2 *REVIEW OF LITERATURE*

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2.1. THE EFFECTS OF FOOD COLOURANTS, THEIR LIMITS AND THEIR APPLICATION

2.1.1 Perspectives of History and Basis of Food Colour Regulations

Naturally occurring colour additives from vegetable and mineral sources were used to colour foods, drugs and cosmetics in ancient times. Paprika, turmeric, saffron, iron and lead oxides and copper sulfate are some examples. The early Egyptians used artificial colours in cosmetics and hair dyes. Wine was artificially coloured beginning in at least 300 BC. In 1856, William Henry Perkin discovered the first synthetic organic dye, called mauve. Discoveries of similar dyes soon followed and they quickly became used to colour foods, drugs and cosmetics. Because these dyes were first produced from by-products of coal processing, they were known as "coal-tar colours." Federal oversight of colour additives began in the1880s. The assessment of colour-imparting ingredients in foods was among the first public initiatives undertaken by the U.S. when, in 1881, the U.S. Department of Agriculture's (USDA) Bureau of Chemistry began research on the use of colours in food. Butter and cheese were the first foods for which the federal government authorized the use of artificial colouring (*Hema Kanekar et al., 2014*).

By 1900, many foods, drugs and cosmetics available in the U.S. were artificially coloured. However, not all of the colouring agents were harmless and some were being used to hide inferior or defective foods. A careful assessment of the chemicals used for colouring foods at the time found many blatantly poisonous materials such as lead, arsenic and mercury being added. In many cases, the toxicities of the starting materials for synthesizing colouring agents were well known and could be toxins, irritants, sensitizers, or carcinogens (*Drugcontrol.org: GSR, 2014*).

Food and Drugs Act. In 1906, Congress passed the Food and Drugs Act, which prohibited the use of poisonous or deleterious colours in confectionery and the colouring or staining of food to conceal damage or inferiority. The USDA had initial enforcement authority for this act. In 1907, the USDA issued Food Inspection Decision (F.I.D.) 76, which contained a list of seven straight colours approved for use in food. Subsequent F.I.D.'s in the early part of the century established a voluntary certification program and listed new colours.

In 1927, responsibility for enforcing the Food and Drugs Act of 1906 was given to the newly established FDA. (The agency was first called the Food, Drug and Insecticide Administration and

was given its current name in 1930.) By 1931, there were 15 straight colours approved for use in food, including six of the seven in use today: FD&C Blue No. 1 (Brilliant Blue FCF), FD&C Blue No. 2 (Indigotine), FD&C Green No. 3 (Fast Green FCF), FD&C Red No. 3 (Erythrosine), FD&C Yellow No. 5 (Tartrazine) and FD&C Yellow No. 6 (Sunset Yellow) (*James Harvey Young, 2014*).

Federal Food, Drug and Cosmetic Act of 1938. In the 1920s and 1930s, it became clear that the Food and Drugs Act of 1906 did not go far enough to protect the public health from misbranded, adulterated and even toxic products, including an eyelash dye that blinded some women. The Federal Food, Drug and Cosmetic Act of 1938 further increased government oversight of food and drugs and, for the first time, passed legislation for the regulation of cosmetics and medical devices.

For colour additives, the 1938 FD&C Act mandated the listing of those coal-tar colours (other than coal-tar hair dyes) that were "harmless and suitable" for use in foods, drugs and cosmetics. In addition, the act: contained adulteration and misbranding provisions for the use of coal-tar colours in foods, drugs and cosmetics; required the listing of new colours; and made mandatory the previously voluntary certification program for batches of listed colours, with associated fees. Colour additive lakes were in use by this time and were included in the provisions of the 1938 FD&C Act. The initial listing of lakes for food use under the act restricted their use to colouring shell eggs (egg dyeing).

In response to the 1938 Act, through public hearings FDA created nomenclature for certifiable colour additives. FDA also established labeling and recordkeeping provisions, identified diluents that could be added to colour additives and established procedures for requesting certification of colour additives and adding new colour additives to the permitted list.

Colour Additive Amendments of 1960 highlights on the fall of 1950, many children became ill from eating an orange Halloween candy containing 1-2 percent FD&C Orange No. 1, a colour additive approved for use in food. That same year, U.S. House Representative James Delaney began holding hearings on the possible carcinogenicity of pesticide residues and food additives. These events prompted FDA to reevaluate all the listed colour additives. In the next few years, FDA found that several caused serious adverse effects and proceeded to terminate their listings. During that time, it also became clear that coal was no longer the primary raw material source for the manufacture of colour additives (*Xaq Frohlich, 2022*).

The Colour Additive Amendments of 1960 defined "colour additive" and required that only colour additives (except coal-tar hair dyes) listed as "suitable and safe" for a given use could be used

in foods, drugs, cosmetics and medical devices. The 1960 Amendments prescribed the factors that FDA must consider in determining whether a proposed use of a colour additive is safe, as well as the specific conditions for safe use that must be included in the listing regulation. FDA updated the procedural regulations for the petition process in response to these amendments. Under these amendments, the approximately 200 colour additives that were in commercial use at the time were provisionally listed and could be used on an interim basis until they were either permanently listed or terminated due to safety concerns or lack of commercial interest. Permanently listing a colour additive for a proposed use was prohibited unless scientific data established its safety (*Benjamin Cohen, 2019*).

The 1960 Amendments also contained a "Delaney Clause" that prohibited the listing of a colour additive shown to be a carcinogen. The clause states that "A colour additive shall be deemed unsafe. . . if the additive is found to induce cancer when ingested by man or animal, or . . . after other relevant exposure of man or animal to such additive." After 1960, FDA gradually removed colour additives from the provisional list either by permanent listing or by termination of listing. Today about half of the "1960" colour additives remain listed; only colour additive lakes remain provisionally listed and initiatives are underway to permanently list them (*Burrow et al., 2019*).

Food colour additives are vital to how it tastes and perceives food, yet it generally remains mysterious to the common consumers. First, it uses recent examples to illustrate the importance of colours to our enjoyment of food. It then recounts the early history of food colours and the emergence of regulation to prevent their unsafe and fraudulent uses. The margarine war of the late 19th and early 20th centuries is described, as well as the 1906 and 1938 Food and Drug Acts. The article then enters the modern era of colour additive regulation, beginning with the Colour Additive Amendments of 1960. The debate over the Delaney anti-cancer clause is assessed, as well as other recent safety and regulatory controversies (*Coultate et al., 2018*).

Colour is an often overlooked sensory character that certainly influences flavour perception. Pigments colouring food are generally unstable and are modified during processing. To maintain or restore product colour uniformity, colouring agents, considered worldwide as food additives, are intentionally added to food products. The natural food additives market has been growing extensively since the last century due to the potential hazards of artificial food additives and the potential benefits of biologically active compounds (*Solymosi et al, 2015).*

Synthetic food colours are produced by full chemical synthesis or by chemical modification of several precursor compounds and are therefore in contrast to natural food colours, which are usually extracted from several natural sources and purified (*König, 2015*). As there is increasing awareness about the harmful effects of usage of synthetic colours and the chemicals obviously demand for natural food colours in the international market abruptly increases. Colours from plant, animal and mineral sources also sometimes called as biocolours, which were used in earlier times, had their own drawbacks like heat, pH and light instability, against oxidizing agents in food, which made synthetic colours gain popularity in food industry. In India according to 'The Prevention of Food Adulteration Act of India' the use of eight synthetic colours in specified food commodities at a uniform level of 100 mg kg^{-1} or mg l^{-1} is permitted (*Chaitanya, 2014).*

Natural food grade colours demand is increasing progressively because of consumer awareness as they are eco-friendly as well as have various pharmacological benefits for human health. It is need of the day to develop viable technologies as well as they should be cost effective to extract food grade colours from natural sources especially utilizing waste of organic compounds. For consumers and manufacturers to attain knowledge about the extraction techniques either conventional or recent technologies of food colours with respect to natural colour application. To extract food grade pigments from natural source such as plants and animals scientists have develop different techniques (*Stich, 2016*).

Numerous optimized extraction techniques were developed in past years to extract natural pigments from animal and plant sources because consumers among worldwide demanded for natural food dyes not just because of their functional and safe to use properties but as well as their positive impact on the human health and environment in a long term manner if compared to the synthetic food dyes had make them popular in this era. To meet the growing demand and attention of the population technologies such as conventional, advanced and combination of two or more technologies are made to gain more yield and higher quality dyes (*Nawaz, 2019*).

2.1.1 Application of Food Colourants in Foods

According to *Medeiros (2012), in bakery* catering to the needs of bakery manufacture or small bakery and bakers, they provide a colour for use in bakery such as biscuits, cookies, cakes and pastries etc. These colours are formulated using the best grade raw materials. These colours possess high quality standard and purity levels. Further, these are offered in various packaging options as per the demands of their clients.

Beverage industries use colours that are used in the manufacturing of cold drinks, juice, dry mixes etc. Cold drinks look and taste are changing due to adding these colours. Formulated using the highest quality organic substances, these colours are highly effective and safe to use. These colours are provided in proper packing in order to retain their purity and composition for long period of time (*Arslan et al., 2018*).

Human provide a range of colours to the large manufacturer of confectionery items such as jellies, chewing gum, cream/paste, gums and chews etc. They make sure that these colours are harmless and accurately fresh. These colours are carefully regulated by the experts. Moreover, these are offered in highly appropriate packing options (*Sohaib et al., 2016*).

According to *Lone (2016)*, study revealed that colours are highly used in fresh comminute meat and seasonings etc. to increase the appetite. It is very important for food colours to be heat resistance these colours make the foods items more delightful. Human offers a wide spectrum of pharmaceutical colours that meet the requirement of clients. These colours are most used as colouring agents in food and pharmaceutical industries as they are highly adaptable and versatile. The pharmaceutical colours are available in different types such as injections, tablets, mouth wash, gels etc. These colours are ideal for colouring products including fats, oils or items lacking sufficient moisture to dissolve dyes. The range of colours should be safe in use.

The concept in the performance of the alternative solvents in the downstream process to recover natural pigments in a more sustainable way. Conventionally, pigments marketed on an industrial scale are produced through chemical synthesis by using petroleum derivatives as the main raw material. Also, the current production chain of the synthetic dyes is linear, with no solvent recycling and waste generation. Thus, the most promising processes of extraction and purification of natural pigments and strategies on the polishing of the solvents are required.

The use of alternative solvents, namely, ionic liquids, eutectic solvents, aqueous solutions of surfactants and edible oils, for recovering natural pigments was reviewed. Works discussing higher extraction yields and selectivity, while maintaining the stability of the target pigments, were reported. Also, a panorama between Sustainability and Circular Economy prospection was discussed for better comprehension of the main advances in the field. Behind the analysis of the works published so far on the theme, the most important lacunas to overcome in the next years on the field were pointed out and discussed. Also, the future trends and new perspectives to achieve the economic viability and sustainability of the processes using alternative solvents will be scrutinized (*Leonard et al., 2020*).

Food colour is any substance incorporated to food or beverage to alter its colouring effects. Adding colour to food and drink has been a normal practice over many centuries. Colour was generally added to serve as a visual cue for quality, induce recognition of flavor and meet consumer expectations. Today, colour is still added during the manufacture of products such as biscuits, pastries, cakes, processed meats, cheese, margarine, confectionery, ice cream, cordials and soft drinks. The addition of colour to food and beverage also serves many technological purposes, such as offsetting colour loss caused by processing conditions, including temperature, time and heat, enhancing colour that is already present in food, ensuring batch to batch uniformity, which preserves food identity and protecting flavors and vitamins that may be sensitive to sunlight during storage. The chemical industry boomed after World War II due to enhanced collaboration within the food industry. This led to incorporation of artificial petroleum based ingredients in artificial food colours, which became widely popular over natural colours due to their manufacturing benefits, such as reduced costs and prolonged shelf life (*Martin et al., 2016*).

A red pigment from the leaf sheath of a sorghum variety (*Sorghum bicolour)* with a high content of apigeninidin is widely used as a bicolourant in processed foods in West Africa. Sorghum bicolourant applied in fermented and heated foods. Traditional extraction methods predominantly differed in two aspects, namely the use of an alkaline rock salt and the temperature of the extraction water. Cool extraction using the alkaline ingredient is said to be efficient than hot alkaline and hot aqueous extraction in extracting anthocyanins. The apigeninidin content is three times higher in the cool and hot alkaline extracts than in the aqueous extract (*Folachode et al, 2016*).

2.1.3 Use of Lake Colours as Colourant in Foods

All colour additives required to be listed by FDA fall into two categories: those that are subject to FDA's certification process and those that are exempt from the certification process. Colour additives subject to batch certification are synthetic organic dyes, lakes, or pigments. Those for food use are chemically classified as azo, xanthene, triphenylmethane and indigoid dyes. Although certifiable colour additives have been called coal-tar colours because of their traditional origins, today they are synthesized mainly from raw materials obtained from petroleum (*BP Harp et al., 2015*).

Colour additives exempt from certification generally include those derived from plant or mineral sources. One, cochineal extract (and its lake, carmine) is derived from an insect. Most are straight colours; one exception is carmine as described above. Certification exempt colour additives

must comply with the identity and purity specifications and use limitations described in their listing regulations. Users of these colour additives are responsible for ensuring that the colour additives comply with the listing regulations. Straight colours subject to batch certification are listed in 21 CFR part 74 and lakes subject to batch certification are listed in 21 CFR part 82. Colour additives exempt from certification are listed in 21 CFR part 73 (*Simon et al., 2017*).

Colour additives are classified as straight colours, lakes and mixtures. Straight colours are colour additives that have not been mixed or chemically reacted with any other substance (for example, FD&C Blue No. 1 or Blue 1). Lakes are formed by chemically reacting straight colours with precipitants and substrata (for example, Blue 1 Lake). Lakes for food use must be made from certified batches of straight colours. (One exception is carmine, which is a lake made from cochineal extract.) Lakes for food use are made with aluminum cation as the precipitant and aluminum hydroxide as the substratum. Mixtures are colour additives formed by mixing one colour additive with one or more other colour additives or non-coloured diluents, without a chemical reaction (for example, food inks used to mark confectionery) (*David R Schoneker, 2013*).

