Review Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.5327/fst.556

Effect of toxicity of artificial colors present in foods and their replacement with natural colors: a review

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Abstract

With technological innovations in industrial foods, food colorings have become part of the formulations of these products, as they have a stimulating effect on the consumer's appetite. However, its use is surrounded by controversy due to the development of allergies and diseases, such as attention-deficit hyperactivity disorder, especially in children. Thus, spurred by potential health effects, coupled with consumer concerns about safety, industry efforts have led to replacement of artificial food colors with natural ones. However, this is not an easy task. Thus, the aim of this study was to carry out a survey of scientific work on the toxicity of artificial dyes and the possible replacement by natural dyes on an industrial scale. To construct this research, a search was carried out for articles and various works published in the area of natural and artificial dyes in the databases, where 40 studies were selected to compose this review. In conclusion, it is noted that despite the food industry's current preference for artificial colorings, there is a high demand for research to transform natural colorings into more stable and low-cost substances, using different sources of raw materials in addition to new technologies and products arising from agro-industrial waste.

Keywords: recommended daily ingestion; regulatory organs; vegetable extracts; toxic.

Practical Application: The food industry is searching for ways of substituting artificial additives with natural ones, as in the case of dyes.

1 INTRODUCTION

Due to urbanization and the accelerated day-to-day rhythm, a change has occurred in the food habits of the Brazilian population, the consumer tending to prefer ready-to-eat or semi-ready-to-eat foods, offering practicality for consumption. With the technological innovations in industrial foods, food additives have become a part of the formulations of these products. It is important to point out that recommendations exist for the use of food additives that evaluate the type, safety level, and technological aspects of these products, permitting their addition to foods or otherwise (Aun et al., 2011; Souza et al., 2019).

Observe that, due to the greater use of processed and ultra-processed foods, the use of these substances has been generating some controversies in relation to their consumption, such as clinical manifestations, metabolic complications, and carcinogenic effects. There has also been a greater incidence of non-transmittable chronic diseases such as systemic arterial hypertension, diabetes mellitus, obesity, and allergies, among others (Bissacotti et al., 2016; Polônio & Peres, 2009; Souza et al., 2019).

Of the strategies used by industry to enrich and favor the processed product, the color exerts a stimulating effect on consumer appetite as an integral part of the feeding process (Azeredo et al., 2016). This class of food additives improves the visual sensory characteristics of the product, producing greater consumer acceptability, such that individuals frequently choose their foods according to the color (Johann & Gräff, 2014; Santo et al., 2013).

Up to the nineteenth century, most food dyes and pigments were obtained from natural sources, together with spices and condiments, which had the function of coloring foods. Vegetable dyes present serious limitations due to their lack of shininess, weak fixation, restricted color scale, and instability to alteration in the medium (Souza et al., 2019).

In general, industry prefers to use artificial dyes since they are cheaper, process resistant, do not easily lose their color even under extreme conditions, and have a higher calorific value (Azeredo et al., 2016; Queiroz & Nabeshima, 2014). However, some studies have related their use to the development of allergies and diseases, such as attention-deficit disorder and

Received: Aug. 27, 2025.
Accepted: Sep. 04, 2025.
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Conflict of interest: nothing to declare.
Funding: nothing to declare.

hyperactivity, especially in children (Gawai et al., 2017; Santos et al., 2022).

Recent studies, stimulated by potential health effects together with consumer safety concerns, have led to efforts to substitute artificial food dyes with natural dyes. This is not an easy task, since natural dyes are generally less stable, more expensive, and not as easy to use as synthetic dyes, as well as presenting interactions with food components, having a limited color spectrum, and having a lower coloring potential (Rodriguez-Amaya, 2016). On an industrial scale, the exchange of artificial dyes for alternative vegetable sources creates challenges from both technological and financial perspectives. However, there is considerable engagement in research using new techniques and technology, optimizing the extraction process to make it more feasible, as well as obtaining more stable natural pigments for industrial use (Martins et al., 2016).

