Re-evaluation on the safety of Erythrosine as a food additive: Structural Mechanism Comparison of the phased-out Red No. 3 and Natural Colorants as alternatives

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Abstract. The safety of artificial food colorants, particularly the Red Dye No. 3 (erythrosine), has become the centre of public health concerns. This type of artificial dye was once widely used in the food industry due to its vivid color and photostability. It has now been connected to potential health risks. Free iodine may be released when the metabolic breakdown of Red Dye No. 3 occurs due to the tetraiodinated fluorescein backbone. This potentially leads to endocrine disturbance and even cancer. Natural colorants, on the other hand, have several chemical issues, including instability when exposed to light, heat, and changes in pH, which makes them less suitable for industrial use. Switching from synthetic to natural food colorants is not only a technological step forward, but it also means that food additives need to be chemically redesigned to make them safer and more environmentally friendly. This article compares the chemical structures, metabolic routes, and safety profiles of synthetic and natural food colors. The focus is on the chemical reasons why Red Dye No. 3 is hazardous and the structural benefits of natural dyes in making things less toxic.

Keywords: Erythrosine, Natural Colorants, Food Safety, Synthetic Food Colorants, Food Additive.

1. Introduction

Food colorants, as an indispensable part of the food industry, play a crucial role in enhancing the appearance of food and increasing customer consumption. However, the increasing public concern about the safety of food additives has brought health issues raised by synthetic dyes to the forefront. Red Dye No. 3, also known as erythrosine, is a petroleum-based synthetic dye that has been widely used in food products in the United States since 1907 (Magazine and Osborne). However, in recent years, it has been strictly restricted or banned in several countries worldwide due to its potential endocrine-disrupting effects and carcinogenic potential (Anand Paramasivam et al.). The tetraiodinated fluorescein structure poses a metabolic risk due to the release of iodine during biotransformation, while contributing to its vivid color and outstanding industrial stability. The release induces interference with thyroid function and toxicology concerns at the DNA level.

Against the global backdrop of the gradual enforcement of a ban on artificial dyes, such as Red No. 3, the investigation and application of natural dyes have become a crucial pathway for development in the food industry. Natural dyes, such as Betanin (from beetroot), Monascus (from fermented rice), and Anthocyanins (from various fruits and plants), with their polyphenolic frameworks and hydroxyl-rich structures, can offer good biocompatibility and antioxidant function (Shoji). These characteristics have attracted widespread attention from the public to natural dyes.

Nevertheless, natural dyes are not just simply “harmless replacements”. Their chemical structures, stability, and coloring mechanisms are significantly different from those of artificial dyes. These differences not only influence the coloring application performance of dyes, but also directly determine their health risks on food application and metabolic behavior.

This article focuses on the current situation of substitutes for Red No. 3, in conjunction with the chemical structure and functional characteristics of food colorants, to analyze the chemical advantages and risks of natural dyes. By systematically comparing the chemical structures and functional group activities of artificial and natural dyes, this essay will provide theoretical support for the safe substitution strategies of food colorants, while also exploring future development pathways in the field of food safety chemistry.

2. Chemical structure and safety risks of synthetic dyes

2.1 Classifications and structures of synthetic food colorants

Synthetic dyes, most widely used in the food industry, can be categorized into four classes: Azo dyes, Xanthene dyes, Triarylmethane dyes, and Indigoid dyes (Center for Food Safety and Applied Nutrition). Among these four categories of artificial dyes, most share a common aromatic π-conjugated system, which allows for the delocalization of electrons across multiple benzene rings, producing visible colors.

Take Azo dye as an example. Azo dye, as one of the widely used groups of food colorants, is characterized by the presence of -N=N- double bond connecting to aromatic rings, forming an effective electron resonance system, as shown below. By modifying the substituent on aromatic rings, azo dyes can emit a wide range of spectra, from 350 nm to 650 nm, resulting in a color range from yellow to red (Kim and Son).

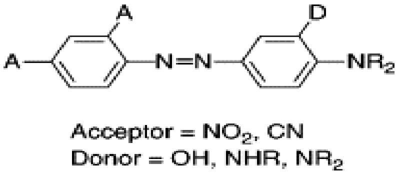

Fig.1 Basic structure of Azo Dyes

In contrast, xanthene-derived dyes represent another significant class of synthetic food colorants. Xanthene itself, reflecting yellow color, is a solid that dissolves in organic solvents. However, many of its derivatives are useful dyes (Gessner and Mayer).