Aluminium Lake of Sunset Yellow FCF may be used in powdered dry beverages mix (powdered soft drink concentrate) up to a maximum limit of 0.04 per cent by weight. The maximum limit of colour content in final beverage for consumption should not exceed 8.3 ppm and that of aluminium content shall not exceed 4.4 ppm of the final beverage for consumption: Provided that the powdered dry beverages mix (powdered soft drink concentrate) label shall give clear instruction for reconstitution of product for making final beverage (*Kumari et al., 2016*).

Colour is a key component to increase the ultimate appetizing value and consumer acceptance towards foods and beverages. Synthetic food colours have been increasingly used than natural food colours by food manufacturers to attain certain properties such as low cost, improved appearance, high colour intensity, more colour stability and uniformity. Varied foods and beverages available in the market may contain some non-permitted synthetic colours and over use of permitted synthetic colours. This may lead to severe health problems such as mutations, cancers, reduced hemoglobin concentrations and allergic reactions. Moreover, 60 percent of the beverages violated the label requirement without including proper colour ingredients. The study concluded that there is a high tendency to use synthetic food colours in confectioneries and beverages and some confectioneries contain unidentified colours including a textile dye. Therefore, the implementation of regulations and

awareness programs of food colours for consumers and food manufacturers are highly recommended (*Subhashish Dey et al., 2022*).

Three foodstuffs (surimi, minced meat and milk) were dyed with carminic acid and carminic aluminum lake. The effects of protein, metal ions and food additives on the colour of carminic acid and carminic aluminum lake were investigated. After being dyed by carminic acid, the colours of surimi, minced meat and milk were light purple, red and gray- green, respectively. When using carminic aluminum lake, surimi and milk were magenta and minced meat was red. Regarding the carminic acid solution, the presence of myofibrillar protein (MFP), whey protein isolate (WPI) and soy protein isolate (SPI) turned it red by changing the pH, while the presence of casein made it orange. The carminic aluminum lake solution turned magenta in all four cases, which were not affected by protein. The colour of carminic acid and carminic aluminum lake was significantly affected by 0.001–0.1 mol/L Fe^{3+}, 0.001–0.1 mol/L Fe^{2+}, 0.001–0.1 mol/L Cu^{2+} and 0.1 mol/L Ca^{2+}, limiting their application in iron, copper and high- calcium foods (*Qian Liu et al., 2020).*

Upon receipt of the sample, FDA personnel evaluate its physical appearance and chemically analyze it. At least 10 analyses are performed, for purity (total colour content), moisture, residual salts, unreacted intermediates, coloured impurities other than the main colour (called subsidiary colours), any other specified impurities and the heavy metals lead, arsenic and mercury. The evaluation and analyses typically take less than five working days. The results are reviewed for compliance with the identity and specifications described in the listing regulation for the colour additive. If the sample is found to meet these requirements, FDA issues a certificate for the batch that identifies the colour additive, the batch weight, the uses for which the colour additive is certified, the name and address of the owner and other information as required. FDA also assigns a unique lot number for the batch and the name of the batch changes. For example, a batch of "tartrazine" becomes "FD&C Yellow No. 5."

Analytical and informational components of the certification program have been automated to the fullest extent possible. Currently, an on-line web-based system allows colour additive manufacturers to submit and access information about individual samples, including receipt of FDA's certificates. Owners of certified batches are subject to FDA inspections of their establishments. During these inspections, FDA examines records of use of the colour additives and takes samples from certified batches for analysis for comparison with FDA's original results (*Sari Lehto et al., 2017*).

2.1.4 Effects of Artificial Food Colours

Ahmed et al., (2021), has quoted that nutritional risk in children is associated with food safety. The study is to identify the food type consumed by 6–17 year old school going children in Saudi Arabia. Eight permitted artificial food colour additives, including Tartrazine (E102), Sunset Yellow (E110), Carmoisine (E122), Allura Red (E129), Indigo Carmine (E132), Brilliant Blue (E133), Fast Green (E143) and Black PN (E151) and two non-permitted ones, Erythrosine (E127) and Red 2G (E128), were determined using 24 hours dietary recall questionnaires. Artificial colour additives in 839 food products were divided into nine categories, including biscuits, cakes, chocolates, chips, ice cream, juices and drinks, candy, jelly and chewing gum are determined using high performance liquid chromatography and diode array detector. The results indicated a high intake of juices and drinks, ice cream and cakes, but low consumption of chewing gum among school-going children. Among the permitted artificial food colour additives, Brilliant Blue (E133) (54.1 percent) and Tartrazine (E102) (42.3 percent) were the most commonly used. Sunset Yellow (E110) in one chocolate sample, Tartrazine (E102) and Sunset Yellow (E110) in one and two juice and drink samples, respectively and Brilliant Blue (E133) in two candy samples exceeded the permitted level. Therefore, further investigations are needed to provide insights into the possible adverse health effects of high intake of these additives in artificial food colouring on the test population are warranted.

Studies concerning children have specified that 73 percentage of children with attention deficit hyperactivity disorder (ADHD) showed a decrease in symptoms when artificial food dyes and preservatives were eliminated. Another study found that food dyes, along with sodium benzoate, increased hyperactivity in both 3-year-olds and a group of 8 and 9 year-olds. However, because these study participants received a mixture of ingredients, it was difficult to determine what caused the hyperactivity. Tartrazine, also known as Yellow 5, has been associated with behavioral changes including irritability, restlessness, depression and difficulty with sleeping. The artificial food dyes do increase hyperactivity in children. Yet it appears that not all children react the same way to the food dyes. In multiple studies, Yellow 5 also known as tartrazine it causes hives and asthma symptoms. Interestingly, people who have an allergy to aspirin seem to be more likely to also be allergic to Yellow 5. In a study conducted in people with chronic hives or swelling, 52 percent had an allergic reaction to artificial food dyes. Most allergic reactions are not life-threatening. However, if you have symptoms of an allergy, it may be beneficial to remove artificial food dyes from your diet. Red 40, Yellow 5 and Yellow 6 are among the most consumed dyes and are the three most likely to cause an

allergic response. Some artificial food dyes, particularly Blue 1, Red 40, Yellow 5 and Yellow 6, may cause allergic reactions in sensitive individuals (*Bigwood et al., 2008*).

World Health Organization (*WHO, 2015*) estimated that foodborne hazards about 600 million food-borne diseases (FBD) and 420,000 deaths. The major causes for FBD are parasites, chemicals and toxins. Artificial food colours are the main source of food toxins. Colours are used during the process of food preparation. Among those colours, there are several chemicals that are not permitted in food preparation. The usage of prohibited artificial food colouring is assessed through post-market surveys regularly in some countries. The usage of prohibited food colouring is strictly controlled in many countries such as the European Council in 1994 and the United Sates' Food and Drug Administration in 2004. Moreover, prohibited artificial food colours such as Rhodamine B and Auramine O can cause the genotoxicity and carcinogenicity in both humans and animals. Rhodamine B has effects of carcinogenicity, reproductive and developmental toxicity, neurotoxicity and acute toxicity in humans (*Singh et al., 2017*).

Artificial food colouring attracts and enhances the appearance of food and can preserve the colour of the original food for a longer duration. Artificial colours are industrial products, which have potential adverse health effects. A meta-analysis study on synthetic food colour additives reported that the consumption of artificial colours was controversially associated with attention deficit hyperactivity disorder (ADHD) among children (*NSW Food Authority, 2014*).

The regulation of food safety in Myanmar is mainly carried out by the Ministry of Health and Sports' Department of Food and Drug Administration (FDA). The Myanmar Food and Drug Act was first developed in 1928. With the guidance of the World Health Organization's model for food law, the Myanmar National Food Law was promulgated in 1997. The Myanmar Food and Drug Board of Authority chaired by Minister of Health and Sports is the steering body for food safety policy and guidelines. The Food and Drug Board collaborates on food control measures with other related ministries such as the Customs Department, the Municipal Health Department and the Consumer Affairs Department (*Zaw, 2015*).

The FDA controls quality and safety of all imported food and local food production. In addition, the FDA also conducts food safety programs for school children and post-market surveys of school canteens. Laboratory tests of school food are performed annually to determine the presence of prohibited colours, harmful chemical substances and pathogenic organisms. After completion of post-market surveys, the FDA makes available a list of prohibited foods and photos on its website

and announces these to the public through social media and newspapers. The FDA has reported that the most available prohibited artificial colours in school food were Auramine O, Rhodamine B and Orange II (*FDA - Myanmar, 2016*).

United States study reported that the environment of the school influences student's food choice. Therefore, the school environment is an important factor in the promotion of accessibility to healthy and nutritious food (*Wang et al., 2014*). Although school children may have good knowledge on nutrition and food choice, they are less likely to apply this knowledge in their eating practices. Relevant and precise knowledge plays a critical role in behavior change in eating practices (*New et al., 2019*).

Several types of dyes are available in the market as colouring agents to food commodities. Some commonly used synthetic food dyes include: brilliant blue, indigo carmine, citrus red, fast green, erythrosine, allura red, tartrazine and sunset yellow. The main food biocolourants are carotenoids, flavanoids, anthocyanidins, chlorophyll, betalain and crocin. There has been a rising concern over the health implications of the use of food dyes in human diets. This has led to a lot of studies, both by individual researchers, corporate organization-sponsored and even government-sponsored researches, to authenticate the benefits or risks associated with the use of food colourants (synthetic and natural). The critically evaluated scientific research from various published journal articles and reports, with a view of clarifying the health implications of using these food dyes. Various studies have shown that synthetic food colourants have considerable toxicological effects, including but not limited to carcinogenicity, hypersensitivity reactions and behavioral effects. However, natural food colourants have been found to be relatively safe to humans. Besides the colouring property, they have been found to possess a number of pharmacological properties like strong antioxidant, antimutagenic, anti-inflammatory, antineoplastic and antiartheritic effects (*Sunday et al., 2016*).

Ponceau 4R (E124) and tartrazine (E102) are synthetic azo colourants widely employed for imparting an appealing colour in many foods such as drinks and sweets. However, due to some adverse health effects related to migraines, anxiety an even mutagenic action and carcinogenicity, their employment must be strictly controlled. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) the maximum daily intake of ponceau 4R (P4R) and tartrazine (TR) is 4 mg/kg and 7.5 mg/kg, respectively. In order to guarantee the consumer's safety and respect the published regulations, accurate and reliable methods for the determination of

these dyes are required. Widely employed methodologies are spectrophotometry, liquid chromatography and capillary electrophoresis but they are demanding in equipment and technical expertise. In contrast, electrochemical techniques are affordable and well-suited for in-situ measurements. Since P4R and TR can be found together in food, providing an orange colour, their simultaneous determination is mandatory (*Qin Cheng et al., 2015*).

2.2. REGULATIONS AND ENFORCEMENTS OF FOOD COLOUR ADDITIVES PROCLAIMED BY FOOD DRUG ADMINSTRATION

2.2.1 Regulations of Food Colour Additives

FDA has regulatory oversight for colour additives used in foods, drugs, cosmetics and medical devices. FDA lists new colour additives or new uses for listed colour additives that have been shown to be safe for their intended uses in the *Code of Federal Regulations* (CFR), conducts a certification program for batches of colour additives that are required to be certified before sale and monitors the use of colour additives in products in the U.S., including product labeling. These activities stem from FDA's role in enforcing the colour additive provisions of the FD&C Act, the Fair Packaging and Labeling Act and other applicable laws, including the recently enacted Public Health Security and Bioterrorism Preparedness and Response Act of 2002 that requires domestic and foreign manufacturers of colour additives used as ingredients in foods to register with FDA by December 12, 2003 (*FDA, 2017*).

Colour additives used in foods, drugs, cosmetics and medical devices must comply with individual listing regulations issued by FDA. The use of an unlisted colour additive, the improper use of a listed colour additive, or the use of a colour additive that does not conform to the purity and identity specifications of the listing regulation may cause a product to be adulterated according to the provisions of the FD&C Act. FDA may take enforcement action against such products. Most products contain only a small amount of colour additive, so it takes only a small quantity to potentially adulterate a large amount of product (*Michael Jelavich, 2016*).

In India, as per the Food Safety and Standards Regulations (*2023*), extraneous addition of colouring matter to be mentioned on the label – where an extraneous colouring matter has been added to any article of food, there shall be displayed one of the following statements in capital letters, just beneath the list of the ingredients on the label attached to any package of food so coloured, namely: Contains Permitted Natural Colour(s) or Contains Permitted Synthetic Food Colour(s)or Contains

Permitted Natural and Synthetic Food Colour(s). In case, both colour and flavour are used in the product, one of the following combined statements in capital letters shall be displayed, just beneath the list of ingredients on the label attached to any package of food so coloured and flavoured.

Regulations emphasis that food colours shall not exceed 100 parts per million of the final food or beverage for consumption, where the maximum limit of permitted synthetic food colours shall not exceed 200 parts per million of the final food or beverage for consumption Many countries have their own specific regulations governing the type, purity, use and amount of artificial colour additives permitted in the food industry. Dietary modeling is an important tool in establishing chemicals from food in the diet and is an important part of the risk assessment process. To estimate dietary exposure to chemicals in food, concentration and consumption data are used, the results are then compared with established health standards. Internationally, dietary modeling has been used in the risk assessment process for many years by food regulatory agencies to establish unacceptable risks to public health. The quality of food concentration and consumption data for dietary modeling determines accuracy of the dietary exposure estimate (*Kettler et al., 2015).*

Food and Drug Administration (FDA) of the United States has permitted the nine artificial colour additives to use in food. In order to contain the improper use, number of countries has developed regulations to limit the quality, purity, use and quantity of permitted artificial colour additives in food. Food and Agriculture Organization (FAO) and World Health Organization (WHO) Joint Expert Committee On Food Additives (JECFA) highly advice to the governments frequently monitor the overall intake of each food additives, in particular, if there use is exploited and/or if the overall intake exceeds the permissible levels. The permissible levels of each artificial food colour additives are determined by the national level dietary studies (*USFDA, 2017*).

"Colour" includes white, black and gray. In addition, any chemical that reacts with another substance and causes formation of a colour may be a colour additive. For example, dihydroxyacetone (DHA), when applied to the skin, reacts with the protein of the skin to impart colour. Even though DHA is colourless, it acts as a colour additive when used for this purpose and is regulated as a colour additive. There is no "generally recognized as safe" (GRAS) exemption to the definition of a colour additive. The Federal Food, Drug and Cosmetic Act (FD&C Act) provides that a substance that imparts colour is a colour additive and is subject to premarket approval requirements unless the substance is used solely for a purpose other than colouring (*Deacon J. Lile, 2023*).

