml distilled water, totaling a volume of 100 ml, along with an extraction solution of 2% ammonia in 70% alcohol. The preparation of the chromatography tank was executed at least four hours before chromatography to ensure solvent saturation within the tank. White wool was introduced into the tank and removed after 30 minutes, allowing the solution to dry. The dried solution, which contained artificial colorants, was spotted onto TLC paper and stained, and the types of colors were identified qualitatively.

Atomic absorption

For the measurement of heavy metals, atomic absorption was employed subsequent to microwave digestion conducted under elevated pressure and temperature conditions (Table 1). Graphite furnace atomic absorption spectrometers, specifically the Varian model 800 atomic absorption spectrometer (Varian, Mulgrave, Australia), were utilized for this analysis.

Table 1. Microwave condition and control setup

Step (N)	Temperture (T) / ^{0}C	Time (t) / min	Power of single vessel (W) / w	Power of multi- vessel (W) / w
1	130	10	400	1000
2	150	5	400	1000
3	180	15	400	1000

Health risk assessment

The mean daily intake of various food groups was considered, and the chronic daily intake (CDI; mg/kg body weight/day) was determined by calculating the average concentration of each food dye or additive along with the amount of food consumed per day. This was calculated using Formula 1:

CDI=∑C×DI/BW

Where C is the concentration of additive content (mg/Kg), DI is the average daily intake (Kg or L/day), and BW is the average body weight set in the study [25].

To assess the overall potential for non-carcinogenic health effects due to synthetic food dyes, the target hazard quotient (THQ) was calculated using Formula 2:

THQ= CDI / RfD

Where CDI is the chronic daily intake of the additive for the average consumer, and reference dose (RfD) is the oral reference dose (mg/Kg/day). A significant risk level is indicated when the THQ value exceeds 1, suggesting a higher likelihood of adverse health effects. The hazard index (HI) for the consumption of synthetic dyes in each food category was calculated as the sum of the THQ values for all food samples:

Total THQ (HI)= ∑THQ [26].

Acceptable daily intake (ADI; mg/kg body weight/day) values were also provided as alternatives to RfD values for the dyes evaluated in this study [9].

Results

Qualitative screening tests

Qualitative screening tests were conducted to determine the presence of artificial or natural colors for each of the five color types. All samples in the jelly powder group, except for one brand (5 samples), contained artificial colors (Table 2). Conversely, in the soft jelly group, three brands (15 samples) used natural colors (Table 2). Samples containing natural colors were excluded from further analysis.

Heavy metal analysis

The heavy metal contents of artificial colors for samples were assessed by atomic absorption. The calibration curve was plotted for copper (Cu), cadmium (Cd), and lead (Pb) (Table 3). By this, the concentration of heavy metals (µg/g) for five different colors (red, blue, green, yellow, and orange) for jelly powder and soft jelly groups was determined. (Table 4). The results demonstrated that Cu is the most and Cd is the least metal observed in all types of colors for both jelly powder and soft jelly groups. Moreover, based on the observed results, the order of the amount of metals found in both groups is as follows:

Pb: Red > Yellow > Orange > Green > Blue.

Cd: Orange > Red > Blue > Green > Yellow.

Cu: Red > Orange > Blue > Green > Yellow.

Therefore, it seems that almost the highest amount of metal was observed in red and orange colors.

Table 2. Qualitative screening tests for jelly powder and soft jelly

Sample Groups	Brands	Colorant	Natural color	Synthetic Color
	A	Red		Negative
		Blue	Positive	Negative
		Green	Positive	Negative
		Yellow	Positive	Negative
		Orange	Positive	Negative
			Positive	
	B	Red		Positive
		Blue	Negative	Positive
		Green	Negative	Positive
		Yellow	Negative	Positive
		Orange	Negative	Positive
			Negative	
	C	Red		Positive
		Blue	Negative	Positive
		Green	Negative	Positive
		Yellow	Negative	Positive
Jelly Powder		Orange	Negative	Positive
			Negative	
	D	Red		Positive
		Blue	Negative	Positive
		Green	Negative	Positive
		Yellow	Negative	Positive
		Orange	Negative	Positive
			Negative	
	E	Red		Positive
		Blue	Negative	Positive
		Green	Negative	Positive
		Yellow	Negative	Positive
		Orange	Negative	Positive
			Negative	