Another challenge for this application is a reduction in the costs involved in obtaining natural dyes. Thus, the extraction of pigments from food industry byproducts, known as agro-industrial residues, is a possible alternative with lower acquisition costs, as well as reducing the volume of industrialized organic residues (Neves et al., 2019).

Considering the above, the objective of this study was to make a literature survey of scientific articles concerning the toxicity of artificial dyes and their possible substitution on an industrial scale by natural dyes.

1.1 Relevance of the work

With technological advances in the food industry, artificial colorants have become common in formulations, enhancing appetite and visual appeal. However, studies indicate health risks such as allergies and neurological disorders, especially in children. Growing consumer concern drives the search for safer, more sustainable natural alternatives. Despite challenges in stability and cost, research is exploring plant-based sources and agro-industrial waste to develop viable natural dyes on a large scale. This scenario highlights the importance of innovations that combine safety, functionality, and commercial appeal.

2 MATERIAL AND METHODS

In this survey, the databases such as Ebsco, Google *Acadêmico*, Scielo, ScienceDirect, Scopus, and other sources shown to be useful for the data collection were used in the search for articles and diverse projects published in the area of natural and artificial dyes to compose this literature review. In all, 90 scientific sources were found in the period from June to August of 2022, and after filtering, 40 research studies were selected to make up this review.

Table 1 shows the keywords used in the survey, in English and Portuguese, which were used both singly and correlating more than one keyword with the joiner-words (ballers) "And" and "Or". Studies published since 2007 were selected and analyzed, and documents with recommendations and regulations concerning ingestion and the use of additives in industrialized foods were also used.

Table 1. Keywords used in the survey of the databases selected.

Corante—*Dye and color*	Artificial—*Artificial*	Toxicidade—*Toxicity*	**Aplicações nas industrias—*Industrial applications***
Pigmento—*Pigment*	Natural—*Natural*	Substituição—*Replacement and Substitution*	Reavaliação—*Re-evaluation*
Corante Natural—*Natural Dye*	Corante Artificial—*Artificial Dye*	Tartrazina—*Tartrazine*	Ingestão Diária Aceitável (IDA)—*Acceptable Daily Intake (ADI)*
Extratos Naturais—*Natural Extracts*	-	-	-

3 LITERATURE REVIEW

3.1 Food additives

The Brazilian National Health Surveillance Agency (ANVISA) (Brasil, 1997) defines a food additive as any ingredient intentionally added to foods during the manufacture, processing, preparation, treatment, packaging, storage, transport, or handling of a food, with no intention to nourish, but with the objective of modifying the physical, chemical, biological, or sensory characteristics. They are classified as preservatives, antioxidants, emulsifiers, stabilizers, flavorings, sweeteners, and colorings.

Although these substances are evaluated by the Joint Food and Agriculture Organization (FAO) / World Health Organization (WHO) Expert Committee on Food Additives (JECFA), the specific rules and regulations vary from country to country. More than 3,000 food additives can be found on the Food and Drug Administration (FDA) Everything Added to Food in the United States (EAFUS) list and 2,000 additives can be found on the Chinese Number System (CNS) and on the National Food Safety Standard for the Use of Food Additives, but only 402 molecules have been attributed to the European Union inventory number catalogue as approved by the European Food Safety Authority (EFSA) (Almeida et al., 2018).

In Brazil, ANVISA is responsible for regulating the use of food additives, and more than 1,000 food additives and dyes have been regulated. The Ministry of Health decree No. 50.040 of January 24, 1961, was the first technical regulatory norm passed concerning the use of chemical additives in foods. This decree determines in which foods they can be used, and the maximum limits allowed (Brasil, 2010).

In addition, Resolution No. 259 (Brasil, 2002) recommends that the additive should appear on the label at the end of the product list of ingredients, indicating the function carried out by the additive in the food, the complete name, and/or the INS (International Numbering System) number according to the FAO/WHO Codex Alimentarius.

Despite the necessary legal requirements, regulated by the specialized organs of each country for the use of any additive in foods, there is an increasing consumer demand for clean-label foods, with the inclusion of no ingredients produced synthetically. Thus, the food industry is currently searching, increasingly, for additives from natural sources (Carocho et al., 2015; Maruyama et al., 2021).