2.2.2 Regulations Enforced for Food Colour Additives

When FDA investigation determines that a colour additive violation has occurred, the agency can take a number of actions to enforce the FD&C Act and to protect the public health. In the absence of voluntary action (e.g., product recall) by the responsible firm to correct the problem, FDA has several advisory, administrative and judicial options which include warning letters, detentions, issuance of import alerts and seizures. FDA has recently sent warning letters for undeclared FD&C Yellow No. 6 in dehydrated papaya, for undeclared FD&C Red No. 40 and FD&C Yellow No. 6 in bakery products and for undeclared FD&C Blue No. 1 and FD&C Yellow No. 5 in noodle products. FDA also recently reported recalls for undeclared FD&C Red No. 40 and incorrectly declared FD&C Yellow No. 6 in bottled food colour and for presence of the unapproved colour additive Ponceau 4R in strawberry filling (*Priyadeep Bhutani et al., 2021*).

FDA frequently offers guidance on the appropriate use of colour additives. One example of a long-standing policy since the early 1900s concerns the use of small, silver balls or "silver dragees" sold for decorating cookies, cakes, etc. As expressed in a Compliance Policy Guide (CPG), "When small silver balls known as 'silver dragees' are sold exclusively for decorating cakes and are used under conditions which preclude their consumption as confectionery, they are not considered to be in the category of a food or confectionery." In summary, the federal regulation of colour additives has a long history and remains an important program for the FDA in assuring that consumers have safe and properly labeled products (*Daniel J. Klionsky et al., 2021*).

Table – 2.2.2: Listed Colour Additives Permitted for Use in Food*

21 CFR Section	Straight Colour	Uses and Restrictions
74.101	FD&C Blue No. 1	Foods generally
74.102	FD&C Blue No. 2	Foods generally
74.203	FD&C Green No. 3	Foods generally
74.250	Orange B	Casings or surfaces of frankfurters and sausages, NTE 150 ppm (by weight)
74.302	Citrus Red No. 2	Skins of oranges not intended or used for processing, NTE 2.0 ppm (by weight)
74.303	FD&C Red No. 3	Foods generally
74.340	FD&C Red No. 40	Foods generally
74.705	FD&C Yellow No. 5	Foods generally

21 CFR Section	Straight Colour	Uses and Restrictions
74.706	FD&C Yellow No. 6	Foods generally
73.30	Annatto extract	Foods generally
73.35	Astaxanthin	Salmonid fish feed
73.40	Dehydrated beets (beet powder)	Foods generally
73.50	Ultramarine blue	Salt for animal feed
73.75	Canthaxanthin	Foods generally, NTE 30 mg/lb of solid or semisolid food or per pint of liquid food; broiler chicken feed; salmonid fish feed
73.85	Caramel	Foods generally
73.90	ß-Apo-8'-carotenal	Foods generally, NTE 15 mg/lb solid, 15 mg/pt liquid
73.95	ß-Carotene	Foods generally
73.100	Cochineal extract; carmine	Foods generally
73.125	Sodium copper chlorophyllin	Citrus-based dry beverage mixes, NTE 0.2% dry mix
73.140	Toasted partially defatted cooked cottonseed flour	Foods generally
73.160	Ferrous gluconate	Ripe olives
73.165	Ferrous lactate	Ripe olives
73.169	Grape colour extract	Non beverage food
73.170	Grape skin extract (enocianina)	Still and carbonated drinks and ades; beverage bases; alcoholic beverages
73.185	Haematococcus algae meal	Salmonid fish feed
73.200	Synthetic iron oxide	Sausage casings, NTE 0.1% (by weight); dog and cat food, NTE 0.25% (by weight)
73.250	Fruit juice	Foods generally
73.260	Vegetable juice	Foods generally
73.275	Dried algae meal	Chicken feed
73.295	Tagetes (Aztec marigold) meal and extract	Chicken feed
73.300	Carrot oil	Foods generally

21 CFR Section	Straight Colour	Uses and Restrictions
73.315	Corn endosperm oil	Chicken feed
73.340	Paprika	Foods generally
73.345	Paprika oleoresin	Foods generally
73.355	Phaffia yeast	Salmonid fish feed
73.450	Riboflavin	Foods generally
73.500	Saffron	Foods generally
73.575	Titanium dioxide	Foods generally, NTE 1% (by weight)
73.600	Turmeric	Foods generally
73.615	Turmeric oleoresin	Foods generally

*****21 CFR Section 73.615, Tumeric oleoresin, has been added to Table

2.2.3 Synthetic Food Colour Additives and GRAS Listed and Authorized by FDA

FDA has established regulations for colour additives in Title 21 of the CFR, parts 70-82. The regulations in 21 CFR parts 73, 74 and 82 identify each listed colour additive, provide chemical specifications for the colour additives and identify uses and restrictions, labeling requirements and the requirement for certification. The regulations in 21 CFR part 71 describe the premarket approval process for new colour additives or new uses for listed colour additives. 21 CFR part 80 pertains to colour additive certification (*Indumathy Jagadeeswaran et al., 2022*).

Additional regulations that provide specific requirements for colour additives in foods, drugs, cosmetics and medical devices are found in other parts of the CFR. For example, the labeling of food products is found at 21 CFR 101.22(k) and cosmetic products at 21 CFR 701.3. Colour additives are sometimes called "artificial colour" or "artificial colouring" (21 CFR 101.22(a)(4)). From the regulatory standpoint, the term "colourant" refers to a dye or pigment used in a food contact material such as a polymer and doesn't migrate to food. These materials are regulated not as colour additives but as food additives (21 CFR 178.3297(a)) (*John E. Lincoln, 2012*).

Colour Additives and GRAS: The FD&C Act provides for an exemption of some substances from the definition of "food additive" if they are generally recognized as safe for their intended uses. Such an exemption does not apply to colour additives. However, a substance that is listed as GRAS

also may be listed as a colour additive. An example is ferrous lactate (21 CFR 184.1311 and 21 CFR 73.165) (*Cameron Faustman et al., 2020*).

A mixture of carotenoid xanthophyll esters ("lutein esters") is the subject of a recent GRAS notice submitted to FDA in support of its use as a food ingredient. The compound is dark orange-brown and may be capable of imparting colour to a food. FDA's response letter to the notice reminds the manufacturer that use of the substance as a colour additive, in addition to use as a GRAS substance, would require premarket approval by FDA (*Joy L. Frestedt, 2018*).

2.2.4Certification of Food Additives and Reviewing of Petition Process for Food Additives

Colour additive certification is the process by which FDA assures that newly manufactured batches of colour additives meet the identity and specification requirements of their listing regulations. During fiscal year 2002, FDA certified batches representing a total of 16.5 million pounds of colour additives, much of it for food uses. The decision about the need for batch certification is made during the agency's review of a petition requesting a listing for the colour additive. Batch certification is required when the composition needs to be controlled to protect the public health. Some colour additives may contain impurities of toxicological concern, such as carcinogenic constituents (*Daniel Bobo et al., 2016*).

The requirements for colour additive certification, as well as storage, fees, recordkeeping and inspection for owners and manufacturers, are described in detail in 21 CFR part 80. Regulations in 21 CFR part 70.25 prescribe labeling requirements for colour additive batches before and after certification. Under the certification process, a sample from each manufactured batch of a certifiable colour additive must be sent to FDA's Colour Certification Branch accompanied by a "Request for Certification" that provides information about the batch including the name of the colour additive, the name of the manufacturer, the batch weight, storage conditions for the batch and the use for which it is being certified. FDA charges a fee for certification based on the batch weight. Prior to certification, the batch cannot be used in food, drug, cosmetic, or medical device products and must be stored separately from batches already certified (*Becky A. Briesacher et al., 2013*).

Petition Review Process: When evaluating the safety of a new colour additive or a new use for a listed colour additive, FDA considers such factors as probable consumption or exposure from its use, cumulative effect in the diet, evaluation by experts qualified by scientific training and

experience and the availability of analytical methods for determining its purity and acceptable levels of impurities (*Asher Mullard, 2024).*

Any interested person may petition FDA for the use of a new colour additive or to amend the listing of a colour additive for a new use. The petitioner for a new colour additive must provide information on the following:

- Identity of the proposed colour additive
- Physical, chemical and biological properties
- Chemical specifications
- Manufacturing process description
- Stability data
- Intended uses and restrictions
- Labeling
- Tolerances and limitations
- Analytical methods for enforcing chemical specifications
- Analytical methods for determination of the colour additive in products
- Identification and determination of any substance formed in or on products because of the use of the colour additive
- Safety studies
- Estimate of probable exposure
- Proposed regulation
- Proposed exemption from batch certification
- An environmental assessment or claim for categorical exclusion

The petitioner must submit data demonstrating the safety and suitability of the new colour additive or new use. FDA will then evaluate the data in the petition, public comments to the petition and other relevant data in FDA's files. Upon approval of the petition, FDA will issue a new listing regulation or alter an existing regulation for the new colour additive or new use. The process for submitting petitions is described in detail in 21 CFR parts 70 and 71, which describe the format, the administrative requirements and the information and data required. The data that are appropriate for support of a colour additive petition will vary depending on whether the petition is for a new colour additive or for a new use for a listed colour additive, the level and type of use of the proposed colour additive and the amount of colour additive and its impurities that may enter body tissues (*Ciociola Arthur et al., 2014*).

Table provides information on recently listed colour additives and pending colour additive petitions. Once a new colour additive is listed, FDA continually monitors its safe use, assuring the consideration of new data and safety information. Historically, this activity has resulted in regulatory changes for colour additives that were necessary to protect the public health (*Dharma Rao Tompa et al., 2021*).

Table –2.2.3: Recently listed colour additives and pending colour additive petitions

Colour additive	Petitioned use	Petition status
Sodium copper chlorophyllin	Colouring citrus-based dry beverage mixes	Listed in 21 CFR 73.125, effective June 20, 2002 (67 FR 35429)
Mica-based pearlescent pigments	Colouring contact lenses	Listed in 21 CFR 73.3128, effective November 26, 2002 (67 FR 65311)
Luminescent zinc sulfide	Colouring externally applied facial makeup preparations and nail polish	Listed in 21 CFR 73.2995, effective September 8, 2000 (65 FR 48375)
Tomato lycopene extract	Colouring foods	Pending
Carbon black	Colouring cosmetics including in eye area	Pending
Pearlescent pigments	Colouring foods	Pending
Pearlescent pigments	Colouring drugs	Pending

2.3. MEDICINAL PROPERTIES, EXTRACTION AND ISOLATION OF NATURAL FOOD COLOURANTS

2.2.1 Madder Root (*Rubia cordifolia*) and its Properties

In the 1700s, the Ottoman Empire met two-thirds of the world's madder needs and until 1875, the revenue of madder only in Izmir exceeded 500,000 gold liras. However, the great transformation toward industrialization in the world economy has encouraged the use of chemical products instead of natural products (*Kilic, 2018*).

When evaluated in terms of the island of Cyprus in the 18th century, it has been noted that the most basic economic activity especially in Famagusta, was root dyeing/madder dyer. It is stated in historical documents that the production of the plant from which madder is obtained is concentrated around the Famagusta castle. Especially the red type of buckthorn was considered very valuable for

colouring both cotton fabrics and leather products. In that century, 318 acres of land were planted for the production of madder. When it comes to the Republican period, one of the most important development moves of the Turkish economy was the Izmir Economy Congress, which was held in 1923. An exhibition was also organized within the congress and products considered prestigious for the domestic economy were exhibited at the exhibition. One of these products was madder. Madder is an ingredient used in the manufacture of carpets and rugs which derives its colours from various plant and animal sources. One of the vibrant red colour sources among these colours is madder (*Rubia tinctorum* L.) also known as Turkey Red (*Sanli et al., 2017*).

Rubia cordifolia (Indian Madder) is growing most often near streams and rivers along the upper Ghats in evergreen forests up to 3750m above sea level. It is a perennial, prickly or scabrous, climbing herb belongs to rubiaceae. Leaves variable, arranged four in awhorl, cordate-ovate to ovate-lanceolate, base slightly cordate, petioles are quadrangular, sometimes prickly on the angles, glabrous and shining. Stipules are absent. Stems is slender, rough, four angled with sharp recurved prickles on the ridges, which are often many yards long, becoming slightly woody at the base. Flowers are incymes, greenish white. Fruits are didymous or globose, smooth, shining and purplish black when ripe. In ancient world, manjistha is reputed as an efficient blood purifier and hence is extensively used against blood, skin and urinary diseases. The root is sweet, bitter, acrid, astringent, thermogenic, antidysenteric, anti-inflammatory, antipyretic, analgesic, anodyne, anthelmintic, antiseptic, constipating, diuretic, galacto-purifier, febrifuge, rejuvenating and tonic. It is useful invitiated conditions of kapha, the body fluid principles relates to mucus and pitta, an energy principle which uses bile to direct digestion. In modern pharmacopoeia, the plant has been used to treat variety of ailments (*Devi Priya et al., 2012*).

The importance of madder in Turkey and the World's foreign trade will be analyzed with statistics. The data for the evaluation specific to Turkey were obtained by using the code 320,300,109,000 with the customs tariff statistical position HS12 which includes madder. However, data regarding the world trade of madder could not be obtained in international sources for further classifications than 6-digit codes. Today, although the colouring of products such as textiles and carpets has surrendered to chemicalization, the return to organic and natural colourants is rapidly becoming widespread. Such products are often the subject of rural production. Regional products come to the fore not only with their natural features but also with the fact that they trigger development in the economies of many countries. Especially in European economies, regional

products such as madder have a high economic value and are considered to be one of the driving factors of rural development (*Joosse, 2016*).

Madder, used as a red dye, contains anthraquinones and some of these can react with DNA possibly causing mutagenic effects. This is especially true for 1,3-dihydroxyanthraquinones with a hydroxymethyl (e.g., lucidin, ibericin) or methyl group (e.g., rubiadin) at C-2. In this research a new process was developed through which the concentration of mutagenic compounds is minimized by adapting extraction and fractionation parameters. The process was tested on lab scale but also on 5000 L industrial scale. The first step is the key biotechnological step. Root are stirred in water and the concentration of lucidin is reduced to (near) zero by endogenous enzymes. When lucidin is absent, the formation of mutagenic ibericin by a reaction with ethanol as extraction solvent, is not possible. Mutagenicity can be further reduced by heat treatment, which is common in industrial downstream processing, e.g., in spray drying. Removal of rubiadin is possible by flash chromatography. All madder root fractions were tested in the *Salmonella* microsome assay (Ames test, TA100) for mutagenicity, which was correlated with the anthraquinone concentration (*Goverdina et al., 2021*).