Xanthene dyes contain a xanthene core, which can be categorized into three further groups: fluorescein, eosin, and rhodamine. The functional groups on the xanthene moiety control their fluorescent color.

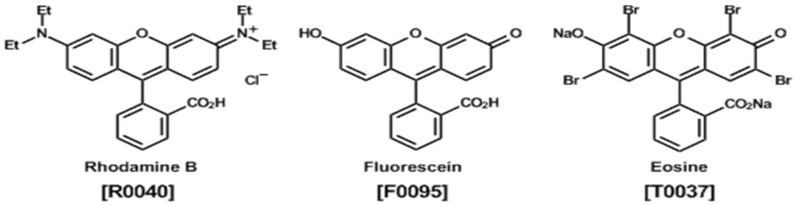

Fig.2 Structures of Rohodamine B, Fluorescein and Eosine

Beyond the classes of azo dyes and xanthene dyes, other types of artificial dyes with distinct structures are also used in the food industry. Triarylmethane dyes are characterized by their brilliant color and high tinctorial strength. A central carbon atom is connected to three aromatic rings, usually with hydroxyl, amino, and substituted amino substituents in the para-position functioning as auxochromes, to form the chromophoric system (Damant).

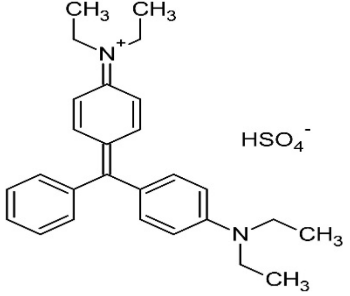

Fig.3 Example compound of Triarylmethane dyes

Indigoid dyes derive from modified indigo structures, gaining features of high melting point and low solubility in organic solvents from chemical structure modification (Fabian and Hartmann)

3. Health risks of Red Dye No.3

The color of food has always been a crucial factor influencing consumers' choices. To obtain stable, vivid, and storage-resistant food colorants, synthetic pigments emerged as a result. Compared to natural pigments, which often suffer from poor stability and color inconsistency, synthetic pigments have rapidly taken over the mainstream of the food industry due to their controllable molecular structures and noticeable lightfastness (Sterza). However, potential health risks may still exist while achieving advantages.

One example of a Xanthene dye is Red Dye No. 3 (Erythrosine). An artificial dye that has been officially announced to be banned in California in January 2025 (FDA). Besides food coloring, erythrosine is also widely used in printing ink, biological stains, dental plaque disclosing agents, as well as visible light photoredox catalysts, according to its specific chemical properties (Rogers et al.). Erythrosine has a tetraiodinated fluorescein structure, with a molecular formula $C_{20}H_6I_4Na_2O_5$.

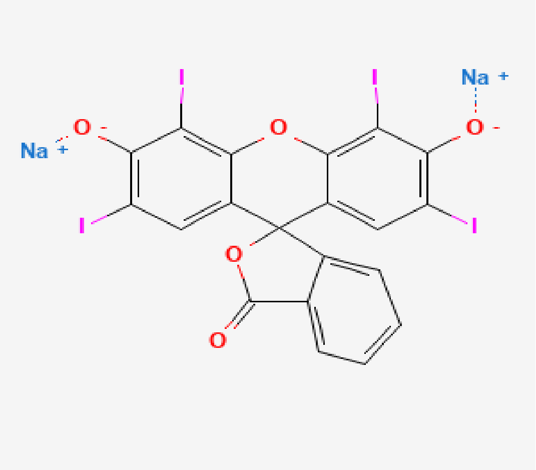

Fig.4 Structure of Erythrosine

The four iodine atoms on the xanthene rings induce a bathochromic shift, relocating the absorption maximum to approximately 524 nm, which results in a distinctive bright pink to red hue (Wang et al.).

Despite all the industrial advantages mentioned above, when the tetraiodinated structure enters the human body, it may release iodine, which could alter metabolic activity. Thyroid follicular hyperplasia, hypothyroidism, and other endocrine diseases may result from an abrupt rise in iodine levels that interferes with the production of thyroid hormones and the equilibrium of triiodothyronine (T3) and thyroxine (T4) (Walton et al.).