3.2 Food dyes

Of the food additives most used, the dyes have a crucial role in developing food preferences and sensory perception. The color is responsible for 62–90% of consumer evaluation, and hence the use of dyes by the food industry is of major importance (Jantathai et al., 2014; Pires et al., 2018; Teixeira et al., 2022).

According to the FDA (2023), a dye or color additive is any dye, pigment, or substance, which, when added or applied to a food, is capable (alone or by reacting with other substances) of conferring color, being recognized as an important part of practically all the processed foods we ingest.

Regulation (CE) No. 1333/2008 states that a food additive can only be used as a dye for one of the following intentions: (a) compensate for a loss of color of the food due to processing, storage, packaging, or distribution; (b) correct natural color variations; (c) make the foods visually more attractive; or (d) provide color to colorless foods (FDA, 2023; Galdeano et al., 2022; Meléndez-Martínez et al., 2022).

Figure 1 shows the classification of food dyes as regulated by Resolution No. 44 of 1977 of the Brazilian National Food Norms and Standards Commission (CNNPA), where natural organic is that obtained from plants or animals; synthetic organic is that obtained by organic synthesis and can be divided into two categories: identical to the natural compound (chemical structure similar to that of the natural compound) or artificial (not found in natural products); inorganic, produced from mineral compounds; and caramel, which can be divided into natural (obtained from heating sugars) or synthetic, identical to the natural compound (produced via processing with ammonia).

3.2.1 Artificial dyes

The artificial synthetic organic dyes are the dyes most used by the food industry due to their lower production costs, greater chemical stability, and ease of use for application in different food matrices (Neves et al., 2019).

According to ANVISA (Brasil, 2015), 16 artificial dyes can be used in Brazil, as listed in Table 2. Resolution No. 04/88 of the CNS/MS (Brasil, 1988) lists the eight artificial dyes permitted for use in foods, as follows: sunset yellow, brilliant blue, Bordeaux S or amaranth, erythrosine, indigo carmine, Ponceau 4R, tartrazine, and red 40 (6). After the adequation of the Brazilian legislation with the member countries of the Southern Common Market, in 1999 the dyes azorubine, patent blue V, and fast green were also approved for use in some foods (Brasil, 1999) and in 2005 (Brasil, 2005) quinoline yellow, brilliant black BN, and chocolate brown HT. In addition, Article 13 of Decree Law No. 986/69 (Brasil, 2015) determined that foods containing artificial dyes present the declaration "artificially colored" on the label, as well as declaring the dyes in the product ingredient list.

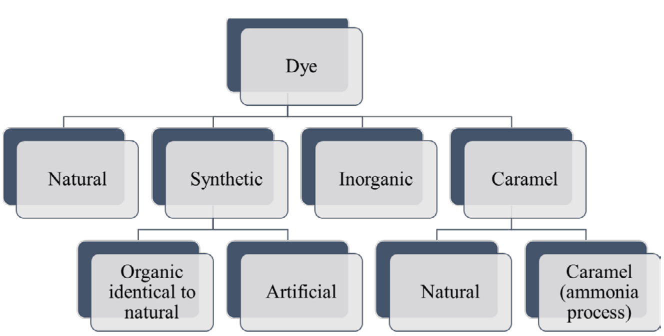

Figure 1. Classification of dyes (Brasil, 1977).

Most of the dyes cited above are the targets of research regarding their toxicity on ingestion. Amaranth (red color), erythrosine (pinkish color), and tartrazine (yellow) are the most studied, since they have presented toxicological and mutagenic potentials in various studies. They manage to alter genetic material, causing cytogenic and genotoxic damage as well as significantly modifying the body's metabolism and interfering with the function of various components in the human body. In addition, the dye tartrazine should be used with great care due to its allergenic characteristics (Anastácio et al., 2016).