2.2.2 Annatto Seeds (*Bixa orellana)* and its Properties

Modern investigations on this plant have revealed the presence of natural reddish-yellow dye in seeds of *B. orellana*. The fruit of the *B. orellana* tree consists of 10–50 seeds of the size of grape seeds covered with a thin layer of soft, slightly sticky vermilion pulp. Seeds are characterized by substantial amount of carotenoid compounds mainly apocarotenoid bixin, nor-bixin and other less important cryptoxanthin, lutein, zeaxanthin and methylbixin. Numerous pieces of research have been conducted on *B. orellana* plant over the last few years; however, there is a paucity of comprehensive review articles on this potential natural dye plant. Keeping in view the tremendous interest in this dye containing plant, we herein summarize up-to-date information on the phytochemistry and biological activities of annatto (*Shahid et al., 2016*).

Annatto (*Bixa orellana* L) seed has been used as a natural colourant in many traditional foods found in Asia. Annatto ranks second place in economic importance worldwide among all natural colourants and its extract shows antimicrobial and antioxidant properties. The colour of the pigment from the outer layer of annatto seeds ranges from yellow to red and is affected by the concentration of the colour compounds. The main colour pigments of annatto seeds are bixin and nor-bixin, extracted from the outer coating of the seeds (*Taham et al., 2015*).

Several studies have measured the antioxidant activities and colour properties of different annatto extracts. On the other hand, numerous studies were performed based on the extraction of bixin compounds from annatto seeds using different techniques (Rodrigues *et al.*, 2014). Although most of the studies focused on the determination or optimization of the extraction methods for the compounds and their antioxidant activities, studies on the antioxidative and antimicrobial effects of annatto seed extracts on meat and meat products are limited (*Yolmeh et al., 2014*).

Annatto is one of the world's oldest natural dyes used to give colour to ranging from red to yellow. It is obtained from the pericarp of Bixa Orellana L. seeds. The carotenoids bixin (methyl ester) and norbixin (carboxylic acid) are the main colouring agents of this dye. The bixin is liposoluble and norbirxin is soluble in alkaline media. Both of these carotenoids have strong colouring capacity, e.g. a solution of norbixin at one percent is sufficient to colour 16 tons of cheese. In fact, annatto is categorized by the FDA as a colourant exempt from certification and suitable for use in foods, drugs and cosmetics. Due to their stability and low cost, synthetic colourants have come to constitute the first option for processed food products; however the consumers have become aware of the adverse health effect associated to them besides that legislation on the use of synthetic dyes have increased restrictions in different countries. For example, annatto is considered to be a potential alternative to replace tartrazine, which is banned in many countries (*Giridhar et al., 2013*).

2.2.3 Eucalyptus (*Eucalyptus grandis*) and its properties

Eucalyptus globulus is the most important hardwood for the pulp and paper industry worldwide due to several features, such as fast-growing, easy adaptability to various types of soil and climate conditions (temperate, subtropical and tropical) and the high-quality papers produced thereof. The plantations of this hardwood genus cover some 18 million hectares worldwide in 90 countries with emphasis to *Eucalyptus globulus* Labill. being the dominant species in Southern Europe. It should be noted, however, that there are more than 700 different eucalyptus species, but only approximately a dozen species, including *Eucalyptus globulus*, are used for pulp production. In Europe, in 2020, the wood consumption for the pulp and paper industry was 146.4 million m^3 and 8.6 percent of this value, 12.6 million m^3, were eucalyptus (CEPI. *Key Statistics 2020 European Pulp and Paper Industry, 2020*)

Such *Eucalyptus globulus* forest residues as bark and branches resulting from debarking and pruning activities do not find industrial use and represent a considerable volume of wastes. These wastes are used to some extent for energy production, but often end up in landfills There are

countless studies on the chemical composition of *Eucalyptus globulus* management wastes such as bark, stumps and branches. Thus, eucalyptus bark can be used for the extraction of phenolic compounds, since they are natural antioxidants with a lot of applications in food and pharmaceutical sectors. Several studies have been carried out with the aim of improving the amounts of extractives removed from *Eucalyptus globulus* bark by supercritical fluid extraction or using ultrafiltration membranes for the recovery of polyphenolic compounds. Other studies revealed the waste biomass from *Eucalyptus globulus* pulp production (e.g., bark) as a source of high-value triterpenic compounds. However, after extraction, a lignocellulosic residue with unaltered macromolecular compounds is left as a residue. Recently, several studies on the chemical composition of eucalyptus wastes related to the evaluation of the industrial bark as a biorefinery feedstock have been carried out (*Neiva et al., 2018*).

Eucalyptus is a large tree belonging to the family Myrtaceae. It is a tree of multiple uses and benefits. The essential oil extracted from its leaves, branch tips and fruits is rich in phytochemicals like eucalyptol, 1,8-cineole, limonene, citronellal, citral, eudesmol, α and β-pinene, p-cymene, terpinen-4-ol, terpineol, α–phellanderene and 9β-sitosterol etc. These compounds can be extracted by various methods like solvent extraction method, hydro-distillation and supercritical fluid extraction. The essential oil from the eucalyptus possesses anti-bacterial, anti-viral, anti-fungal, antioxidant, antimalarial, analgesic, antiseptic, anti-diabetic, anti-cancerous, anti-inflammatory and cytotoxic properties. The historical records of the food and medicinal applications of eucalyptus are available and the recent studies on its phytochemical composition further strengthen its claim for application in the modern food and pharmaceutical industry (*Surbhi et al., 2021*).

Eucalyptus (*Eucalyptus spp*.) is a large genus belonging to the *Myrtaceae* family which has about 900 species and subspecies. This is an evergreen tall tree native to Australia and Tasmania and is being cultivated in a majority of sub-tropical and warm temperate regions of the world. In India, this tree was first introduced in the 1790s at Nandi Hills, Mysore and since then it has been spread to the different regions of Andhra Pradesh, Kerala, Tamil Nadu, Mysore, Gujarat, Punjab and Haryana. The species that belong to India are Eucalyptus citriodora and E. globulus. It has been known by various local names like Blue-gum (English), Taliparna (Sanskrit), Safeda (Hindi), Harit Parn (Gujarati) and Karpuramaram (Tamil) in different regions of India.This plant has been planted extensively for the prevention of water and wind erosion and as a source of timber, fuel, paper pulp and essential oil. Eucalyptus is rich in bioactive compounds such as eucalyptol, eudesmol, limonene, α- and

β-pinene and terpineol etc. Eucalyptus oil particularly from E. citriodra is a major source of citronellal which has been used universally in fragrance and chemical industries. The major applications in the fragrance industry include the preparation of creams, deodorizers, detergents, lotions, perfumes, soaps etc. The essential oil is also used in food and beverage preparations, aromatherapy and phytotherapy in countries like China, India, South Africa, Portugal, Brazil and Tasmania.In the food industry, eucalyptus oil is used as a flavouring agent in chewing gums, ice-creams, confectionery and the liquor industry. It is also used in water disinfection plants. The essential oil also possesses anti-diabetic, anti-bacterial, anti-inflammatory and antioxidant properties and is used for cleaning of external wounds and skin infections (*Vecchio et. al., 2016*).

2.4. EXTRACTION PROCESSES OF NATURAL FOOD COLOURANTS

Colour of a food is one of the major factors influencing its acceptance by consumers. At presently synthetic dyes are the most used food colourant in food industry by providing more esthetically appearance and as a means to quality control. However, the growing concern about health and environmental due to associated toxicity with synthetic food colourants has accelerated the global efforts to replace them with safer and healthy food colourants obtained from natural resources (plants, microorganisms and animals). Further, many of these bio colourants not only provide myriad of colours to the food but also exert biological properties, thus they can be used as nutraceuticals in foods and beverages (*Monika et al., 2022*).

2.4.1 Traditional Extraction Process of Aqueous and Fermentation Extraction Methods of Food Colourants

Aqueous extraction is one of the ancient traditional processes to extract dyes from the natural sources with water as the base of soluble phase. Other mode of extraction with solvents, alcohol and alkaline were not carried out because dyes with phenolic groups have a better yield when they undergo alkaline extraction. Some colours get destroyed when they undergo alkaline conditions that are pH sensitive. When changing the pH of the extraction medium by adding acid or alkali leads to a different constituent differing in colour properties. Solvent extraction process requires a very low temperature thus degrading a few plant substances. Presence of toxic residues, their greenhouse effects, readily insoluble substances which requires aqueous medium for complete extraction, other sub-extracted products like chlorophyll and waxy substances, create a problem of disadvantage in this method of extraction (*Rimsha Nawaz et al., 2019*).

According to *Wang (et al., 2013)*, the immersed food colour sample along with the water, were boiled in water bath at 60°C for 60 minutes. The extracts were allowed to cool and then were filtered into a sterilized glass bottles. As reflux extracts are highly prone to contamination, the extracts were stored in room temperature at 37^{0}C and the other in the refrigerator at 5^{0}C.

Aqueous extraction is a traditional extraction process used to extract dyes from plants and other natural sources. In this method, the dye material is first put into small pieces or powdered and sieved to improve efficient extraction. It is then soaked with water in a vessel for over a night to loosen the cell structure and then boiled to get the dye solution which is filtered to remove non-dye plant particles. The process of boiling and filtering is repeated over and over again to remove as much dye as possible. In case of large scale production of dyes, the dye extraction particle is soaked for over a long period of time (for a day or two). Generally, centrifuges are used to remove residues and also trickling filters to remove very fine non-dye insoluble plant particles. The aqueous media has its own disadvantages like longer extraction time, larger water requirement, usage of high temperature, but very low dye yield and only water-soluble components will be extracted whereas many dyes have low water solubility level. Heat sensitive dyes will get reduced if they are extracted in high boiling temperature, therefore lower temperature should be used to extract these substances (*Guilherme Sorita et al., 2023*).

The microorganisms present in the atmosphere or the enzymes of the microorganisms present in the natural resources play an assisting role in this method. Fermentation is similar to that of aqueous extraction process with an exception that this method does not require high temperature. In warm water of around 32 degree Celsius, for about 10-15 hours the natural particle is fermented. Then it is left open to get oxidized with air, compressing them in vats. The residues settles at the bottom are collected, washed and excess water is removed from the pressed cakes. The natural colour binding is disintegrated by the microorganisms. Few disadvantages of this method are that: longer extraction period, needs an immediate extraction process right after the harvest and foul smell due to microbial reaction (*Yanjun Feng et al., 2017*).

2.4.2 Solvent and Enzymatic Extraction Method of Food Colourants

Commercially available enzymes like cellulose, amylase and pectinase are used to loosen the plant tissues like cellulose, starch and pectin, where the molecules under mild conditions are used to extract the dye. This process id used for the extraction of colours from hard plant materials like barks and root.In this method, naturally available organic solvents including: acetone, petroleum ether,

chloroform, ethanol, methanol or mixture of solvents like: ethanol and methanol or water and alcohol are used to extract the dyes. This process extracts both water-soluble and water-insoluble substances and the extraction yield is high than compared with aqueous method. Purifying of the extracted colours are easily removed by distillation and redoing it. This extraction process requires a very low temperature thus degrading a few plant substances. Presence of toxic residues, their greenhouse effects, readily insoluble substances which requires aqueous medium for complete extraction, other sub-extracted products like chlorophyll and waxy substances, create a problem of disadvantage in this method of extraction (*H. B. Sowbhagya et al., 2010*).

Dyes are in the form of glycosides that can be extracted through dilute alkali or acidic conditions. Adding alkali or acid enables the hydrolysis of glycosides resulting in higher yield and better extraction of colours. To prevent oxidative degradation, acidified water is also used for extraction. Dyes with phenolic groups have a better yield when they undergo alkaline extraction. Some colours get destroyed when they undergo alkaline conditions that are pH sensitive. When changing the pH of the extraction medium by adding acid or alkali leads to a different constituent differing in colour properties (*Adrija Saha et al., 2022*).

2.4.3 Electric Field Assisted Extraction and Ohmic Heating Processes of Food Colourants

Electric field (EF)-based technologies are emergent processes with the potential for the rapid and uniform thermal treatment of materials. Ohmic heating (OH) and pulsed electric fields (PEF) are included in this category. Although it is not common to use these methods to extract natural colourants, some studies have addressed the potential of EF on the stability, functionality and application of biomolecules. In addition, the electric field's non-thermal effects (mainly electroporation) seem to enhance the extraction of compounds (*Pereira et al., 2024*).

Microwaves and ultrasound are used to reduce the quality of solvent, time and temperature for extraction. While using the microwave extraction method, the natural source is treated with minimal amount solvent, in the presence of microwave energy where the rate of extraction process is higher and completed in a short period of time. When the natural particle with dye is treated with either water or other solvents and they come in contact with the ultrasound, small bubbles or cavities are formed in the liquid. These bubbles they get larger and larger every second when they are exposed to ultrasound, which do not get back to its original form. But they burst open forming millions of bubbles, which increases the pressure and temperature of the dye, making the extraction process more efficient in a short period of time. Lowering the temperature makes the heat-sensitive

dye molecules better for extraction. Ultrasonic extraction process is also known as sonication process. Microwave and ultrasonic extraction methods are considered as “Green-Processes”, as they reduce the extraction time, temperature and solvent usage resulting in very lower energy consumption (*Sakr et al., 2014*).

Ohmic Heating: With this method, an alternating electrical current is passed through a material. Consequently, the material is internally heated from the core to the outer material’s surface due to the food’s electrical resistance. This feature is the main innovation of OH, making it a highly energy-efficient process suitable for rapid and uniform plant material processing. Using this technology, plants are not over-processed and minimal changes in phytochemicals and colour are produced (*Brochier et al., 2019*).