Erythrosine's extreme stability due to its highly halogenated structure is one of the reasons for its popularity as an artificial dye. While enjoying its stability, it is essential not to overlook the fact that this characteristic makes degradation in the environment and within the body difficult, leading to a high risk of forming persistent pollutants.

Additionally, the presence of large amounts of iodine substituents may lead to distortion of the electron cloud distribution, thereby increasing the molecule's affinity for electron donors or electron acceptors and enhancing its chemical reactivity (Clark et al.).

One of the central features of the Red Dye No.3 molecular structure is its highly conjugated aromatic system. Its main chain consists of a xanthene skeleton formed by the thickening of triphenyl rings, together with side-chain benzene rings, which form an extensive π-electron leaving region. The conjugated system reduces the HOMO-LUMO energy level difference of the molecule, making it easier to be excited to a higher energy state and possessing a certain photochemical activity (Wu et al.). In addition, the aromatic conjugated structure is prone to π-π stacking interactions with other molecules in the environment, and under certain conditions, may promote non-covalent binding of dye molecules to polymeric materials, proteins or DNA (Abdulwahid et al.). Although such binding is not a biotoxic reaction, it may trigger adsorption accumulation, catalytic reaction or photodecomposition chain reaction in materials chemistry and environmental chemistry. Also, electron delocalization may lead to the formation of excited state radicals or electron transfer processes under specific conditions, UV or high-energy light irradiation, which in turn induce redox reactions (Mansha et al.).

Overall, the food industry is progressively turning to natural colors as safer alternatives. Although natural dyes are often limited in terms of stability and hue intensity, they have relatively simple molecular structures. They are often free of halogenation, azo linkages, or other reactive molecules, which reduces the risk of toxicity and genotoxicity events. Bentamin, Monascus, and Anthocyanin, as natural dyes, possess alternative colors similar to Red No. 3, and are expected to replace Red Dye No.3.

4. Alternative natural dyes

4.1 Betanin

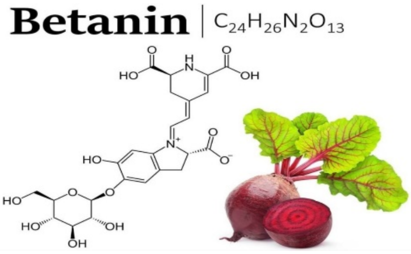

Fig.5 Structure of Betanin posted by chemistry concepts

Betanin is a naturally occurring red-violet dye. It is extracted from the juice of red beetroot, and it is also the compound responsible for the vibrant red color of the Beetroot (Beta vulgaris). Betanin belongs to the betacyanin group, specifically as a glycosylated betacyanin. The betacyanin group is a category of red to violet pigments that belong to a wider class of betalains. Betalain is a vibrant class of water-soluble, nitrogen-containing pigments found in certain plants—especially those in the Caryophyllales order, like cactus fruit as well as beets (Martínez-Rodríguez et al.).

Betanin's structure consists of five main components: an indole ring, cyclo-Dopa, a glucose moiety, a carboxyl group, and a conjugated pi-system. Each structure contributes to a function that results in the production of a beneficial natural pigment. To begin with, the indole ring is a fused structure composed of a pyrrole ring with a nitrogen atom and a benzene ring. Due to the fact that both rings are aromatic, they form an aromatic system together, which allows for electron delocalization and the formation of a stable, electron-rich cloud that can interact with other molecules. The delocalized electrons in the ring enable it to absorb light and donate electrons, which is key to Betanin's red-violet hue through its ability to absorb visible light. Additionally, the ability to donate electrons gives betanin its identity as an antioxidant; the donated electrons can neutralize free radicals that attack our body cells. In addition, the nitrogen atom can form hydrogen bonds or interact with metal, which leads to the ability to bind with metal ions like iron or copper, which further prevents oxidative damage. Furthermore, Betacyanins incorporate a cyclic form of 3,4-dihydroxyphenylalanine, otherwise known as a cyclo-Dopa residue, as it condenses with the nearby structure, the cnjugated system is extended to the diphenol ring of cyclo-Dopa, meanwhile allowing the delocalisation of electrons to extend into its aromatic ring with two hydroxyl groups, this extra conjugation shifts the absorption maximum from 480nm, which is the yellow betaxanthins to about 540nm of violet betacyanins giving the betanin its red color .