Mpountoukas et al. (2010) evaluated the genotoxic effects of the dyes such as amaranth, erythrosine, and tartrazine in peripheral blood cells, applying some in vitro tests such as evaluation of the proliferation rate and mitotic index (MI), DNA mobility and displacement in agarose gel electrophoresis, DNA-binding studies using spectroscopic titration, and the genic amplification test in the polymerase chain reaction (PCR). The three dyes negatively affected the proliferation rates and mitotic indexes (reproduction) of the cells tested in concentrations above 4.0 mM, erythrosine and tartrazine being so toxic that there was no more cell division. They are also bound to the DNA retarding the agility of the nucleic acids and degrading the genetic material.

In addition, some studies showed evidence of affecting the fetus when the mother was exposed to these dyes during pregnancy. Doguc et al. (2012) used specific tests to analyze the cognitive and learning abilities and behavior of mice born to mothers fed

Table 2. Artificial dyes permitted by Brazilian legislation (Brasil, 2015).

International numbering system	Name of food additive
102	Tartrazine
104	Quinoline yellow
110	Sunset yellow
122	Azorubine
123	Amaranth or Bordeaux S
124	Ponceau 4R
127	Erythrosine
128	Red 2G
129	Red 40
131	Patent blue V
132	Indigo carmine
133	Brilliant blue FCF
143	Fast green FCF
151	Brilliant black BN
155	Chocolate brown HT
180	Lithol rubine BK

on amaranth (0.5 mg/kg/day), erythrosine (0.1 mg/kg/day), and tartrazine (7.5 mg/kg/day). The results obtained were very similar for the three dyes studied, with no expressive changes in behavior and no adverse effects on the memory or learning abilities. However, due to small changes in locomotory and exploratory activities and anxiety levels of the descendants of those exposed to the dyes, the authors raised the hypothesis that such exposure could be correlated with attention-deficit/hyperactivity disorder (ADHD).

Matsuo et al. (2013) carried out a trial to prove the allergenic effect of tartrazine and its potential to liberate histamine. They took blood samples from patients with idiopathic urticaria and exercise- or food-dependent induced anaphylaxis (FDEIA) and isolated the basophils present. Subsequent tests proved the inductor action of liberating histamine by tartrazine.

Although the synthetic dyes used have long reports of the safety evaluations carried out and rigid regulation, note that the consumption of synthetic dyes is constantly connected to allergenicity and hyperactivity in children. Thus, the food industry is searching for alternatives for renewal, aimed at substituting artificial dyes with natural ones (Sigurdson et al., 2017; Teixeira et al., 2022).

3.2.2 Natural dyes

Consumer habits are currently changing due to the appearance of new tendencies, including the search for more natural, less processed foods, and which provide health benefits due to their bioactive properties, such as an antioxidant effect. Hence, one alternative to updating industrial products is the use of natural dyes in the production (Amarante et al., 2020).

Diverse beneficial effects are associated with the ingestion of these natural substances, such as an increase in the resistance of low-density lipoprotein to lipid peroxidation, the chelation of transition metals that cause oxidative reactions, and the blocking of enzymes involved in oxidative stress (Teixeira et al., 2022).

In addition, considering current transformations occurring with the environment, there is greater concern with sustainable development, whereby there is a harmonious interaction between man and nature, thus contributing to a change in the current consumption tendencies (Azeredo et al., 2016). Natural dyes also act as agents allied to "green chemistry" since they are biodegradable sources and do not therefore cause environmental damage to effluents and phreatic sheets (Aguirre-Joya et al., 2020; Martins et al., 2016; Muthusamy et al., 2020).

In Brazil, according to Resolution No. 44 of CNNPA in 1977 (Brasil 1977), the natural dyes shown in Table 3 have been liberated for use. Cochineal carmine, turmeric, cupric chlorophyll, caramel, and annatto are the natural additives most used by the industries, generally used as dye mixtures.

The dye carmine is widely used in the food industry due to its wide scope of colors, from red to purple (Ferreira et al., 2022), as well as being stable to light, heat, and the presence of ascorbic acid, and non-toxic. It is extracted from females of the insect cochineal (*Dactylopius coccus*), and the hue depends on the pH value of the medium (Santos et al., 2022). It is indicated for use in foods with pH values above 3.5, since it is slightly soluble at pH values below 3.0 (Hamerski et al., 2013).

Table 3. Natural dyes permitted for use by Brazilian legislation (Brasil, 2015).