Previous studies have used OH to extract phenolic compounds, mainly ANC, from different plant tissues. The best conditions to extract polyphenols from wheat bran using OH were set at 20 V/cm, 80 °C and 10 min, using water instead of the solvents commonly used to extract phenolic compounds (*Nwoba et al., 2020*). A recent study also evaluated the extraction yield of ANC from *Solanum tuberosum* L. var. Vitelotte, a coloured potato with blue and violet tones, using OH at different temperatures and voltages. An 85 percent recovery of total anthocyanidins (TA) was achieved at 90 °C and 15 V after 10 min holding time, compared with a conventional thermal treatment that yielded 73% recovery. This effect depended on the time and temperature applied, but enhanced by the non-thermal effects that might cause the potato tissues’ permeabilization due to an electroporation phenomenon. The main pigments extracted from potatoes were petunidin glucosides, malvidin and delphinidin, responsible for the purple colour. Given the antioxidant nature of ANC, this extract may be a functional colourant in foods and beverages, although its stability has not yet been studied (*Al-Hilphy et al., 2020*).

A colourant powder was obtained from black rice bran (*Oryza sativa* L.) with ohmic heating-assisted solvent extraction. The colourant yield obtained (up to 20.63%) was significantly higher compared to a steaming extraction process (17.64%). Dark purple ANC (cyanidin-3-O-glucoside or C3G, delphinidin, cyanidin and pelargonidin) were successfully extracted with OH and tocopherols such as γ-Oryzanol. Moreover, the solubility and colour of the powder were not affected by the OH process; essential parameters used when evaluating the feasibility of a method for industrial applications. ANC extraction was enhanced by the electric field that causes cell wall permeabilization, allowing a higher and homogeneous release of intracellular components. This

effect was also observed for ANC ethanolic extraction from red grape pomace after OH application, with a 36 percent yield at 400 V/cm (*Loypimai et al., 2015*).

By-products such as peels and Vine Pruning Residues (VPR) can also be used as sources of colourants. VRP is a good source of polyphenols. The OH technology has been used to obtain ethanol-water polyphenol extracts from this material (840 V/cm, 80 °C and 60 min extraction). Moreover, VP extracts may have beneficial effects on health such as antioxidant capacity, antimicrobial and anticancer activity against several cancer cell lines including HepG2, MDA-MB-231, MCF-7 and Caco-2 (*Jesus et al., 2020*).

Colour stability is an important feature when evaluating the feasibility and application of industrial systems. Processing conditions significantly affect this stability, as reported for ANC, carotenoids and fungal pigments exposed to OH. Typically, the OH methods reach a temperature higher than 70 °C, at which phenolic compounds degrade. As shown for blueberry (*Vaccinium* spp.) pulp treated by OH, ANC degradation depends on the temperature-electric field combination. At high voltages (>200 V), degradation was larger than the observed with conventional heating and depended on the total solids content. In another study, a red extract produced by *Penicillium purpurogenum*incorporated in a beverage model system was processed with OH for microbial inactivation at 30 V and 0–80 min holding time. The degradation kinetics showed lower stability for the samples treated with OH compared with conventional pasteurization. These observations indicate that the thermal effect and the concomitant influence of electric field and matrix compositions must be taken into account to maximize ANC yield and stability (*Aguilar et al.,2017*).

2.4.4 Supercritical Fluid Extraction Method of Food Colourants

Supercritical fluid extraction (SFE) is a process used for separating one component, named the extractant, from another known as the matrix, using supercritical fluids as the extracting solvent (*Ibáñez et al., 2016*). The conditions of these fluids are above their critical point of pressure and temperature, their density is similar to liquids, their viscosity is comparable to gases and their diffusivity is between both gases and liquids. The main advantage of a supercritical fluid is that its density can be modified by changing its pressure and temperature. The properties mentioned earlier allow supercritical fluids to penetrate deeper and faster to solid matrices because they diffuse easily through them (*Silva et al., 2016*). Carbon dioxide (CO_2) is the most used solvent due to its low cost, safety and moderate critical temperature (31.2 °C) that enables the preservation of bioactive compounds in extracts (*Abhari et al., 2020*).

Other remarkable advantages in comparison with standard extraction techniques are the use of solvents generally recognized as safe (GRAS), lower extraction times, increased yields meaning higher efficiency of the extraction process and the option of direct coupling with analytical chromatographic techniques such as gas chromatography (GC) or supercritical fluid chromatography (SFC). Likewise, the authors carried out the extraction of ANC by the conventional method using water, intending to compare the yields obtained. A relevant aspect of this work was the combination of water and CO_2. The highest total ANC yield (52.7%) from berry pulp paste using CO_2 was achieved using 45 MPa, 65 °C, 5.4 g water to 3.2 g paste, 15 min static and 20 min dynamic time. In conclusion, compared with conventional extraction, using CO_2 as solvent and water use as co-solvent offered higher ANC extraction efficiency (52.7% vs. 38.3%) (*Jiao et al., 2018*).

The recent work conducted by *Idham et al*. (2020), aimed to evaluate the effects of different particle sizes, flow rates and modified ratios on the extraction yield and ANC content of Roselle (*Hibiscus sabdariffa*) by using the supercritical carbon dioxide (SC-CO_2) method. The pressure and temperature were kept constant at 10 MPa and 70 °C respectively and 75% ethanol was used as a modifier. Different solvent flow rates were studied: 4 mL/min, 5 mL/min and 6 mL/min. Three different ground dried Roselle sizes were used: 200–355 μm, 355–500 μm and 500–710 μm. Finally, three percentages of modifiers ratios were compared: 5%, 7.5% and 10%. The effect of these three parameters showed different results of overall extraction yield and total anthocyanin content (TAC), demonstrating that these conditions are key to obtain the highest ANC concentration. The optimal parameters that allowed reaching the highest ANC concentration were extraction time of 120 min, flow rate of 4 mL/min obtaining a TAC of 5 mg equivalents of C3G (EC3G)/L, particle size of 200–355 μm showing a TAC of 4.95×10^4 mg EC3G/L and a 10% of modifier ratio obtaining a TAC of 3.84×10^4 mg EC3G/L.

2.5. PHYSIOCHEMICAL AND MICROBIAL ANALYSIS OF THE NATURAL FOOD COLOURANTS

2.5.1. Physiochemical Properties of selected Natural Food Colourants

Food colour contributes to food acceptability. Hitherto, colours for foods are obtained from artificial sources or chemicals. However, there is a gradual shift in sourcing food colouring materials from aritical sources to natural pigments. This was meant to utilize functional properties in natural pigments such as bioactive activities, anticancer potentials, production of vitamin A and so on in addition to enhancing consumers' acceptability. Some of the functional compounds in natural pigments are polyphenols, antocyanins, chlorophyll a and b, carotenoids, betalains and so on. These

compounds possess potent antioxidants, antidiabetics, vasoprotective, anti-inflammatory, anti-cancer, chemoprotective and anti-neoplastic properties. Carotenes serve as precursor of vitamin A. Isolation and untilization of natural pigments will prevent side effects notable in artificial colouring agents in addition to reducing the prevalence of some diseases like diabetics, cancer and cardiovascular diseases. The functionalities of these natural compounds in foods promotes health of the consumers (*Olugbenga et al., 2021*).

A chemical added to a particular food for a certain reason during processing or storage which could affect the characteristic of food or become part of the food. Preservatives are additives that inhibit the growth of bacteria, yeasts and molds in food items. Food preservation involves treating and then handling food to stop or slow down food spoilage, loss of quality, edibility or nutritional value and thus, allow for longer food storage. Preservation usually involves preventing the growth of bacteria, fungi (such as yeasts) and other microorganisms although some methods work by introducing benign bacteria, or fungi to the food. Food antioxidants in the broadest sense are all the substances that have some effect on preventing or retarding oxidative deterioration in foods (*Amin et al., 2020*).

Textile and food industries wastewaters are characterized by a strong colouration due to use of dyes, among which azo dyes are the most frequently used. These compounds are harmful and their discharge without an adequate treatment causes a negative impact on the environment. Given that conventional biological treatment processes are usually not sufficient to degrade azo dyes, other treatment alternatives must be studied. Enzymes have demonstrated their effectiveness to degrade several types of recalcitrant compounds. Enzymatic degradation of Crystal Ponceau 6R (CP6R) azo dye by turnip (*Brassica rapa*) peroxidase was assessed in the presence of 100 μm redox mediator (1-hydroxybenzotriazole). The treatment performance was evaluated at different pH values (3, 4, 5, 6 and 7) and the most appropriate pH and contact time were determined. Moreover, a kinetic model of the enzyme-catalyzed reaction was developed by mathematical description of the degradation mechanism, being further validated with experimental data. For the estimation of the kinetic parameters and model validation, a central composite design of type “start points” was made. The results showed that the enzymatic treatment was very effective. CP6R dye degradation higher than 97 percent was achieved in less than one minute of reaction at the optimum pH (i.e., 4), at which the maximum reaction rate (43 μM min^{-1}) was obtained. At the other tested pH values, degradation performance was substantially lower (<70%) in the same timeframe, but still satisfactory. Moreover, the proposed kinetic model successfully predicted the CP6R dye degradation under different experimental conditions in terms of dye and enzyme concentrations (*Almaguer et al., 2018*).

Synthetic dyes can cause many health problems and their use as food additives is rigorously regulated worldwide. Two methods for the determination of synthetic dyes in food are described in this article. The visual qualitative expression method was based on the extraction of synthetic dyes using a liquid anion exchanger (0.01 M solution of trioctyl methyl ammonium chloride in chloroform). Using this reagent, an optimal transition of 15 anionic synthetic dyes from the aqueous to the organic phase was achieved (R > 99.8%). It was applicable for testing food that must not contain synthetic dyes (wines, juices, etc.) in a very short time (5–10 min). In the case of colouring of the organic phase, identification and quantification was carried out using the HPLC-DAD method described. The rapid and simple method allows for simultaneous determination of 16 synthetic dyes from all food types. The LOD and LOQ ranged from 0.026 to 0.086 μg mL^{-1} and from 0.077 to 0.262 μg mL^{-1} respectively and recovery was 83.7–107.5 percent (*Alena et al., 2022*).

The physicochemical properties of food are mainly responsible for the final quality of the product. Moreover, the measurement of the properties of food colourants is essential to design the quality control limitations of the food during the process of preparation. Physical and chemical changes result from physical, sensory, nutritional changes in food and therefore in the quality (*Marta et al., 2022*).

Organoleptic characteristics aid in determining the acceptance, selection and consumption of foods. Colours added during food processing in foods undergo changes in level of oxygen content, presence or absence of metals, hydrogen ion concentration and even water activity. To overcome the problem of characteristic changes in food and to ensure the good manufacturing practices and consumer safety, physical and chemical properties of the natural food colourants are analyzed (*Novais et al., 2022*).

The effect of the powder produced by ball-milling the outer layer of annatto (*Bixa orellana* L.) seeds on the physicochemical properties as well as the antioxidant and antimicrobial activities of pork patties over 14 d of refrigerated storage (4±1°C). Five pork patty treatments were produced containing three different concentrations of annatto seeds, 0.1, 0.25 and 0.5 percent (ANT0.1, ANT0.25, ANT0.5), 0.1 percent ascorbic acid (AA0.1) and a control (CTL). Based on the results, annatto seed powder appeared to show antioxidant activity. The Hunter colour values of pork patties were affected by the addition of annatto seed powder, which increased the redness and yellowness values, but decreased the lightness of the patties (p<0.05). To evaluate the antioxidative effects of annatto on pork patties, thiobarbituric acid reactive substances (TBARS) and peroxide values (POV) were analyzed over 14 d of refrigerated storage. Treatments containing annatto seed

showed lower TBARS and POV than control (CTL) samples (p<0.05). The volatile basic nitrogen (VBN) of the pork patties containing annatto seeds were lower than that of CTL at the end of storage (p<0.05). Although no differences in total bacterial counts were observed between control and treated patties, those containing annatto seeds had lower microbial counts for *Enterobacteriacease* than CTL or AA 0.1 percent. Therefore, annatto seed powder might be a good source of natural antioxidants for the production of meat products (*Cuong et al., 2016*).

Bixa orellana seeds (Annatto) have been used in food colouring and cosmetics. The main commercial processes to extract the pigment from annatto seeds were direct extraction into oil or aqueous alkali and indirect extraction using organic solvents. This research was held to determine the colour and physicochemical properties of annatto extracts using aqua dest as a solvent. The extraction was carried out by maceration at various pH of aqua dest (distilled water) (4, 7 and 9) at 80^{o}C for 10 minutes. Colour property of brightness (L), redness (a) and yellowness (b) was determined using a colour reader. Physicochemical characterization was conducted by observing pH value, total titrated acid, total dissolved solids and total solids. The results showed that the colour and physicochemical properties of annatto extract were influenced by the pH of aqua dest influences. The highest brightness (43.8), redness (9.37), yellowness (42.5) and total soluble solid (1.67^{o} Brix) was produced by extraction using pH 7 of aqua dest, while the highest of total titrated acid (4.2%) of extract produced in pH 4. The lowest pH of the extract (4.83) was resulted in extraction using aqua dest pH 4. The higher the pH of the solvent, the higher the pH of the extract. The different pH of aqua dest used to extract annatto seeds resulted in various colour intensities and physicochemical property (*Handayani et al., 2023*).

Annatto is a natural dye extracted from the pericarp of *Bixa Orellana* L. seeds. The main colouring agents of this dye are bixin and norbixin. In this study, it was evaluated the effect of light and temperature on the stability of an aqueous formulation of norbixin. Both studies were carried out in controlled conditions. Photostability studies were conducted exposing samples in different concentrations under irradiated for 6 hours using a xenon lamp at 1000 W/m2 . The effect of temperature was assessed by analyzing the samples exposed at 30±2ºC during 12 months, in order to simulate the natural storage condition. Norbixin concentration during storage after exposure to various conditions was measured by spectrophotometry at 455 nm. This was correlated with colour, which was measured by sensorial analysis, where perceptible changes in colour were identified. Results from forced photostability studies showed that samples at high norbixin concentration (5.58%) did not suffer decomposition. The decay of norbixin promoted by temperature was fitted a linear model and a significant change of colour was observed at 12 months when the remaining

norbixin concentration was 4.42%. The findings showed that high concentrations are a protective factor against photo-degradation and the shelf life of norbixin in aqueous formulation was 12 months at 30ºC (*Gallardo et al., 2015*).