Sample Groups	Brands	Colorant	Natural color	Synthetic Color
	A	Red	Positive	Negative
Soft Jelly		Blue	Positive	Negative
		Green	Positive	Negative

	Yellow	Positive	Negative
	Orange	Positive	Negative
B	Red	Positive	Negative
	Blue	Positive	Negative
	Green	Positive	Negative
	Yellow	Positive	Negative
	Orange	Positive	Negative
C	Red	Negative	Positive
	Blue	Negative	Positive
	Green	Negative	Positive
	Yellow	Negative	Positive
	Orange	Negative	Positive
D	Red	Negative	Positive
	Blue	Negative	Positive
	Green	Negative	Positive
	Yellow	Negative	Positive
	Orange	Negative	Positive
E	Red	Positive	Negative
	Blue	Positive	Negative
	Green	Positive	Negative
	Yellow	Positive	Negative
	Orange	Positive	Negative

Table 3. Analytical parameters of calibration curves of the heavy metals (μg/L)

Analytical Instrument	Heavy metals	Linear range	Regression equation		Optical condition	
			Slope	R^2	Wavelength	Slit width
Atomic Absorption	Pb	0.0-40.0	0.00654	0.9990	283.3	0.5
	Cd	0.0-.0.8	0.11900	0.9992	228.8	0.5
	Cu	0.0-30.0	0.01523	0.9974	324.8	0.5

Table 4. Concentration of heavy metals (μg/g) in different types of jelly powders and soft jelly candies for color types

Jelly powders:

Metals	Pb		Cd		Cu	
Color Types	Mean	Range	Mean	Range	Mean	Range
Red jelly powder	1.195	0.0815-1.6950	0.0059	0.0001-0.0012	19.0325	15.4501-21.0031
Blue jelly powder	0.086	0.0092-1.0032	0.0001	0.0042-0.0137	17.0001	15.3122-20.0123
Green jelly powder	0.063	0.0024-1.0014	0.0005	0.0031-0.0011	18.0256	15.1113-20.6547
Yellow jelly powder	0.100	0.0081-1.0038	N.D	0.0019-0.0259	14.1365	14.1654-19.0000
Orange jelly powder	0.954	0.0243-1.0409	0.0029	0.0026-0.0415	18.8314	15.4256-20.0328

Soft jelly:

Metals	Pb		Cd		Cu	
Color Types	Mean	Range	Mean	Range	Mean	Range
Red soft jelly	1.031	0.0645-1.4950	0.0046	0.0001-0.0012	18.9645	15.0300-20.1201
Blue soft jelly	0.098	0.0075-1.0010	N.D	0.0042-0.0137	16.2369	14.8012-19.0359
Green soft jelly	0.079	0.0019-1.0003	N.D	0.0031-0.0011	17.0003	14.5962-20.0178
Yellow soft jelly	0.102	0.0074-1.0012	N.D	0.0019-0.0259	13.0698	13.0247-18.0236
Orange soft jelly	0.921	0.0143-1.0376	0.0025	0.0026-0.0415	18.0364	14.9256-20.0059

Quantitative detection of synthetic colors

HPLC was used to detect synthetic colors quantitatively. Four synthetic colors (Carmoisine (E122), Brilliant Blue (E133), Quinoline Yellow (E104), and Sunset Yellow (E110)) were detected in red, blue, yellow, and orange, respectively. The green color included both *Quinoline* yellow and Brilliant blue. The chromatogram for four synthetic colors, retention time, calibration data, and instrumental information of HPLC method are illustrated in Figure 1 (Table 7). It was also observed that the amount of all four types of synthetic colors, E122, E133, E104, and E110 (mg/kg), in group jelly powder is more than soft jelly (Table 5). Moreover, the order of artificial color concentration (mg/kg) in both soft jelly and jelly powder groups is as follows:

Brilliant Blue (blue-E133) > Carmoisine (red-E122) > Quinilone Yellow (yellow-E104) > Sunset Yellow (orange-E110).