International numbering system	Name of food additive
100 i	Turmeric
101 i	Riboflavin
101 ii	Riboflavin 5' sodium phosphate
120	Cochineal carmine, Na, K, NH4, and Ca salts
140 i	Chlorophyll
140 ii	Chlorophyllin
141 i	Cupric chlorophyll
141 ii	Cupric chlorophyllin, Na and K salts
150a	Caramell—simple
153	Charcoal
160a ii	Crotenes: natural extracts
160b	Annatto, bixin, norbixin, annatto extract & Na and K salts
160c	Paprika, capsorubin, capsanthin
160d	Lycopene
160e	Beta-apo-8'-carotenal
160f	Methyl or ethyl esters of beta-apo-8' carotenoic acid
161b	Lutein
161g	Canthaxanthin
162	Beetroot red betanin
163 i	Anthocyanins (from fruits and vegetables)
163 ii	Grape skin extract

Another dye of great industrial importance is annatto, obtained from the lipstick tree (*Bixa orellana*). This pigment can be commercialized both as a liposoluble and hydrosoluble extract, making it easy to use in different food matrices. It has a yellowish-orange color and is susceptible to light, oxygen, and heat (Santos et al., 2022), and for this reason, it is frequently used in dye mixtures together with turmeric.

Curcumin is a constituent isolated from turmeric rhizomes and is the main active principal in the turmeric used as a dye, with a color varying from brilliant yellow to dark orange (Codevilla et al., 2015). Its stability is altered by pH values out of the range from 4 to 7, by the incidence of light, metal ions, ascorbic acid, and heat treatment (Paramera et al., 2011).

Cupric chlorophyll is a synthetic derivative of chlorophyll, which is green and is the principal choice by industry for this color spectrum. Chemically, it is highly resistant to heat and light, more resistant to oxidative agents in general, and highly water soluble (House & Schnitzer, 2008). It differs from chlorophyll due to the substitution of an Mg atom by a Cu atom and for not containing phytol (Lanfer-Marquez, 2003). Thus, this dye, together with chlorophyll in nature, has been widely investigated for its beneficial biological activities including skin healing, anti-inflammatory properties, and anti-mutagenic activity (Ferruzzi & Blakeslee, 2007; Ferruzi et al., 2002; López-Carballo et al., 2008).

Caramel I stands out as the main choice by the industry for use in darker-colored products. It is a complex mixture of products produced by caramelization, a process involving the degradation of sucrose, glucose, and other carbohydrates, which, on slow heating, acquire the color and flavor characteristic of caramel. Caramel is considered to be one of the oldest dyes allowed for use by the food industry, used to obtain colors going from straw yellow to dark brown, varying according to the process (Mendonça et al., 2021).

Substances like anthocyanins, for example, are little used by the food industry since they are unstable to pH, concentration, and metal complexation, and also to temperature, light, oxygen, ascorbic acid, and sugars, among others. Nevertheless, anthocyanins are moderately stable at low pH, generating great interest by industry due to their antioxidant, anti-inflammatory, anti-carcinogenic, and antidiabetic properties, coinciding with current consumer preference (Panel on Food Additives and Nutrient Sources Added to Food [ANS Panel], 2013; Pires et al., 2018; Rodriguez-Amaya, 2016). Thus, there are currently various scientific studies aimed at developing stable natural pigments by using new technology or by producing vegetable extracts and concentrates.

3.3 Synthetic organic dyes identical to the natural dye

Table 4 presents a list of synthetic organic dyes identical to natural dyes that are allowed for use by Brazilian legislation according to CNNPA Resolution No. 44 of 1977 (Brasil, 1977), where caramels III and IV are widely used by the food industry, mainly in beverages.

It is important to note that synthetic beta-carotene is the most used form of this dye due to its great stability. It is elaborated by chemical synthesis, having 0.6 µg of beta-carotene, the color varying between yellow and orange, and is the synthetic dye that most stands out in terms of production, due to its vast applicability (Grune et al., 2010).