Lima et al. (2018) reported the chemical and anatomical characterization and antioxidant properties of barks from 11 eucalypt species. Similar works were carried out to evaluate the chemical composition and anatomical structure of *Eucalyptus globulus* stumps and different pre-treatments to improve their delignification. Regarding the chemical composition of the bark in 11 eucalyptus species, it was concluded that the chemical composition is specific to each species and differs in extractive content (from 5.5 to 18.6%), in Klason lignin (from 11.6 to 24.3%) and in glucose/xylose ratio (3.8 to 12.1). By applying pre-treatment, in a biorefinery context, *Eucalyptus globulus* stumps (with 11.9% extractives and 22.2% Klason lignin) could be successfully delignified and, therefore, used for the production of cellulosic pulp. Regarding the chemical composition of *Eucalyptus globulus* stumps, it was found that the amount of ash in piled industrial stumps (19.2%) is much higher than in fresh stumps (3.5%) and that the extractive content is different in fresh stumps (7.5%) and on stacked stumps (4.1%). Recently, a study on the structural composition of lignin from *Eucalyptus globulus* bark revealed a H:G:S ratio of 1:26:73. The study also showed some structural units (*p*-coumaric acid, coniferylic acid and sinapylic acid) in addition to the three main groups mentioned above. The identification of the components present in the extractives have also motivated several studies. Recently, 202 compounds were found in the lipophilic fraction of *Eucalyptus globulus*, of which, 189 were fully identified (*Gominho et al., 2021).*

The studied materials are wastes of a Forest management company that efficiently separates *Eucalyptus globulus* bark and branches before sending wood for the cellulose companies. The material dries in the sun and the initial samples had moisture around 17.5% and 10.6% for bark and branches, respectively. Since especially branches are mostly removed in the forest and afterwards transported to the company and chipped, there can be a vast diversity in the composition, such as bark, leaves and possibly some sand, as mentioned before for *Pinus Sylvestris* forest residues shredded chips (*Roman et al., 2022).*

Bark and branches are heterogeneous materials, consisting of non-structural and structural components. Among the macromolecular structural components, the main ones are cellulose, hemicelluloses and lignin. The non-structural components are low molecular weight compounds, which include mainly the extractives and inorganic compounds. It is known that the chemical composition of branches and bark varies from species to species, changing in various parts of the

same tree (trunk, branches, root, bark and needles) and is also affected by the trees growing conditions (*Kilulya et al., 2014*).

Other factors influencing the chemical composition of the bark and branches includes the age of trees, tree cutting season and time elapsed between cutting and carrying out the analyses. The results on the chemical composition of bark and branches. The major component for both samples is cellulose, with 40.5 percent and 41.3 percent for bark and branches, respectively. Similar results for the cellulose content for eucalyptus bark cellulose (41.6%) and for *Eucalyptus globulus* branches (41.2%). 62.6% of holocellulose content in eucalyptus bark, which is not much different from the 63.6 percent obtained.Branches presented 3.0 percent extractives in dichloromethane, 3.1 percent in ethanol and 4.8 percent in water. The similarity between bark and branches chemical composition was to be expected since branches have a high bark content. The high percentage of ashes obtained in this study, 14.2 percent and 10.6 percent for bark and branches, respectively, might be due to some contamination of the samples from some inorganic materials since these wastes are left outside in the field as stated before. Eucalyptus bark has a high mineral content dominated by Ca that corresponds to 75% of the mineral fraction, while in wood is just 19 percent. There is also a high amount of Fe (546 ppm) and Mn (731 ppm). These authors also mentioned that the HHV of eucalypt bark was 18.4 (MJ/kg), lower than the 19.3 (MJ/kg) for wood. The values for branches are difficult to find since they depend on the amount of leaves, bark, or wood in the sample (*Neiva et al., 2020*).

2.5.2 Microbial and Antimicrobial Analysis of Natural Food Colourants

Preservatives are additives that inhibit the growth of bacteria, yeasts and molds in food items. Some additives have been used for centuries; for example, preserving food by pickling (with vinegar), salting, preserving sweets or using sulfur dioxide as in some wines. Food preservation involves treating and then handling food to stop or slow down food spoilage, loss of quality, edibility or nutritional value and thus, allow for longer food storage. Preservation usually involves preventing the growth of bacteria, fungi (such as yeasts) and other micro-organisms although some methods work by introducing benign bacteria, or fungi to the food. Preservatives or antimicrobial agents play an important role in today's supply of safe and stable foods. Increasing demand for convenience fast foods and reasonably long shelf-life of processed foods make the use of chemical food preservatives imperative. Some of the commonly used preservatives such as sulfites, nitrate and salts used for centuries in processed meats and wines (*Carter et al., 2018*).

To determinate the antibacterial activity of the studied extracts, the cup-plate agar diffusion method was used. BHI agar was autoclaved for 15 min at 121 °C and cooled to about 55 °C. The

medium was then inoculated with the prepared bacterial suspension, mixed gently and finally poured into sterile Petri dishes. Sugar tubes containing molten agar (10 ml) were sterilized and cooled to about 40–42 °C. The tubes were then inoculated with 0.1 ml of the appropriate culture suspension of bacteria. These agar plates were incubated under sterile condition for 8 hours at room temperature. The Petri dishes were incubated under the same growth conditions mentioned above. At the end of the period, the inhibition zones formed were measured in millimeters using a vernier. The inhibition zones with less than 12 mm in diameter were not considered for the antibacterial activity analysis. For each extract, 12 replicates were assayed. *B. orellana* methanolic extract *in vitro* antibacterial effect was measured on *S. mutans* and *S. sanguinis* strains and inhibition zones over 12 mm were considered positive. For *S. mutans* the seed extract produced an inhibition zone of 15.11 mm and the leaves extract an inhibition zone of 19.97 mm. Moreover, the Petri dishes with *S. sanguinis* showed an inhibition zone of 16.15 mm and 19.97 mm for seeds and leaves extract, respectively. In both bacterial cultures a larger inhibition zone was observed in the leaves methanolic extracts (*Dyanne et al., 2016*).

The microbiological analysis of food is part of food safety management and conformity tests that define microbiological criteria or assess the performance of control strategies based on the Hazard Analysis and Critical Control Point. For microbiological testing of foods, rapid and conventional methods can be used. The conventional methods are used because they were developed many years ago and have been in use ever since as the official methods of most food microbiology laboratories. The traditional methods have disadvantages associated with excessive laboratory work, time consumption, culture media and laboratory glassware requirement. Other limitations should also be taken into account, such as technique failures related to high agar temperature and high risk of contamination because of all the stages involved in culture medium preparation and inoculation. The limitations of the traditional methods have encouraged the development of alternative methods for microbiological analysis of foods. To identify the presence of microorganisms in the natural food colour extract following assay was carried out in traditional assaying method (*Pariza et al., 2015*).

For detecting the presence of bacteria, Nutrient agar medium was prepared and culture was inoculated in sterilized petric plates, using standard pour plate method was followed. For the period of 24 hour growth, bacterial colonies were identified. The bacterial colonies formed were counted and recorded (*Bonnet, 2020*).

The pour plate method is based on the fact that when an agar medium mixed with microorganisms is incubated, each of the viable microorganisms will multiply forming a separate colony. In this method, a certain volume, usually 1 ml, of the serially diluted liquid sample is mixed properly with approximately 15 ml of specific molten agar medium of about 40 – 45°C (less than 50°C) in a petri plate. The medium is allowed to solidify and is incubated, usually at 37°C for 24 – 48 hours. Following the incubation, the viable microorganisms in the sample will grow into visible colonies on the surface of and within the medium. The visible colonies can be counted and CFU/ml can be calculated using the following formula *(Ahmed E. Yousef et al., 2003)*.

2.6. STUDY OF NATURAL AND SYNTHETIC FOOD COLOURANTS IN *IN-VIVO* MODELS

2.6.1 *In-Vivo* Models used for Analyzing Natural Food Colourants

Albino rats (200- 230g) of both sexes were used. They were housed under standard environmental conditions at temperature (25±2º C) and light and dark cycles (12/12 h). Rats were fed standard balance diet and water. Twenty healthy Wister albino rats were randomized into 2 groups of 10 animals each. Group1 which served as the control was administered with distilled water, the control vehicle, Groups 2, was orally administered daily with 5000 mg/kg b. wt. of the Ethanolic extracts of *E.camaldulensis* for 30 days. The test animals were observed for lethargy, restlessness, weight loss, appetite and deaths. After 30 days, the control and surviving rats were sacrificed and organs (the heart, liver, kidney, intestine, lung and spleen) were collected in sterile saline. Freshly dissected organs from each animal were isolated rapidly and fixed in buffered neutral formalin (10%) for at least 24 hours and then were used for histopthological study. The results demonstrated that ethanolic extract of *E.camaldulensis* leaves is practically Nontoxic when administered orally safety and nontoxic effect (*Azza et al., 2015*).

Aqueous extract of bixin (Annatto E) was fed for 28-day for four groups of five male and five female Control CDBR rats. Three groups received the test material by dietary administration for a 4-week period and one control group received untreated diet alone. The test material complied with the specifications for aqueous extract of bixin (Annatto E) an actual content of the tested batch of 27.2%. Mean group achieved dosages during the fourth week for Group 2 receiving 30,000 mg/kg diet were 2,872 mg/kg body weight per day for males and 2,886 mg/kg bw per day for females. For Groups 3 and 4, which both received 40,000 mg/kg diet during week 4, mean group achieved dosages were 3,339 and 4,570 mg/kg bw per day for males and 3,234 and4,526 mg/kg bw per day for females,

respectively. No deaths occurred. The only signs observed were orange staining of the coat, faeces and cage tray paper, which were reported for all animals receiving the test material. These were due to the intense colour of the annatto extract. Overall body weight gains were decreased for all treated groups, but the effect was more marked in females than in males. When compared with the controls, reduced food intake was seen in all treated groups throughout, but most markedly in the first week of the study. Food conversion efficiency values were not significantly reduced in any of the groups. When compared with the controls, increased, but not in a dose-related manner, absolute and relative liver weights were recorded in all male and female groups. Orange discolouration of the tongue and the gastrointestinal tract was reported for all animals given the test material. Orange staining of the mesentery was also observed in the majority of treated animals. Histopathological examination revealed diffuse hepatocellular hypertrophy in the livers of the Group 2 females and Group 3 males (*Scientific Opinion, 2016*).

To investigate the effect of beet (BE) and curcumin (CE) extracts as natural red and yellow colour, edicol erythrosine and sunset yellow as recommended synthetic colours in addition to two unknown commercial colouring agents (red and yellow) on the balance of four hormones in the serum of male rats. Rats were divided into 11 groups and administrated daily for 60 days with 1 mL colour solution contained the admissible daily intake (ADI) and overdose (5 times) of the synthetic recommended or commercial colours. BE (0.31 mg) and CE (7.87 mg) were also tested as natural colourants. No significant ($p=0.05$) changes were noticed in brain and testes weight due to treatment with the ADI dose of all studied colourants. Significant reduction in the testes weight was recorded when rats were treated with the overdose. The overdose applied in the present study led to elevation in the level of dopamine and noradrenaline and reduction in the concentration of testosterone and interstitial cell-stimulating hormone (ICSH) in blood serum compared with the control group. These changes were associated with alterations in the histological architecture of brain and disturbance in the exploratory behavior. Correlation coefficient matrix indicated strong relationship between changes in the balance of studied hormones and histopathological alterations in brain induced by consumption of the artificial food colourants. The obtained results emphasis that, there is a need to re-evaluate use of edicol erythrosine and sunset yellow as synthetic food colourants. Moreover, beet and curcumin extracts could be used as alternative natural red and yellow colourants, respectively (*Khiralla et al., 2015*).

2.6.2 *In-Vivo* Models used for Analyzing Synthetic Food Colourants

FD & C Blue No. 1, water soluble brilliant blue of artificial food dye was fed to Charles River CD rats and CD-1 mice as a dietary admixture in lifetime toxicity/carcinogenicity studies. The rat study was conducted with an *in utero* phase in which the compound was administered to the F_0 generation rats (60/sex/group) at dietary concentrations of 0.0%, 0.0%, 0.1%, 1.0% or 2.0%. After randomly selecting the F_1 animals, the lifetime phase was initiated at the same levels with 70 rats/sex/group, including two control groups. The maximum exposure times were 116 and 111 weeks for males and females, respectively. The no-observed-adverse-effect levels are dietary concentrations of 2.0% for males (1072 mg/kg body weight/day) and 1.0% for females (631 mg/kg/day) based on a 15.0% decrease in terminal body weight and decreased survival in the high-dose females compared with the combined control groups. Charles River CD-1 mice (60/sex/group) were fed Blue No. 1 as a dietary admixture at levels of 0.0%, 0.0%, 0.5%, 1.5% or 5.0% in a lifetime toxicity/carcinogenicity study. The maximum exposure time was 104 weeks for both males and females. No consistent, significant compound-related adverse effects were noted. The no-observed-adverse-effect level established in this study is a dietary concentration of 5.0% (7354 mg/kg/day and 8966 mg/kg/day for male and female mice, respectively) (*Darsaut et al., 2012*).

The food additives thiabendazole (TBZ), monosodium glutamate (MSG) and brilliant blue (BB) are commonly used in many daily-consumed food products worldwide. They are widely used in major agricultural and industrial applications. Yet, many of its toxicological aspects are still unclear, especially immune modulation. This research was therefore intended to investigate the effects of male Wistar rats' daily oral exposure for 90 days to TBZ (10 mg/kg b.wt), MSG (20 mg/kg b.wt), or BB (1.2 mg/kg b.wt) on the blood cells, immunity and inflammatory indicators. The three tested food additives showed varying degrees of hematological alterations. Initially, megaloblastic anemia and thrombocytopenia were evident with the three tested food additives. At the same time, TBZ showed no significant changes in the leukogram element except eosinopenia. An obvious increase in $CD4^+$ but a lessening in $CD8^+$ immune labeling was evident in TBZ and MSG groups. The cytokines, including interferon gamma, tumor necrosis factor alpha and interleukin 1β, 6, 10 and 13, were significantly up regulated in the spleen of rats exposed to TBZ, MSG and BB. These results concluded that TBZ, MSG and BB negatively affect hematological parameters, innate and humoral immune functions together with inflammatory responses. TBZ achieved the maximal negative impacts followed by MSG and finally with BB. Given the prevalence of these food additives, TBZ

and MSG should be limited to a minimal volume use, or natural food additives should be used instead (*Motwadie et al., 2021*).

