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Review Article

A COMPREHENSIVE REVIEW OF VARIOUS ANALYTICAL METHOD AVAILABLE FOR THE DETERMINATION OF FOOD COLOURS CONSIDERING DIFFERENT REGULATORY ASPECTS

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ABSTRACT

Food additives have been an integral part since ancient times in the preservation and enhancement of food. Ancient civilization had evolved from simple preservation techniques to sophisticated synthetic compounds that transformed over thousands of years. This review article focuses on various food additives such as: acidity regulators, emulsifying and stabilizing agents, antioxidants, colorants, flavouring agents, leavening agents, bulking agents, and preservatives. Particular emphasis is placed on the colorants in food and divided it into three main categories: natural, nature-identical, and synthetic. Food colours have a different regulatory framework across all the countries. However, few differences have been seen among FDA of United States, EFSA of European Union, and FSSAI of India. India has approved only nine synthetic colour additives while the FDA allows 19, EU allows food colours of 43 altogether-natural and synthetic. The analytical techniques employed for the analysis of food colours are validated HPLC, UV-Visible spectrophotometry, and Mass spectrometry methods. Coupled Ultra-High-Performance Liquid Chromatography (UHPLC) with tandem mass spectrometry (MS/MS) techniques have led to highly sensitive methods for determination of food colours in different food matrices. For this reason, the understanding of such analytical methods has ensured compliance toward regulatory limits; Inturn, food safety standards within the global food chain supply.

Keywords: Colorants, FSSAI, HPLC, Mass spectrometry, UHPLC, UV-Vis Spectrophotometry

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INTRODUCTION

Food system evolved from hunter-gatherer society to agricultural revolution between 2.5 million years ago and 10,000 BCE, transforming human societies forever. Domestication of plants and animals, in turn, led to the period of Neolithic times. Early foods were preserved through drying, fermentation, and salting. The Egyptians (3000 BCE) implemented organized food production and storage; the Romans perfected preservation, many times using saltpeter. Then, during the turn of the 18th century, processing and preservation techniques ushered in the food systems of today [1]. Evolution of food regulatory bodies and their development includes early regulation era (1906-1908) where, pure food and drug act got established for modern food safety [2], creation of Food and Drug Administration's (FDA) predecessor agency marked systemic food regulation, in 1950-1960's international framework was developed, In 1970-2000's, modern regulatory structure was developed such as European Food Safety Authority (EFSA) and Food Safety and Standards Authority of India (FSSAI), In 2000's EFSA establishment transformed European food safety approach [3]. The food colorants were classified into certified and non-certified or exempted from certification. From the perspective of FDA, it was classified as natural or synthetic colourants [4]. Typically, the natural pigments were derived from the natural sources but, main drawback of this natural colouring was its limitations. For e. g., Naturally derived colouring agents are highly expensive than synthetic colourants and it requires a huge quantity of raw materials for the production of natural pigments, they are more sensitive to heat and light and oxygen etc., [5,6]. Therefore, the chances of replacing of synthetic dyes with natural pigments are very less. The current regulatory framework requires evidence that the colour additive is safe at its intended level of use before it is incorporated to the food materials. Each countries have their own regulatory department such as FDA for United States and EFSA for European countries and their belts and FSSAI for India to monitor and control over safety and permissible limits of synthetic food colorants which was added to the foods.

Introduction about food additives

Food additives can be deliberately incorporated in foodstuffs for serving different technological purposes which includes preservation, enhancement of colour, flavour, texture, and appearance. These can be obtained from natural sources or synthesized artificially. The above categories include different kinds of food additives; Emulsifying, glazing and bulking agents, colourants, flavour enhancers, acidity regulators, antioxidants, sweeteners, glazing emulsifiers and preservatives etc., Despite the safety that emanates from the FDA and EFSA, food additives are currently faced with more and significant critique concerning their long-term impact on human health. More and more studies indicate a link between some food colorants and hyperactivity in kids [7], and some possible carcinogenic effects due to consumption of food preservatives. Generally, the regulatory authorities are expected to consider food additives safe by just following empirical evidence. For every additive, an acceptable daily intake is established to ensure the safety of consumers. Food additives play a critical role in modern food processing for increased shelf life, improved food safety, and sensory characters [8, 9].

Importance of food colours

Food colorants are among the most fundamental ingredients in the food industry. They do have empirical evidence to validate their relevance to consumer behaviour, where pleasing colours enhance palatability and influence much food choice [10], at the same time, they change the appearance and richness of taste [11], They are also applied for the recognition of products and brands [12], and therefore, can fill the lost colour due to processing when trying to satisfy the expectations of consumers [13], some of the specific natural food colorants like carotenoids and anthocyanins may possibly offer health benefits due to their antioxidant activities [14]. Colours in food further add to food safety. They aid in identification of spoilage or contamination by consumers [15].