Depending on the process used to obtain them, caramel-type dyes can be divided into four categories, as shown in Table 5. Caramel I (containing no additive), caramel II (containing the additive sodium sulfite), caramel III (containing the additive ammonium hydroxide), and caramel IV (containing the additive ammonium sulfite). The dye caramel I is the natural dye, and caramels II, III, and IV are the synthetic organic dyes (Ferreira et al., 2022).

Since the caramel dyes III and IV use ammonia in their caramelization process, 4-methyl-imidazole (4-MEI) is formed, a substance that has been associated with diverse health problems such as the development of intestinal and lung cancer (Moon & Shibamoto, 2011). Hence, the World Health Organization (WHO) has included caramels III and IV in their list of possibly cancerous substances (Jacobson, 2012).

Table 4. List of synthetic organic dyes identical to the natural dye permitted by Brazilian legislation (Brasil, 2015).

International numbering system	Name of food additive
160a i	Beta-carotene (synthetic, identical to natural dye)
150b	Caramel II—caustic sulfite process
150c	Caramel III—ammonia process
150d	Caramel IV—ammonia sulfite process

3.4 Toxicity of artificial food dyes versus substitution by natural dyes

The toxicity of artificial food dyes still causes considerable controversy and discussion concerning their use and the amount present in foods, since this is not specified. Thus, the responsible regulatory organs, in addition to quantifying a maximum limit for each additive, recommend an acceptable daily ingestion (ADI) value.

According to RDC No. 295 of July 29, 2019 (Brasil, 2019), the ADI is defined as the estimated amount of a substance present in foods that can be ingested daily throughout a person's lifetime, without offering a considerable health risk, and is expressed in milligrams of the substance per kilogram of body weight (mg/kg b.w.).

However, to maintain consumer safety, the previously expressed values have been submitted to a technical review due to new studies presented to the regulatory organs. Thus, Table 6 shows the ADI values before and after review by EFSA, with the corresponding name and number.

After the reviews were carried out, there were no changes in the ADI values for the following dyes: azorubine (ANS Panel, 2009b), erythrosine (ANS Panel, 2011b), indigotin (ANS Panel, 2014), brilliant black (ANS Panel, 2010b), red 40 (ANS Panel, 2009c), tartrazine (ANS Panel, 2009f), and the caramels (I, II, III, and IV) (ANS Panel, 2011a). The technical report presented by EFSA concerning these dyes indicated insufficient motives to revise the previously recommended ADI values, since these dyes did not demonstrate health risks, and, in fact, the values found for the ingestion of these additives were well below the values cited.

Table 5. Classification of caramel dyes according to mode of preparation (Brasil, 2015).

INS	Name	Manufacture
150a	Class I: Simple caustic caramel	Prepared by heating carbohydrates, with or without acid or alkaline substances. Ammonia and sulfite components are not used
150b	Class II: Caustic sulfite caramel	Prepared by heating carbohydrates, with or without acid or alkaline substances, in the presence of sulfite compound Ammonia components are not used
150c	Class III: Ammonia caramel	Prepared by heating carbohydrates, with or without acid or alkaline substances, in the presence of ammonia. Sulfite compound are not used
150d	Class IV: Ammonia sulfite caramel	Prepared by heating carbohydrates, with or without acid or alkaline substances, in the presence of ammonia and sulfite compounds

Table 6. Name of additive, international numbering system No., and acceptable daily ingestion before and after review by the European Food Safety Authority.

Name of dye	INS	ADI (mg/kg b.w./day) before review	ADI (mg/kg b.w./day) after review
Azorubine	122	4 (1984)	4 (2009)
Quinoline yellow	104	10 (1984)	0.5 (2009)
Sunset yellow FCF	110	2.5 (1984)	1 (2009)
Brilliant blue FCF	133	10 (1984)	6 (2010)
Bordeaux S or amaranth	123	0.5 (1984)	0.15 (2010)
Patent blue V	131	15 (1983)	5 (2013)
Caramel I, II, & IV	150 (a, b, c, & d)	300 (2011)	300 (2011)
Caramel III	150b	200 (1984)	100 (2011)
Erythrosine	127	0.1 (1990)	0.1 (2011)
Indigotin	132	5 (1984)	5 (2014)
Lithol rubine BK	180	1.5 (1983)	Not established (2010)
Brown HT	155	3.0 (1984)	1.5 (2010)
Ponceau 4R	124	4 (1984)	0.7 (2009)
Brilliant black	151	5 (1984)	5 (2010)
Red 40	129	7 (1989)	7 (2009)
Tartrazine	102	7.5 (1984)	7.5 (2009)

INS: International numbering system; ADI: acceptable daily ingestion.