Moving on to the glucose moiety, otherwise known as the glucoside linkage, it consists of a glucose molecule attached to betanidin via an O-glycosidic bond at the C-5 position. The sugar makes the molecule hydrophilic, which gives Betanin a relatively high solubility and enables it to mix easily in juices, blood, or cell fluids. It also enhances the bioavailability of betanin, allowing it to travel smoothly through the body and be effectively absorbed (Sadowska-Bartosz and Bartosz; Harris et al.). Not only is it water-soluble, but the composition of the network of hydrogen bonds stabilizes the pigment. With stability, the pigment is protected from degradation in acidic environments. Betanin also contains carboxyl group, as this type of acidic group can donate a proton and carry a negative charge when deprotonated, which allows them to bind with metal ions, preventing metals from catalyzing harmful reactions. It can also participate in acid-base reactions and hydrogen bonding, which helps neutralize reactive oxygen species, making it an antioxidant. The hydrogen bonding enhances this pigment's water compatibility. The conjugated pi-system creates a delocalized electron cloud, absorbing light in the visible spectrum, which is responsible for the color of betanin and makes it an antioxidant, similar to the indole ring.

Betanins are widely used in various areas, including food colourings, pharmaceutical products, cosmetics, and personal care, etc. Betanin is added to functional foods, such as nutrition bars or beverages, as an antioxidant supplement. The key reasons why it is suitable are that, as a natural antioxidant, it neutralizes free radicals, which makes it able to protect our body cells, lipids, and proteins from oxidative damage, and it can also protect DNA from oxidative stress. This is why studies have shown that betanin helps prevent cell aging, inflammation, and chronic disease; it may also reduce the risk of cardiovascular diseases or certain cancers.

4.2 Anthocyanin

Anthocyanins, otherwise known as anthocyanins, are a class of water-soluble pigments that contribute to various colors such as red, purple, blue, or black in different conditions, and are responsible for the colors in many fruits and vegetables. They are glycosylated derivatives of anthocyanidin and belong to the flavonoid family. Anthocyanins are distributed broadly in nature and can be extracted from fruits such as blackberries, raspberries, cherries, and blackcurrants, and vegetables like red cabbage (Ahmadiani et al.)

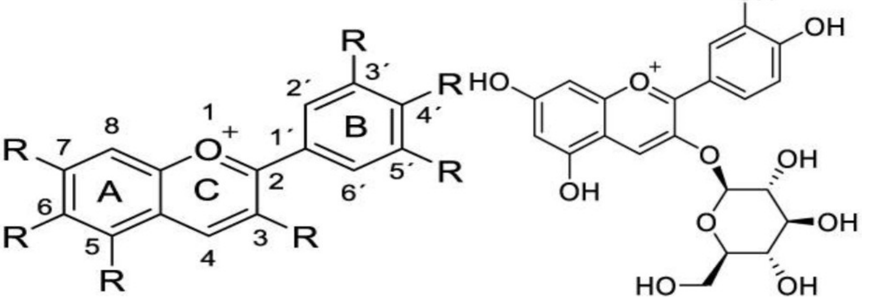

Fig. 6 anthocyanidin backbone on the left with atom numbering and ring label (R = H, OH) and chemical structure of anthocyanin on the right.

Just like betanin, anthocyanins possess structural features that contribute to their effective functions. The core structure is a flavylium cation, also called a 2-phenylbenzopyrylium ion, made of two aromatic rings A and B, as shown in Figure 6, joined by the heterocyclic C ring (Dong et al.). The conjugated system of pi-electrons enables the absorption of visible light, which makes this structure responsible for the color expression of red, purple, or blue (Zhao et al.). However, it is also worth mentioning that the color of anthocyanins are also affected by substitutions and pH(see the next paragraph). The structure also contains hydroxyl (–OH) and methoxyl (–OCH_3) groups on the B-ring at positions 3', 4', and 5'. The purpose of the hydroxyl groups is to increase electron density, which leads to better metal chelation, shifts absorption towards blue, and enables blue complex formation. Methylation, on the other hand, stabilizes the molecule by reducing polarity and oxidative reactivity, resulting in increased pigment stability, which is crucial for maintaining long shelf lives in food applications (Yoshida et al.).