Many pharmaceutical drugs like acetaminophen, amoxicillin, ciprofloxacin, etc. cause hepatotoxicity in humans leading to severe liver diseases, representing a serious public health issue. This study investigates the ability of the anthelmintic and antifungal drug thiabendazole to cause cell death by apoptosis and metabolic changes in primary cultures of rat hepatocytes. Thiabendazole (200–500 μM) induced apoptosis in hepatocytes after 1 to 24 h, causing loss of mitochondrial membrane potential, cytochrome c release from mitochondria, Fas-associated death domain (FADD) translocation from the cytosol to membranes and activation of caspases-3, -8 and -9. Thus, thiabendazole activated both the mitochondrial and death receptor pathways of apoptosis. Under these conditions, cell death by necrosis was not detected following exposure to thiabendazole (100–500 μM) for 24–48 h, measured by lactate dehydrogenase release and propidium iodide uptake. Furthermore, thiabendazole increased activities of cytochrome P450 (CYP) isoenzymes CYP1A and CYP2B after 24 and 48 hours, determined by 7-ethoxyresorufin-O-deethylase (EROD) and 7-pentoxyresorufin-O-dealkylase (PROD) activities, respectively. An important finding is that thiabendazole can eliminate hepatocytes by apoptosis, which could be a sensitive marker for hepatic damage and cell death. The study improves understanding of the mode of cell death induced by thiabendazole, which is important given that humans and animals are exposed to this compound as a pharmaceutical agent and in an environmental context (*Séïde et al., 2016*).

Thiabendazole (TBZ) has been extensively employed as a pesticide and/or a fungicide in agriculture, while its residues would threaten to public health and safety. Simple, rapid and sensitive probes for detection of TBZ in real food samples are significantly desirable. In present work, a highly selective and sensitive luminescent sensor for monitoring TBZ in oranges has been constructed based on a Tb^{3+}-functionalized Zr-MOF (Tb^{3+}@**1**). Tb^{3+}@**1** exhibited many attractive sensing properties toward TBZ, including broad linear range (0–80 μM), high selectivity, low LOD (0.271 μM) and rapid response time (less than1 min). Moreover, the probe was employed to determine TBZ in real orange samples, in which good recoveries from 98.41 to 104.48% were obtained. It only takes 35 minutes for the whole process of detection TBZ in real orange samples combined with QuEChERS method. Therefore, it has provided a reliable and rapid method for monitoring the TBZ in real orange samples (*Peng et al., 2021*).

Methanol extract showed potent activity against *L. amazonensis* isolated from a patient with diffuse cutaneous leishmaniasis with IC_{50} = 22 μg/mL. Additionally further research findings on *in vitro* and *in vivo* effects of the essential oil (Ishwarane and geranylgeranoil) of *B. orellana* seeds showed potential activity against intracellular amastigote form with IC_{50} value of 8.5 μg/mL . The effect of methanol extract of *B. orellana* leaves on diuretics was demonstrated in Wister rat models and results showed that the extract at a dose level of 500 mg/kg possessed diuretic effect with a significant increase in urine volume (2.4 ± 0.02 ml) and levels of sodium (82 ± 3.07 mEq/L), potassium (12.3 ± 0.47 mEq/L) and chloride (71 ± 2.52 mEq/L) as compared to control group 0.7 ± 0.04 mL, 62 ± 2.01, 11.4 ± 1.90 and 56 ± 1.90, respectively. Additionally, various extracts of this plant have been used to neutralize snake venom and prevents associated adverse effects and prove its use in folk medicine. Also, ethanol extracts (LD_{50} = 44 μg) offer partial protection against the edema forming activity and lethality in mice against *Bothrops atrox* venom. Root and leaf extract of this plant have been found to have potent antigonorrheal activity with zones of inhibition 6.0 mm and 17.40 mm respectively. The foliage of Bixa is used to treat skin problems and hepatitis and also used as aphrodisiac, antidysenteric and antipyretic. The binding of naringenin-7-O-glucoside isolated from the fruit shell of *B. orellana* with calf thymus DNA (ct DNA) and the influence of cyclomaltoheptaose (b-cyclodextrin, b-CD) on the binding were studied by absorption and fluorescence spectroscopic techniques (*Shahid et al., 2017*).

To evaluate the broadest toxic effect of some synthetic additives of colourants and/or flavors on different body organs and metabolic aspects in rats.A number of chemical food colour and flavor additives are routinely added during processing to improve the aesthetic appearance of the dietary items. However, many of them are toxic after prolonged use. In this experiment, a total of 100 male albino rats of Spargue Dawley strain were divided into 10 groups: G_1 was fed basal diet and served as control, G_2: basal diet + Brilliant blue (blue dye, No. 2, 124 mg/kg diet), G_3: basal diet + carmoisine (red dye, No. 3, 70 mg/kg diet), G_4: basal diet + tartrazine (yellow dye, FD & C yellow No. 5, 75 mg/kg diet), G_5: basal diet + trans-anethole (4.5 g/kg diet) G_6: basal diet + propylene glycol (0.25 g/kg diet), G_7: basal diet + vanillin (1.25 g/kg diet), G_8: basal diet + Brilliant blue + propylene glycol, G_9: basal diet + carmoisine + trans-anethole, G_{10}: basal diet + tartrazine + vanillin for 42 successive days. All food colourants mixed with or without flavor additives induced a significant decrease in body weight, hemoglobin concentration and red blood cell count. Also there was a significant decrease in reduced glutathione content; glutathione-*S*-transferase and superoxide dismutase activities in both blood and liver compared to control group. On the other hand, a significant increase in serum alanine aminotransferase, aspartate aminotransferase, alkaline

phosphatase activities, bilirubin, urea, creatinine, total protein and albumin were observed in all test groups when compared to control group. Finally, it is advisable to limit the uses of these food colourants and/or food flavor additives especially those used by children (*Hanan et al., 2012*).

The interest in food toxicology is evident by the dependency of human kind on nutrition by virtue of their heterotrophic metabolism. By means of modern biochemistry, molecular and cell biology, computer science, bioinformatics as well as high throughput and high content screening technologies it has been possible to identify adverse effects and characterize potential toxicants in food (*Alexander Gosslau, 2016*).

To evaluate the toxic potential of tartrazine, a food colour, in different tissues in adult rat: blood, liver, kidneys and spleen. Tartrazine was administered orally at a dose of 300 mg/kg of body weight to adult male Wistar rats during a period of 30 days. Tartrazine treatment led to an increase in platelets count, a reduction in peripheral lymphocytes and in spleen T CD8-lymphocytes. Furthermore, tartrazine increased the activities of hepatocellular enzymes and promoted changes in kidney biomarkers. In order to explore the possible mechanism involved, oxidative-stress assessment was performed. Results identified critical oxidative alterations in all tested organs, as shown by the promotion of lipid peroxidation and the modification of endogenous antioxidant-defense enzymes. Thus, tartrazine is able to induce in adult rats' hematotoxicity, immunotoxicity and liver and kidney injuries by changing the whole balance between oxidants and antioxidants (*Narges et al., 2016*).

2.7. APPLICATION OF AI IN FOOD AND FOOD COLOURS

Artificial intelligence (AI) has embodied the recent technology in the food industry over the past few decades due to the rising of food demands in line with the increasing of the world population. The capability of the said intelligent systems in various tasks such as food quality determination, control tools, classification of food and prediction purposes has intensified their demand in the food industry. Therefore, the diverse applications in comparing their advantages, limitations and formulations as a guideline for selecting the most appropriate methods in enhancing future AI- and food industry–related developments. Furthermore, the integration of this system with other devices such as electronic nose, electronic tongue, computer vision system and near infrared spectroscopy (NIR) is also emphasized, all of which will benefit both the industry players and consumers (*Nidhi et al., 2021*).

As the population in the world is rising, food demand is predicted to rise from 59 to 98 percent by 2050. Thus, to cater for this food demand, AI has been applied such as in management of

the supply chain, food sorting, production development, food quality improvement and proper industrial hygiene (*Garver, 2018*).

Sharma (2019) stated that the food processing and handling industries are expected to grow about CAGR of 5 percent at least until 2021.Food safety is a great public concern and outbreaks of food-borne illnesses can lead to disturbance to the society. Consequently, fast and nondestructive methods are required for sensing the safety situation of produce. As an emerging technology, hyperspectral imaging has been successfully employed in food safety inspection and control. After presenting the fundamentals of hyperspectral imaging, the application in determination of physical, chemical and biological contamination on food products. Additionally, other studies, including detecting meat and meat bone in feedstuffs as well as organic residue on food processing equipment, are also reported due to their close relationship with food safety control. With these applications, it can be demonstrated that miscellaneous hyperspectral imaging techniques including near infrared hyperspectral imaging, fluorescence hyperspectral imaging and Raman hyperspectral imaging or their combinations are powerful tools for food safety surveillance. Moreover, it is envisaged that hyperspectral imaging can be considered as an alternative technique for conventional methods in realizing inspection automation, leading to the elimination of the occurrence of food safety problems at the utmost.

Colour and appearance are perhaps the first attributes that attract us to a fruit or vegetable. Since the appearance of the product generally determines whether a product is accepted or rejected, measuring the colour characteristics becomes an important task. To carry out the analysis of this key attribute for agriculture, it is recommended to use an artificial vision system to capture the images of the samples and then to process them by applying colourimetric routines to extract colour parameters in an efficient and nondestructive manner, which makes it a suitable tool for a wide range of applications. The purpose of this chapter is to give an overview on recent development of image processing applied to colour analysis from horticultural products, more specifically the practical usage of colour image analysis in agriculture. A quantitative values of colour are extracted from Habanero Chili Peppers using image processing; the images from the samples were obtained using a desktop configuration of machine vision system (*Raul et al., 2018*).

Colour and moisture content are two most important attributes of the commercial food product. Estimation of moisture content is very important to know the storability of the food product. It also relates to the process of drying in a fruit or vegetable. Extra drying and shrinkage deteriorates the quality of food product. The goal of the experiment was to examine the changes in RGB values of

an apple during drying at different temperatures. This passage emphasis on how the colour changes when there is a significant change in the moisture content of the apple. Three randomly chosen varieties of apples were sliced to 8 mm thickness and dried in vacuum oven at 60°C, 70°C and 80°C. The loss of moisture was recorded for every 30 min interval and corresponding digital images were taken to determine the change in RGB value. The images that were captured during the study was analysed in MATLAB image analysis computer software. The analysis of moisture content and average colour share with respect to time showed that average colour share value decreases with time at all three temperatures. More than 50 per cent of variation in moisture content was explained by average colour share. There is a significant linear relationship between moisture content and colour changes in RGB and can be used to predict the moisture content of apple during drying process (*Ganesh et al., 2018*).

Voltammetric determination of Tartrazine (Tz) and Brilliant Blue FCF (BB) in their mixture using novel type of carbon black-polyethylene composite electrode (CBPCE) with renewable surface modified by carbon ink (CI) was developed. Electrochemical properties of the tested dyes were investigated in 0.1 mol L^{-1} Britton-Robinson (BR) buffer by cyclic voltammetry (CV) and linear scan voltammetry (LSV). Simultaneous determination of the dyes is based on the application of supporting electrolytes with different pH: 2.0 for Tz and 10.0 for BB. Under the optimum experimental conditions, linear concentration dependences in the concentration ranges from 0.037 to 1.38 μmol L^{-1} for Tz and from 0.025 to 2.52 μmol L^{-1} for BB were obtained by LSV in the first-order derivative mode. Limits of detection (*LOD*s) for Tz and BB were 0.019 and 0.011 μmol L^{-1}, respectively. The modified electrode showed good stability, reproducibility and was successfully applied for the determination of the mixture Tz and BB in a candy and soft drink products (*Lipskikh et al., 2020*).

Azo dyes are commonly added to food in order to improve the appearance and make the food more attractive. Among them, tartrazine (E102), a yellow colourant and allure red (E129) are widely used in drinks, juices, bakery, meat and sweets products. However, some studies claim that their use cause health problems, like frequent headaches in adults, neurotoxicity, genotoxicity and carcinogenicity. Children are especially vulnerable to the consumption of azo dyes for two reasons: they probably eat more candies, particularly rich in azo dyes colourants and they may exceed the acceptable daily intake (ADI) easily due to a lower weight. Actually, there is evidence that the consumption of these additives is related to the increase of hyperactivity in children. Therefore, over the last few decades, many efforts are being made in restriction, or even prohibition, of these dyes in

food industry. The European Union (EU) establishes that the sum of additives of group III, E102 and E129 are here included, should not exceed a limit that goes from 20 to 500 mg L^{-1} or mg kg^{-1}, depending on the food category and most colourants cannot be in more than 50 mg L^{-1} or mg kg^{-1}.

On the other hand, ADI values are 7.5 mg kg^{-1} per body weight (b.w.) for E102 and 7 mg kg^{-1}b.w. for E129. In consequence, methods to determine the concentration of these colourants in food are necessary. A method based on digital image is described to quantify tartrazine (E102), yellow and allura red (E129) colourants in food samples. HPLC is the habitual method of reference used for colourant separation and quantification, but it is expensive, time-consuming and it uses solvents, sometimes toxic. By a flatbed scanner, which can be found in most laboratories, images of mixtures of colourants can be taken in microtitration plates. Only 400 µL of sample are necessary and up to 92 samples can be measured together in the same image acquisition. Results for repeatability and reproducibility are under 12%. These results are slightly worse but comparable to the ones obtained by HPLC. The applicability of both methodologies to real food samples has proven to give the same result, even in the presence of a high concentration of an interfering species, provided that this interference is included in the image analysis calibration model. Considering the colourant content found in most samples this should not be a problem though and, in consequence, the method could be extended to different food products. Values of LODs of 1.8 mg L^{-1} and 0.6 mg L^{-1} for tartrazine and allura red have been obtained by image analysis (*Vidal et al.,2018*).

A sensitive and novel electrochemical sensor for the detection of Allura Red (AR) in the presence of tartrazine (TRZ) was fabricated using a screen-printed electrode modified by functionalized nano diamond covered using silicon dioxide and titanium dioxide nanoparticles (F-nanodiamond@SiO_2@TiO_2/SPE). Scanning electron microscopy (SEM), brunauer–Emmett–teller (BET), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FT-IR) techniques were performed to characterize the as-synthesized Fnanodiamond@SiO_2@TiO_2nanocomposite. The as-fabricated electrode demonstrated two wide dynamic ranges of 0.01–0.12 and 0.12–8.65 µM with a limit of detection (LOD) as low as 1.22 nM. Moreover, the modified electrode exhibits excellent repeatability, reproducibility, reusability, selectivity and stability with high sensitivity of 44.3 µA $µM^{-1}$ cm^{-1}, offering good prospects in the simple, cost-effective and rapid assessment of their total concentration. The successful detection of AR and TRZ, simultaneously and individually in food samples, revealed the applicability of the sensor in the determination of AR and TRZ with satisfactory recovery. Therefore, these advantages provide an excellent possibility for the smart monitoring of AR and TRZ in the future. In the final

step, the preferential intercalative binding mode of Allura red with ds-DNA was approved for the first time by a molecular docking study. The study paves the way for engineering highly sensitive DNA biosensors to monitor azo dye compounds by combining the benefits of nanocomposites and valuable information of a molecular docking study (*Mohammad et al., 2022*).