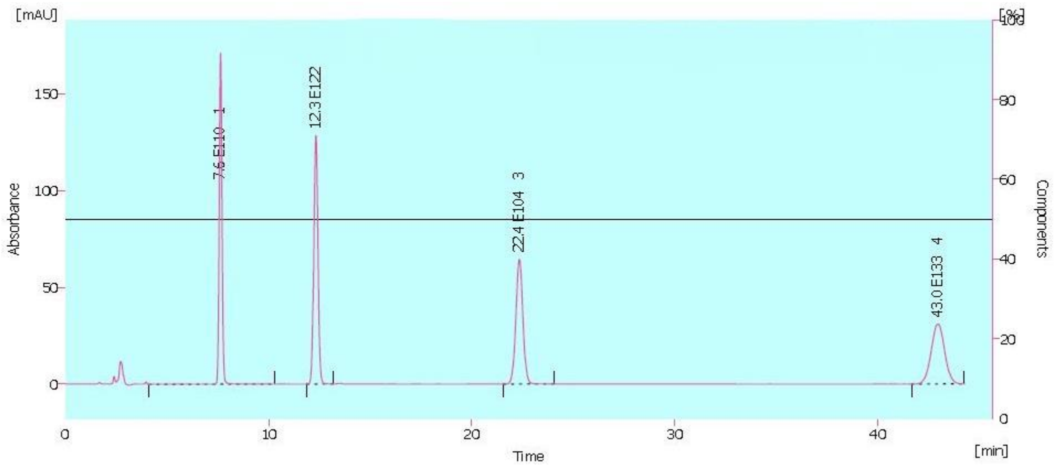

Figure 1. The HPLC chromatogram
Peak and retention time of four color additives
Flow rate 1 ml/min
Peak 1: Sunset yellow, Wavelength (λ)= 506, Retention time (min)= 4.100
Peak 2: Carmoisine, Wavelength (λ)= 526, Retention time (min)= 11.883
Peak 3: Quinilone yellow, Wavelength (λ)= 408, Retention time (min)= 21.567
Peak 4: Brilliant blue, Wavelength (λ)= 610, Retention time (min)= 41.450

Tabel 5. Synthetic color concentration (mg/kg) in soft jelly candies and jelly powders by HPLC method

Sample groups	E110	E104	E122	E133
Jelly powders	10	11.35	13.25	15.46
Soft jelly	4.6	6.71	8.97	14.52

Risk assessment

The risk assessment included various parameters, such as DI, CDI, THQ, HI, RfD, and ADI. The results of the risk assessment are displayed in Table 6.

Table 6. Risk assemssent data information

		ADI	THQ	CDI	DI	RfD	HI total
Jelly powders	E104	5	0.17	0.033	0.3	0.2	0.882
	E110	4	0.58	0.15	0.3	0.26	
	E122	4	0.095	0.19	0.3	1.985	
	E133	12.5	0.037	0.2319	0.3	6.125	
Soft jelly	E104	5	0.115	0.023	0.1	0.2	5.28
	E110	4	0.088	0.023	0.1	0.26	
	E122	4	5.16	0.033	0.1	0.0065	
	E133	12.5	0.11	0.72	0.1	6.125	

E104: Quinilone yellow; yellow
E110: Sunset yellow; orange
E122: Carmoisine; red
E133: Brilliant blue; blue
ADI: Acceptable Daily Intake (mg/kgbw/day)
THQ: Target Hazard Quotient
CDI: Chronic Daily Intake (mg/kgbw/day)
DI: average daily intake (Kg or L/day)
RfD: Oral reference dose (mg/Kg/day)
HI: Hazard Index

Table 7. Calibration data and instrumental information of the HPLC method
The set-up calibration data:

Analyte	Eqution of calibration Graph	R^2	Residuum [mAU.s]
Sunset yellow	Y=33.45015X+13.8555	0.9992376	22.7227
Carmoisine	Y=36.40637X-15.32372	0.9981163	35.84102
Quinilone yellow	Y=30.99968X+13.16351	0.9996874	12.72236
Brilliant blue	Y=28.38363X+10.09265	0.9999596	4.84947

HPLC Instrumental information:

Retention Time (min)	W05 (min)	Asymmetry	Capacity	Efficeincy [th.pl]	Eff/l [t.p/m]	Resolution	Compound name
7.633	0.167	1.125	0.00	11621	232419	11.164	E110
12.333	0.250	0.857	0.00	13483	269663	13.310	E122
22.350	0.383	1.000	0.00	18833	376653	18.663	E104
42.950	0.733	1.025	0.00	19003	380070	21.768	E133

Statistical analysis

A correlation analysis was performed using Prism software to investigate the relationship between atomic absorption and HPLC results. Based on the statistical results, no significant relationship was detected between the two methods.