Furthermore, similar to betanin, anthocyanin also had glycosylation, specifically, sugar such as glucose and rhamnose are linked to the 3-,5-, or 7- position on the C-ring. The increase in water solubility enables vacuolar storage in plant cells and color stability, especially in acidic environments (e.g., in fruits). It also stabilizes the flavylium ion by preventing nucleophilic attack by water, which further improves bioavailability and transport in physiological systems (Lin et al.). Moreover, acyl groups added to sugar promote intramolecular stacking, enhancing heat and light stability. It also acts as a shield from degradation, deepening color intensity, especially in purple and blue colorants.

Anthocyanins are mostly used in the food and Nutraceutical industries. In food, it acts as natural food colorants and are widely used in beverages pastries, candies, jellies, and dairy products for their vibrant red-purple-blue hues, what made it suitable and save is that not only it is a non-toxic pigment, more importantly, their structure had methoxylation shifting towards blue which enhances stability, acylation further deepens color and prevents fading, this makes the color of food more long-lasting, making it more appealing to customers. In addition, as it shares a similar structure and functions with betanins, it is also utilized as a strong antioxidant, anti-inflammatory, and health-promoting ingredient, which has been proven to offer benefits in diabetes management, cancer prevention, and cardiovascular protection (Lin et al.). Finally, due to their ability to absorb UV and visible light, anthocyanins serve as natural photoprotective agents and are increasingly explored in cosmetic applications. Their conjugated aromatic structure allows them to shield skin and protect ocular tissues from light-induced oxidative damage (Zhang et al.).

5. Comparisons of Red Dye No.3 and substitutions

5.1 Structural Stability

Natural pigments are chemical compounds found in living organisms, characterized by diverse chemical structures and properties that influence their color, stability, and applications. Key chemical properties include their molecular structure, solubility, and pH sensitivity.

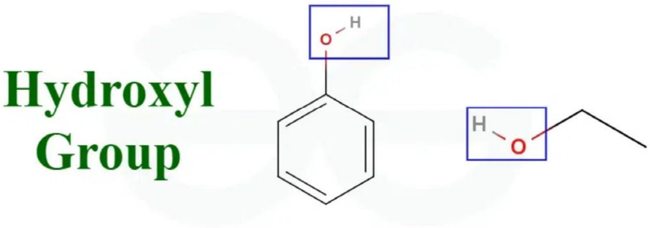

Fig.7. structure of the -OH group

Natural pigments often feature hydroxyl (-OH) groups in their molecular structures. The presence and arrangement of -OH groups can affect how a pigment interacts with light. For example, increasing the number of hydroxyl groups on the B-ring of anthocyanin tends to shift the color towards blue, as it allows for greater delocalization of π-electrons and extends the conjugation system responsible for light absorption. (Andersen & Jordheim, 2021)

For example, industrial pigments are mainly man-made and based on carbon-containing compounds. They often feature conjugated ring structures with elements such as carbon, hydrogen, oxygen, and nitrogen. (González-Laredo & Álvarez-Parrilla, 2024) Industrial pigments are generally more stable, durable, and lightfast than natural pigments. For example, Red No.3 (erythrosine) is an industrial pigment with a fluorine derivative structure where four iodine atoms are substituted on the fluorescein backbone. Its strong and stable molecular structure helps it maintain the same color appearance under various conditions.

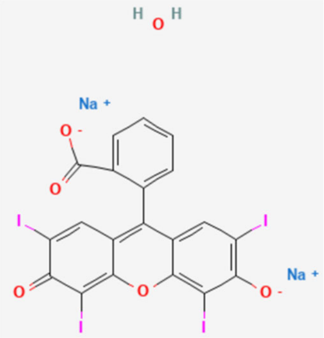

Fig.8 Structure of Red NO.3

Natural pigments, however, are sensitive to external factors such as light, heat, and pH. For instance, anthocyanin exhibits a purple color under neutral conditions but changes color with pH, appearing red in acidic solutions and blue in basic solutions. This color change is caused by the molecular rearrangement under different pH values. Light and heat can also cause oxidation and structural changes, leading to fading or color loss. Industrial pigments, on the other hand, are made with more stable molecular structures that resist environmental influences, maintaining their color consistency and unaffected by pH, temperature, or light exposure.