With respect to caramel III, the published technical report established a new amount for the ADI (100 mg/kg b.w./day) due to new information concerning the immunotoxicity of THI (2-acetyl-4-tetrahydroxybutylimidazole), a toxic substance that only appears in this type of caramel. Sufficient studies have still not been presented to classify caramel III and caramel IV as carcinogenic, and hence there is a need for new studies demonstrating the damage done by THI and 4-MEI. Finally, indications exist that both adults and children can exceed the ADI value, having a greater exposure to this dye (ANS Panel, 2011a).

With respect to yellow-colored artificial dyes, such as quinoline yellow (104) and sunset yellow (110), the recommended maximum acceptable ingestion values were altered (Table 6). For the dye INS 104, the panel concluded, based on studies concerning the long-term semi-chronic and reproductive toxicity that could develop, including a study using rats, that a redefinition of the ADI (0.5 mg/kg b.w./day) was justified (ANS Panel, 2009a).

The ADI of sunset yellow was temporarily altered to 1 mg/kg b.w./day, due to inconclusive studies that reported significant effects on the testicles of rats exposed to high levels of this dye. However, clarifications concerning sperm morphology and mobility were lacking, and hence the panel explained that they would re-evaluate this value, but a new report has not yet been published (ANS Panel, 2009e).

The scientific reports concerning brilliant blue and patent blue indicated the need to alter the values for their acceptable daily ingestions (Table 6), due to data concerning genotoxicity, sub-chronic toxicity, and the long-term development of carcinogenicity. With respect to INS 133, the panel considered a study concerning chronic toxicity in rats to allocate a new ADI value, applying an uncertainty factor of 100, establishing an ADI value of 6 mg/kg b.w./day (ANS Panel, 2010c). Patent blue was treated similarly, generating a new ADI value of 5 mg/kg b.w./day (ANS Panel, 2013).

With respect to the reddish dyes Amaranth and Ponceau 4R, the ADI values were considerably reduced, as shown in Table 6. To re-evaluate the ADI value for amaranth, studies concerning its toxicity were taken into consideration, and a value of 0.15 mg/kg b.w./day was indicated. The panel concluded that exposure to this dye by children (1–14 years) was less than 30 times the ADI value, but that in adults it could be six times greater than the ADI value due to the consumption of beverages such as wine (ANS Panel, 2010a).

Studies carried out with Ponceau 4R indicated neurobehavioral effects and an increase in hyperactivity in 3-year-old children. However, it was negative for in vitro genotoxicity and for long-term carcinogenicity studies, so the panel recommended an ADI value of 0.7 mg/kg b.w./day (ANS Panel, 2009d).

Based on the scientific report for chocolate brown HT involving a study for carcinogenicity and toxicity in mice, an ADI of 1.5 mg/kg b.w./day was recommended by the panel (ANS Panel, 2010d). Finally, the Scientific Committee for Foods recommended an ADI of 1.5 mg/kg b.w./day for the dye lithol rubine (Table 6) (1983). However, the panel considered the database too limited to continue supporting the previously defined value, indicating that this value should be removed, but without establishing a new value. Nevertheless, the panel observed that the greatest exposure predicted for lithol rubine was 1,700 times smaller than the effect level identified in female rats (100 mg/kg b.w./day), and hence a food safety concern for human beings, as the only use currently authorized for this dye, in edible cheese skins, was highly improbable (ANS Panel, 2010e).

Thus, one can conclude that despite the regulatory companies searching to review previous reports, there is a time lag between reviews, corroborating the population's concern about how health prejudicial the accepted levels could be. Table 7 shows the research studies carried out to evaluate the use of natural dyes in foods, with a view to their large-scale use by the industries.