Discussion

The results of this study align with findings from similar research carried out in the European Union and the United States, where the concentrations of colorants in nutritional products designed for children have raised concerns. The examination of artificial food colorants within jelly powder and soft jelly products has yielded critical insights regarding the exposure levels experienced by consumers, particularly children, who represent the most susceptible demographic due to their reduced body mass and habitual intake of such items. There exists no precise correlation between the concentration of heavy metals in synthetic colorants and the ingestion of such colorants. Given the prevalent application of these colorants in gelatinous products, it is crucial to assess the associated risks of their consumption among pediatric populations [27, 28]. Although the precise quantity of gelatinous product consumption among individuals, particularly children, remains indeterminate, approximations regarding daily intake yield several indicators pertinent to daily risk assessments, as delineated in Table 6. Nonetheless, in light of the substantial amount of production and consumption of gelatinous products within the Iranian market, it appears imperative to reformulate the standards governing the quantity and type of synthetic colorants permitted in these products. Our observations indicated that red and orange colorants exhibit the highest concentrations of heavy metals. Furthermore, it was determined that Carmoisine and Brilliant Blue are the most frequently utilized synthetic colorants. The national standards of Iran impose the most stringent restrictions on Sunset Yellow, as referenced by the Codex, relative to other synthetic colorants [29, 30]. This colorant exhibited the highest concentration of heavy metals in comparison to other colorants (Table 4). Nevertheless, the existing rate of utilization is three times higher than what is allowed in the European Union. Sunset Yellow is classified as one of seven authorized synthetic colorants in Iran, although it is essential to estimate its daily intake [31].

In this investigation, we identified a significant concentration of Carmoisine and Brilliant Blue within the gelatin powder samples (Table 5). This finding is significant due to the observation that the highest concentration of heavy metals is associated with this particular colorant. Iran's regulatory standards outline and specify only the synthetic food colors that are permitted; nonetheless, they do not impose restrictions on their application in certain colored food products, including those of a gelatinous nature [30, 32]. According to the Iranian National Standard, the synthetic colorants employed in gelatinous products are positioned within the maximum limit range for Sunset Yellow (as per Codex guidelines) [31]. Nevertheless, this concentration is thrice that of the European Union and is additionally prohibited in gelatin powder [9]. Despite being one of the seven authorized synthetic colorants in Iran, an estimation of its daily intake is requisite. Although Quinoline is not prohibited in numerous countries, including the United States and Europe, it is recognized as another permitted synthetic colorant in Iran [33, 34].

The lowest and highest concentrations of synthetic colorants detected in the gelatinous product samples were Sunset Yellow and Brilliant Blue, respectively (Table 5). Brilliant Blue and Carmoisine colorants were the most prevalent among those observed, while Sunset Yellow and Quinoline Yellow were the least prevalent (Table 5). While the precise daily consumption levels of gelatinous products among children are not ascertained, the elevated consumption rates necessitate a thorough evaluation of the potential risks, particularly in pediatric populations. The daily intake of gelatinous products within the Iranian food industry is considerable [35]. In light of the data presented in Table 6, a re-evaluation of the daily intake standards concerning these products, as well as the types and concentrations of synthetic colorants utilized, is warranted.

The minimal quantities of certain synthetic dyes can lead to accumulation over time, particularly in children, whose developing systems may exhibit heightened sensitivity to chemical exposure. [36]. Synthetic food colorants have been linked to adverse health effects, including behavioral issues in children who frequently consume processed foods. Therefore, while individual dyes may remain within RfD limits, the collective hazard associated with the ingestion of multiple dyes can yield elevated THQ [37]. In our research, this phenomenon was apparent in soft jelly, where the THQ for E122 was as elevated as 5.16, while the RfD for E133 was 6.125, indicating a considerable health risk. Moreover, the HI for jelly products attained a value of 5.28, suggesting that cumulative exposure to various dyes, including E133 and E122, could present long-term health implications (Table 6).