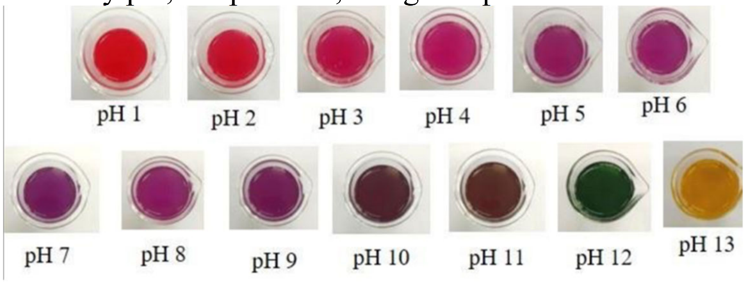

Fig.9 Color of Anthocyanin in different pH

5.2 Toxicology and Metabolism

There are significant differences in toxicity and metabolism between natural and industrial pigments. Natural pigments are derived from plants, animals, and minerals, and generally show lower toxicity and better biocompatibility. They can usually be metabolized by living organisms through redox reactions, producing non-toxic products.(González-Laredo & Álvarez-Parrilla, 2024) For example, anthocyanins, naturally found in berries and red cabbage, can be broken down in the stomach and intestines, and most of them are excreted in the urine or feces. Many natural pigments also exhibit antioxidant activities, as they can donate electrons to neutralize free radicals, thereby providing beneficial health effects (Andersen & Jordheim, 2015).

In contrast, industrial pigments are made from synthetic chemicals and are usually more chemically stable and less biodegradable. (González-Laredo & Álvarez-Parrilla, 2024) Due to their inertness and stability, they are not easily metabolized by the human body and may remain unchanged or even release toxic byproducts under certain conditions, such as exposure to UV light or heat. (Sendri et al., 2023) For example, Red No. 3 has a very limited metabolism and is excreted mostly unchanged in urine and feces. Therefore, natural pigments are generally safer and more environmentally friendly, while industrial pigments may pose higher health and environmental risks due to their low degradability.

5.3 Functional and Application Properties

In terms of functionality and application, natural and industrial pigments each have their advantages and disadvantages. Natural pigments offer soft, natural colors, are renewable and safe for use in foods, cosmetics, and pharmaceuticals, and often provide additional biological benefits, such as antioxidant activity.(Andersen & Jordheim, 2021) However, their major limitation is instability—they are easily affected by light, heat, oxygen, and pH changes, which leads to color fading or alteration. This limits their use in products that require long-term color stability.

Industrial pigments, in contrast, are engineered for high stability, intense color, and excellent resistance to light and heat. (González-Laredo & Álvarez-Parrilla, 2024) They are ideal for applications such as plastics, textiles, and paints, where durability and colorfastness are essential. Their fixed molecular structures ensure consistent color under various conditions. However, industrial pigments often lack the nutritional or antioxidant properties of natural pigments, and some may raise concerns about safety or toxicity when used in food or biological contexts (Andersen & Jordheim, 2015).

Overall, natural pigments have advantages in terms of biodegradability and health safety, while industrial pigments are superior in terms of stability and performance under harsh conditions. Future developments could focus on enhancing the stability of natural pigments through structural modifications or green synthesis methods, thereby combining the advantages of both approaches for broader and safer applications.

6. Conclusion

Red Dye No. 3 may cause the body to release free iodine, which could disrupt thyroid function and potentially increase the risk of cancer. Similar to this, intestinal microbes or reductase enzymes can break down azo artificial colors due to the azo bond (-N=N-) in the molecule, forming aromatic amines, a class of metabolites that have been demonstrated to have genotoxic and carcinogenic potential. Due to these issues, some artificial colors are being phased out by the food industry and regulatory bodies in favor of safer alternatives. Natural dyes, such as beet red, crimson, and anthocyanins, have simpler metabolic pathways and low toxicity of decomposition products, which are more compatible with the human metabolic system.

However, there are still significant problems with natural colorants. Especially the low chemical stability and deterioration caused by light, heat, and pH changes, which can lead to color failure or distortion. In addition, the extraction process of natural dyes are complex and costly. Is difficult to compare the color stability of natural colorants with that of artificial colorants. Therefore, further technical support and process innovation are still required for the development and use of natural dyes in the future.

In conclusion, the use of natural dyes and the banning of Red Dye No. 3 reflect iconic improvements in food safety. Through continuous chemical research, structural optimization, and the application of new material technology, natural dyes will ultimately achieve wider application in the food industry, providing more reliable solutions for the sustainable development of healthy food

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