Food colours are organic substances that have important effects on human health and food safety. While these substances do not pose a problem when used in the daily intake (ADI) amounts, they harm human health when consumed excessively. Amaranth and carminic acid are synthetic and natural food colours ingredients, respectively. Analysis of these substances in food, pharmaceutical, cosmetic and textile samples is extremely important because of their genotoxicity, cytostatic and cytotoxic effects. Electroanalytical methods, which have great advantages over traditional analytical methods, shed light on the scientific world. Electrochemical monitoring modules, which are fast, simple, accurate, reliable and highly selective, are promising for the determination of both substances. Until now, amaranth and carminic acid food determinations have been carried out successfully with electrochemical monitoring techniques in many numbers in the literature (*Anelisa Christ-Ribeiro et al., 2022*).

Voltammetric techniques are the most widely used among these electroanalytical methods. In particular, square wave and differential pulse voltammetric techniques, which have extraordinary properties, have been heavily preferred. Limits of detection (LOD) comparable to the standard analytical method have been achieved using these methods, which have very quick analysis durations, high precision and accuracy, do not require long preprocessing and have great selectivity. In addition, more sensitive and selective analyses of amaranth and carminic acid in natural samples were carried out with numerous indicator electrodes. The merits of powerful electrochemical monitoring studies for the determination of both food colours during the last decade are presented in this study. Moreover, parameters such as analytical applications, detection limits, electrochemical methods, selectivity, working electrodes and working ranges are summarized in detail (*Marzieh et al., 2022*).

In today's world, the stability and affordability of food colourants have caused to attracted considerable attention in the food industry. Food dyes attract the appearance of food by increasing colour of that while the harmful effect of these dyes on living organs is undeniable. Synthetic food colours are becoming more common than natural ones via food manufacturers to achieve specific features like high colour intensity, low cost, improved appearance, more colour uniformity and

stability. Varied beverages and foods obtainable in the market might contain synthetic colour, which in turn leads to serious health issues such as cancers, mutations, allergic reactions and reduced hemoglobin concentrations. Thereby, WHO (World Health Organization) highlight the required control of food dyes in food. Up to now, several analytical methods have been developed for different food dyes determination in various food matrixes. On the other hand, the performance of conventional detection platforms has been limited due to many limitations including time consuming and lack of sensitivity. Recently, cost-efficiency, sensitivity and reproducibility of electro-analytical and optico-analytical approaches have led to the development of many of them for food dyes quantification. The nanoprobes have demonstrated satisfactory results in terms of sensitivity and cost. New kinds of nanoprobe consisting of carbon-based, silica-based and metallic-based composites with nanoscale size might open up new opportunities towards the investigation of colourants in food samples by developing a sensor with better analytical performance. Therefore, an attempt has been made to summarize the recent progress of electrochemical and optical sensors in diverse matrice show how nanoprobes could increase the performance of these approaches (*Sulieman et al., 2023*).

To date, many techniques have been developed for determination of SY in different food samples, such as chromatography, capillary electrophoresis, electrochemical method, immunoassay, spectrophotometric method, Fluorometric method and so on. Though there are some merits in these traditional instrumental methods, such as excellent selectivity and high sensitivity, these methods still suffer from the use of expensive instruments, time-consuming, complicated operations and the demand for technical and professional personnel, making them not to be suitable for real-time monitoring food quality. Among these, fluorescent approaches have attracted great attentions on account of simplicity, cost-effectiveness and rapid response. With the rapid developments in nanotechnology, a variety of nanomaterials with outstanding features have been constructed lots of nanosensors. Over the past years, some nanomaterials like silicon nanoparticlesand carbon quantum dotshave been demonstrated for fluorescent determination of SY with great improvement in detection performance. The fabricated F-AC was utilized for ratiometric fluorescence determination of sunset yellow with good sensitivity by virtue of the ratiometric fluorescence varies at 535 nm and 490 nm and a linear relationship in the range of 0–30 μM was obtained with a LOD of 0.195 μM. Furthermore, the F-AC was utilized for anti-counterfeiting and fabricated as fluorescent gel and membrane, revealing the promising optical applications of F-AC (*Yongming et al., 2023*).

The development of an electrochemical sensor for the simultaneous determination of ponceau 4R (P4R) and tartrazine (TR) dyes in non-alcoholic beverages. The sensor is based on the sequential

modification of a glassy carbon (GC) electrode with WS_2 and diamond nanoparticles (DNP). First, we have performed a computational study to select, among several eco-friendly solvents, the most adequate for exfoliating the 2D nanomaterial from bulk WS_2. From this study, four solvents (triacetin, triethyl citrate, dimethylisosorbide and ethanol/water) were preselected for obtaining WS_2 dispersions that were characterized by scanning electron microscopy (SEM) and atomic force microscopy (AFM) techniques. The sensors obtained with these WS_2 dispersions and DNP were tested for the determination of both dyes, finding the best results for ethanol/water. We have verified that the presence of both nanomaterials gives rise to an enhancement in the response with respect to both the bare GC and the sensor containing only one of them. The response consists of a pair of peaks corresponding to P4R (+0.69 V) and TR (+0.96 V) and a peak at around +0.2 V, coming from both dyes. After optimizing the initial potential in differential pulse voltammetric (DPV) measurements, we found that when −0.30 V is applied, the peak at +0.2 V is only due to P4R. This lower potential value is more adequate than that at +0.69 V to perform the determination of P4R. As a result of the capability to determine P4R at a low potential together with the synergistic effect between both nanomaterials, which leads to improved sensitivities, our methodology allows the simultaneous determination of both dyes with good analytical properties (*Elias et al., 2020*).

Herein, a multi-mode visualization platform was initiated for in-situ detection of food dyes (FDs) by combining colourimetry, fluorometry and smartphone-based digital image analysis, in which water-dispersible quantum dots (QDs) were served as nanoprobes. Colourimetry was achieved by colour comparison, while both fluorometry and fluorescence quantification were performed through inner filter effect (IFE)-induced fluorescence quenching, then colour information (RGB & gray-scale values) of colourimetry and fluorometry was picked by a smartphone to reconstruct digitized alignments. Since IFE mechanism was concentration-dependent but did not rely on the interaction between fluorophore and quencher, the whole process of fluorescence response could be finished within 10 s and both colour gradients and fluorescence changes showed fine mappings to FDs concentrations in the range of 1.0×10^{-3}∼0.035 mg/mL for brilliant blue and 1.0×10^{-4}∼0.1 mg/mL for Allura red and sunset yellow. As a proof-of-concept, the in-situ multi-mode visualization of these FDs in real beverages was experimentally proved to be highly feasible and reliable as compared with instrumental techniques like UV–vis/fluorescence spectrometry, along with HPLC. Finally, this strategy was extended to the multi-mode visualization of non-food dyes in three simulated wastewater samples with high credibility by contrast with the true additive amounts of model dyes *(Shuangshou Wang et al., 2023).*

Food dyes (FDs) have been widely used in the food industry as a functional category of food additives ascribe to some clear advantages like powerful oxidation resistance, fine colouring uniformity and inexpensiveness. However, there are mounting evidences disclose that the excessive consumption of FDs can cause various issues concerned with human health (*SuarezTorres et al., 2021*). From a global vantage point, to remould the appearance of foodstuffs and make them more visually aesthetic to consumers, FDs are still worldwide used as a strategic marketing tool. As a result, environmental pollution, especially water pollution, caused by food industrial dyes is increasingly serious (*Sridhar et al., 2022*) and the global food scandals caused by the abuse of FDs have also never stopped, such as Sudan Ⅰ contaminated chili products in 2003 (*Patra et al., 2017*), malachite green dyed seafoods in 2005, etc., thus many once-favored dyes like Sudan series and Rhodamine B have been banned as food seasonings in many countries and also been categorized as class 3 carcinogens by the International Agency for Research on Cancer (IARC). Admittedly, FDs-related food safety is currently a worldwide concern and endeavors to facilitate the advances of FDs analysis technics for food safety and quality control are very meaningful *(Lim et al., 2020*).

Up to now, instrumental techniques, including spectrometry (*Yamjala et al., 2016*), electrochemical detection (*Cheng et al., 2015*), capillary electrophoresis (*Feng et al., 2021*), high performance liquid chromatography (HPLC) (*Palianskikh et al., 2022*), along with liquid chromatography-mass spectrometry (LC-MS & LC-MS/MS), are still the backbone forces for the detection of FDs thanks to their ionization properties and strong light absorption. Regrettably, these approaches are highly relied on bulky instruments and skilled personnel, leading to enhanced instrumentation cost, time consumption and technical threshold. It is also for these reasons that such techniques are not suitable for portable point-of-care tests (POCTs) of FDs, impeding their practicability as handy tools for FDs supervision. Thus it is of great value to design and construct sample-to-answer platform for instrument-free rapid detection of FDs (*Arrizabalaga et al., 2021*).

Colourimetry is one of practical strategies to fulfill this goal due to some unique merits such as easy to operation, fine portability, intuitive readout and low cost, making it an ideal candidate for field tests ranged across environment monitoring (*Zhang et al., 2022*), medical diagnosis (*Alafeef et al., 2021*) and food safety. Nevertheless, in literatures, most of the existing colourimetric methods were designed by single-signal manner, e.g., test strips, fluorometric nanoprobes (*Huang et al., 2019*), colourimetric pipette tips, transparent polymer optode, etc. In contrast, multi-signal colourimetry can enhance the accuracy and reliability of detection systems by interactive verification

of different detection channels, but it is still very challenging, especially the multi-mode colourimetry with an excellent versatility for various FDs has not been reported yet (*Dudkina et al., 2019*).

The abundance of unsaturated structures containing azo group, aromatic ring and carbon-carbon/-oxygen/-nitrogen double bonds endows FDs with strong absorption in UV–vis light region, while most of them are fluorescence inactive, making them superb fluorescence quencher by virtue of inner filter effect (IFE) (*Pan et al., 2018*) in which fluorescence quenching can be achieved by the absorption of FDs toward excitation or emission light of fluorescence reporter while no need direct interaction between them. Since the IFE-induced fluorescence quenching is concentration-dependent, (semi)quantitative detection system can be established by right of the stoichiometric relationship between fluorometric/fluorescence signals and FDs concentrations. Under this background, herein, a versatile dual-mode (colourimetric & fluorometric) visualization strategy was initiated for semiquantitative POCTs and IFE-based 'turn-off' fluorescence quantification of FDs using water-dispersible CdTe QDs as fluorophores. The schematic was shown in Fig. 1, carboxyl group-capped CdTe QDs were firstly synthesized via one-step solvothermal method. Attributed to their excellent hydrophilicity and fine-controllable fluorescence emission, the as synthesized QDs could be used as eligible nanoprobes with stable and tailored fluorescence and the fluorescence quenching occurred immediately upon the addition of FDs due to their absorption toward excitation/emission of QDs, accompanied by dose-dependent changes of apparent colours and fluorescence. Therefore, a dual-mode visualization platform was built for facile and reliable detection of FDs (*Wang et al., 2022*).

Synthetic colourants added during food processing not only fail to provide nutrients, but also can be harmful to human health when used in excess. To establish a simple, convenient, rapid and low-cost surface-enhanced Raman spectroscopy (SERS) detection method for colourants, an active surface-enhanced substrate of colloidal gold nanoparticles (AuNPs) was prepared in this study. The density functional theory (DFT) method of B3LYP with 6-31G(d) was applied to determine the theoretical Raman spectra of erythrosine, basic orange 2, 21 and 22 and to attribute their characteristic spectral peaks. The SERS spectra of the four colourants were pre-processed using local least squares (LLS) and morphological weighted penalized least squares (MWPLS) and multiple linear regression (MLR) models were established to quantify the four colourants in beverages. The results showed that the prepared AuNPs with a particle size of about 50 nm were reproducible and stable, with a good enhancement of the SERS spectrum of rhodamine 6G at 10^{-8} mol L^{-1}. The theoretical Raman frequencies were in good agreement with the experimental Raman frequencies and the peak position differences of the main characteristic peaks of the four colourants were within 20

cm^{-1}. The MLR calibration models for the concentrations of the four colourants showed relative errors of prediction (REP) of 2.97–8.96%, root mean square errors of prediction (RMSEP) of 0.03–0.94, R^2 of 0.973–0.999 and limits of detection of 0.06 μg mL^{-1}. The present method could be used to quantify erythrosine, basic orange 2, 21 and 22, revealing its wide range of applications in food safety (*Mingyan Cao et al., 2023*).

Aqueous two-phase systems (ATPSs) containing a cationic and anionic surfactants mixture were used for the preconcentration of the synthetic food dyes Allura Red AC, Azorubine, Sunset Yellow, Tartrazine and Fast Green FCF. A rapid, simple, low cost, affordable and environmentally friendly methodology based on microextraction in ATPSs, followed by spectrophotometric/colourimetric determination of the dyes, is proposed. The ATPSs are formed in mixtures of benzethonium chloride (BztCl) and sodium N-lauroylsarcosinate (NaLS) or sodium dihexylsulfosuccinate (NaDHSS) under the molar ratio close to equimolar at the total surfactant concentration of 0.01–0.20 M. The density, viscosity, polarity and water content in the surfactant-rich phases at an equimolar ratio BztCl:NaA were determined. The effects of pH, total surfactant concentration, dye concentration and time of extraction/centrifugation were investigated and the optimum conditions for the quantitative extraction of dyes were established. The smartphone-based colourimetric determination was employed directly in the extract without separating the aqueous phase. The analytical performance (calibration linearity, precision, limits of detection and quantification, reproducibility and preconcentration factor) and comparison of the spectrophotometric and smartphone-based colourimetric determination of dyes were evaluated. The method was applied to the determination of dyes in food samples and food-processing industrial wastewater (*Svetlana et al, 2023*).