Pros and cons-incorporation of food colorants

Scientific evidence shows that the food colorants have both advantages and disadvantages. The advantages include the following: The additives enhance the sensory attraction of products hence improving consumer acceptance and satisfaction. According to Spence (2015), colour influences the perception of flavour, which then impacts food choice [10]. Food colorants may also play a critical role in restoring the colour of products that lose colour during processing and thus meeting consumer's expectations [12]. They are also quality indicators. For instance, the presence of titanium dioxide in milk products may indicate the levels of fat. However, food colouring has been associated with a couple of adverse side effects associated with it. Certain synthetic food colours have been known to cause adverse health effects, but this appears to be more pronounced in paediatrics. McCann *et al.* [7] have indicated that there is an association between the consumption of some artificial colorants and elevated levels of hyperactivity among children. Some synthetic colorants raise questions concerning the matter of safety, whereas in some instances, the time scale is longer like Red 3, proved through animal experiments to induce thyroid tumours. Conversely, food colorants are perceived as safer, but all these present problems concerning stability and uniformity in the matrix of foods composed of different elements [17]. Moreover, consumers seeking "clean label" products would react adversely to the application of food colours, which would also be detrimental to product marketability [18]. Thus, proper equal balancing of the factors involved in food product development and regulation is very important.

Reported banned food colorants

It is normally believed that consumption of natural colorants within a food matrix poses no harmful effects, but this assessment does not hold for synthetic colorants. Thus, it becomes important to determine the amount of synthetic colorants existing in food stuff.

Acceptable Daily Intake: Acceptable daily intake means the total daily dietary consumption level which is thought to pose no risk to human health [19]. Synthetic food which has high concentrations of colorants FD and C Blue 1, Blue 2, Green 3, and Orange B were eliminated in faeces with low intestinal absorption upon oral application to rats, however some food dyes, such as Citrus Red 2, FD and C Red 40, FD and C Yellow 5 (Tartrazine), Yellow 6 (Sunset Yellow) are metabolized by gut microbiota into metabolites, which might be absorbed, thereby leading to human health issues. Most synthetic food colorants were banned in developed countries, but the scenario is entirely different in India, where these colorants are used on the regular basis as an food ingredient for example, azo dyes [20]. Azo dyes have one or more azo groups (–N=N–) in their chemical structures. The most commonly used additive is tartrazine, which features toxic properties. It has been found to be toxic to the Liver function, renal performance, lipid profiles, and behaviour [21]. The metabolism of the azo-dyes is decreased by substitutions in the aromatic ring-sulphonation or carboxylation and such substitutions may reduce genotoxicity as their mechanism of action through decrease in lipid solubility of the dye, consequently of its absorption [22]. At times unscrupulous individuals were adding industrial dyes deceitfully to a vast selection of foods to make it more valuable (for example, colorants act as an quality indicators for the products) or else to cover up the lower quality. Such dyes are readily available, and manufacturing is relatively inexpensive [23].

Scientific studies authenticate the fact that food is the source of energy and is a complex matrix of bioactive compounds that provide needed health and wellbeing to the human being. Modern sciences of nutrition have proved the fact that components in diet, from macro to micronutrients, play vital roles in cellular functions and the regulation of metabolism that prevent diseases. The development of food technology and the development of food additives have been considered essential for preservation of food, make it safer for consumption, and enhance the quality of food. By peer-reviewed studies, it has been found that proper controlled food additives, like colorants, play a crucial role far beyond their apparent functions. Scientific studies in Food Chemistry and Food and Chemical Toxicology journals have shown that colorants in foods play a role in product identification, standardization, and protection against light-sensitive nutrients. Scientists are constantly testing the synthetic and natural colorants using highly advanced analytical methodologies and in-depth safety tests. Currently, research studies are under way on synthetic colorants' natural alternative, safe, and eco-friendly colorants. Recently, there have been various new studies on novel pigments made from natural ones and their stability in food systems. This direction in food science and technology is focused to demonstrate how safe and useful food products meet consumer demand in more natural ingredients, and also increasingly demanding consumers. These findings, coupled with centuries of scientific investigation and regulation, make continued exploration into food components and their potential therapeutic values, as well as the safety of additives, of prime importance. The future of food science will depend on how scientific discoveries are channelled toward the creating more safer, more nutritious, and more sustainable food products that would meet consumer demands and stand up to regulatory requirements.

Types of colorants available in the market

• Synthetic food colorants

These type of colour additives prepared artificially and applied enormously in the food industry as the ingredients are used to increase the visual acceptability. Synthetic food dyes used in food items based on FDA and The EFSA regulation with Acceptable Daily Intake (ADI) with specified dose regulations are presented in the subsequent paragraph [24].

• Natural food colorants

Natural food colours are agents developed from natural origins such as plants, animals, and other organic matter. These are safer to apply on foodstuffs since they do not contain any toxic effect that can harm human health [20]. They produce extensive spectrums of spectrum colours that are added to food products and beverages. Such colours exist in all possible forms, such as liquids, powder gels, and pastes [25].

Regulatory bodies and their approved food colours

USFDA approved food colours

United States Food and Drug Administration approves certain food colours which are used in many processed foods such as snacks, beverages, candies, ice creams, sauces, confectioneries and jellies. Food colours accepted by FDA are; Food Drug and Cosmetics-blue No: 1 and 2; Green No: 3; Red No: 3 and 40; Yellow No: 5 and 6, orange B, annatto extract, cochineal or carmine extract, β-carotene, grape extract, caramel colour, Citrus Red No.2, paprika oleoresin, Saffron, fruits and veggies juice extract [26].