Average local daily intake of Brilliant Blue in jelly products attained a level of 5.55 mg/kgbw/day, which is in proximity to the RfD of 6.125 mg/kgbw/day (Table 6) [38]. This situation raises serious concern over the safety of habitual consumption, particularly among children, who possess a lower body weight and may ingest larger quantities of such products in relation to their body size. Thereafter, due to the correlation between synthetic food dyes and behavioral disturbances in children, such disorders as ADHD can be observed more frequently [36]. In the context of Brilliant Blue and Carmoisine, there exists evidence indicating that prolonged exposure, particularly at concentrations near or exceeding the RfD, may present risks associated with neurodevelopmental and allergic responses [39, 40]. Specifically, Carmoisine, with an average local daily intake of 2.22 mg/kgbw/day in jelly powder, surpasses the RfD of 1.985 mg/kgbw/day (Table 6), implying a potential for detrimental effects with consistent consumption [41].

The European Food Safety Authority (EFSA) has established ADI for food colorants, such as Sunset Yellow quantified at 4 mg/kgbw/day (Table 6) [42]. The chronic daily intake (CDI) of Quinoline, derived from the consumption of jelly powder and soft jelly, was quantified at 0.033 and 0.023 mg/kgbw/day, respectively, both values being below the established permissible daily intake or ADI. These findings indicate that the level of Quinoline consumption among the pediatric population in Iran surpasses ADI. Given that this synthetic dye is prohibited in numerous countries, there exists a paucity of information regarding the CDI of this artificial colorant in food products. The CDI for Sunset Yellow in jelly powder and soft jelly was measured at concentrations of 0.15 and 0.023 mg/kgbw/day, respectively. Concurrently, the CDI of Sunset Yellow within the Iranian market exceeded ADI. Furthermore, the CDI values for Carmoisine and Brilliant Blue were also found to surpass the acceptable ADI in the analyzed products, as detailed in Table 6.

The analysis of the gathered data revealed that the THQ values for the four synthetic colors present in jelly product samples exceeded 1. Notably, the highest THQ values for synthetic colorants were identified among the Iranian pediatric demographic concerning jelly powder and soft jelly. Consequently, these products are associated with significant potential health risks for the pediatric population in Iran. Implications for public health outcomes are imperative for enhanced regulation and oversight regarding synthetic colorants utilized in food products intended for children. Specifically, E133 and E122 emerge as the most concerning agents, as their consumption levels in jelly powder products either approach or surpass established safety limits [43]. Policymakers may need to reevaluate the ADI for these colorants.

Nevertheless, owing to regional and cultural variances in dietary practices, as well as the differing methodologies and locations utilized in exposure assessments, drawing comparisons between the dietary habits in this study and those reported in other studies proves to be a challenging endeavor. Furthermore, the elevated hazard indices for soft jelly products, in contrast to jelly powder, underscore the augmented health risks linked to the habitual intake of these items, particularly in instances where multiple colorants are utilized.

The natural pigments, including anthocyanins and carotenoids, may offer safer alternatives for food coloring devoid of the health risks commonly associated with synthetic dyes. This transition to natural colorants has the potential to significantly mitigate health hazards linked to such products as jelly powder and soft jelly, especially for at-risk groups, notably children [44]. Nonetheless, the results indicated that the HI values for all identified colorants exceeded 1. Despite this, the current child population is subjected to substantial potential health risks, underscoring the necessity for heightened scrutiny regarding the consumption of artificial food colors. Therefore, the advocacy for the utilization of natural colorants and the reduction of food products containing synthetic food colorants is strongly recommended.

Conclusions

Although the concentrations of synthetic food colorants in jelly products do not consistently exceed regulatory thresholds, the cumulative exposure to various dyes, particularly among children, constitutes a notable public health issue. The elevated THQ and HI values documented for Brilliant Blue and Carmoisine highlight the necessity for vigilant monitoring and possible reformulation of these products to safeguard consumer health. Future investigations should prioritize examining the long-term consequences of low-dose exposure to synthetic dyes, particularly within pediatric cohorts, and assess the feasibility of natural alternatives to synthetic colorants.

Conflict of Interests

The authors declare no conflicts of interest with any entities.

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Compliance with Ethical Guidelines

Not applicable.

Authors' Contributions

All authors equally contributed to preparing this article.

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