Table 7. Studies evaluating the use of natural dyes to substitute artificial dyes in foods.

Reference	Objective of study	Dye analyzed	Results
Johann & Gräff (2014)	Sensory acceptance (color & flavor) by an infant population of jellies produced with artificial and natural dyes and vegetable extracts.	Yellow: turmeric and carrot extract; Red: red 40, anthocyanin, and beetroot extract; Green: tartrazine and brilliant blue FCF, chlorophyll, and spinach extract.	The general acceptance with respect to color was greater for the jellies with artificial color, followed by jellies with a natural dye and vegetable extract. However, for flavor, the natural and artificial dyes are tied, and consequently the vegetable extract.
Amarante et al. (2020)	Evaluate the long-term color stability and its antioxidant capacity (after in vitro digestion) of a phycocyanin extract in milk-based ice cream.	Phycocyanin extracted from *Spirulina platensis.*	The samples analyzed presented stable coloring throughout the 6 months of the study, but presented low antioxidant activity before the simulated in vitro digestion. After digestion, the ice creams containing the phycocyanin extract presented 13 times more antioxidant activity than the control ice creams, by the method analyzed.
Teixeira et al. (2022)	The substitution of synthetic food dyes by natural nanoencapsulated compounds using the principal components analysis (PCA).	Nanoencapsulated extracts of turmeric, spirulina, and hibiscus versus egg yolk yellow, strawberry red, apricot yellow, and tartrazine.	The antioxidant capacity of the natural dyes was proven in the trials reproduced. The principal components analysis allowed for a visual description of the proximity between the natural dye components and the artificial dyes when applied to different simulated food models, showing its usefulness in the industry with the other dyes.
Galdeano et al. (2022)	Evaluate the stability of a lycopene-rich concentrate extracted from watermelon, when applied as a natural dye to three types of food products: snack crackers, rice extrudates, and fusilli pasta.	Reddish-purple lycopene concentrate.	The effects of heat processing on the color parameters were not marked during manufacture of the product. However, there was a loss of color during product storage. The lycopene losses depended on the time/temperature exposure, the food matrix, and the initial dose of lycopene.
Bühler et al. (2013)	Pigment production by the fungus *Monascus ruber* using the byproduct glycerin as the substrate.	Pigments produced by *Monascus ruber* (red, orange, and yellow).	Crude glycerin has a high pigment-producing potential, the greatest yield being for red pigments, followed by orange pigments, and finally yellow pigments.
Gomes & Takahashi (2016)	Compare the biotransformation efficacy of sclerothiorin (orange bio-compound produced by *Penicillium sclerotiorum*) by *B. bassiana* using growing cells and those at rest, to obtain new dyes.	Orange and red pigments.	The methodology of using cells at rest was more useful than the use of growing cells for product formation. An intense red pigment was produced at the end of synthesis instead of an orangey one, due to the chemical composition of *B. bassiana.*
Lin et al. (2018)	Feasibility and acceptance of natural pigments as food dyes for coloring dry tofu.	Gardenia yellow, gardenia blue, turmeric, radish red, and canthaxanthin.	The experimental dry tofu obtained visual sensory acceptance and consumer buying intention. Thus, the combined natural dyes could be used as new coloring materials by the dry tofu industry in Taiwan.

In conclusion, despite the current preference of the food industry for artificial dyes, there is a great demand for research to transform natural dyes into more stable substances. There are diverse raw materials varying from vegetable extracts, nano-encapsulates, and pigments derived from fungi, which stand out even more for being low-cost options with good yield.

4 CONCLUSIONS

Considering that *clean-label* foods represent the current consumption tendency, where the composition is as natural as possible, the food industry is searching for ways of substituting artificial additives with natural ones, as in the case of dyes. The main challenges to allowing natural dyes to be used more by industry are reductions in their application costs and instability.

However, various databases are available presenting a variety of research projects aimed at developing stable natural food dyes obtained from vegetable extracts and filamentous fungi. Some agro-industrial residues can also be used, providing low-cost products as well as contributing to a greater sustainable development of the environment.

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