EFSA approved food colours

EFSA (European Food Safety Authority-EU) impose strict regulative restrictions on food colours, which it divides into two basic types: natural as well as synthetic types (E 100 curcumin), (E 101 Riboflavin), (E 102 Tartrazine), (E 104 quinoline yellow), (E 110 sunset yellow FCF) (E 120 carminic acid, carmine), (E 122 Azorubine, Carmoisine) (E 123 amaranth), (E 124 ponceau 4R), (E 127 erythrosine), (E 129 Allura red AC), (E 131 patent blue V), (E 132 Indigotine, indigo carmine), (E 133 brilliant blue FCF), (E 141 Copper complexes of chlorophylls and chlorophyllins, (E 142 green S), (E 151 Brilliant Black PN), (E 155 Brown HT) (E 163 Anthocyanins), (E 180 litholrubine BK) [27].

FSSAI approved food colours

The Food Safety and Standards Authority of India, being the main regulatory body of food color standards in the country, supervises these food colours. This organization classifies food colours into two main categories: natural and synthetic, with the establishment of strict regulations concerning their use, purity, and safety.

Various application of food colours

Food colours give food an added visual appearance that makes them more alluring to the consumer and there are so much uses of food colours which can be imparted on different food stuffs are mentioned below.

Bakery stuffs

Breads, Biscuits, Muffins, Donuts, croissants, sweet pies and cookies etc., [29].

Soft drinks and beverages

Cola beverages, Clear carbonated beverages, Fizzy drinks, Fruit flavored carbonated drinks, Caffeinated energy drinks etc., [30].

Table 1: FSSAI food colours with limits approved food colorants-FSSAI

Natural	Limits
Chlorophyll	No Specific Limits are provided for the usage of Natural Food Colours.
Curcumin or Turmeric	
Canthaxanthin	
Annatto	
β Carotene	
Riboflavin	
β Apo – 8 Carotenol	
Methyl Esters of β Apo-8 Carotenic acid	
Lactoflavin	
Ethyl esters of β Apo-8 Carotenic acid	
Caramel	
Saffron	
Synthetic	Limits [28]
Carmoisine	100 mg/Kg
Erythrosine	
Ponceau 4R	
Tartrazine	
Brilliant Blue FCF	
Sunset Yellow FCF	
Indigo Carmine	
Fast Green FCF	

Confectioneries

Hard candies, Chewy and soft candies, nougats and similars, Chocolate Confections, Traditional/Regional confection [31].

Cosmetic products

Lake colours are used extensively in nail polishes, facial creams, eye liners, moisturizer, lotions, gels and mostly in lipsticks [32].

Dairy products

Dairy products like ice creams and frozen desserts are also colored to make them rich, smooth, and more flavorful, for example, in milk and flavored beverages [33].

Animal-based products

This category includes meat, fish, and poultry. They are preserved in frozen state after being colored red and then packed [34].

Available analytical methods for the estimation of natural and synthetic food colours

Various Analytical techniques are developed, which include spectrophotometry and other chromatographic methods such as thin-layer chromatography, high-performance liquid chromatography, Enzyme-Linked Immunosorbent Assay, and capillary electrophoresis techniques. Hyphenated techniques, for instance LC-MS/MS are examples of the intricacy involved in food safety analysis.

Table 2: Spectrophotometric methods for the estimation of food colours

Spectrophotometric methods				
Food matrix	**Food color**	**Analytical instrument technique**	**Detection**	**References**
-	Azorubin S, Acid Red 18, Food yellow 3, Acid Yellow 23, Acid Blue 9	UV-Visible Spectrophotometric kinetic method	UV-Visible λmax of prussian blue: 760 nm	[35]
Solid food products and Beverages	Food Red 17, Food Yellow 3, Acid Yellow 23	Spectrophotometric method (Bilinear Least Squares/Residual Bilinearization)	λmax (300-600 nm) at different pH	[36]
Solid food products	Tartrazine, Quinoline, Sunset Yellow, Brilliant blue and Carmoisine	Spectrophotometric method	UV-Visible Multiple Wavelength	[37]
Liquid food products	Allura Red AC Dye	Spectrophotometric method	UV-Visible λmax: 506 nm	[38]
Granulated Liquid Drinks	Azoribin and Allura Red AC Dye	Spectrophotometric method	UV-Visible ZCDS method	[39]
Powder	Acid Yellow 23, Acid Blue 9, Food yellow 3, Acid red 18	SERS-Raman	Raman Spectroscopy with confocal microscope	[40]
Liquid (Beverages and Alcoholic Drinks) Fish foe	Allura Red AC, Azorubin S, Erythrosine, Acid Red 18, Food Yellow 3	SERS-Raman	514.5 nm form air cooled argon ion lasers were used for SERS Excitation	[41]

Current trends and future perspective

Today, food colour evaluation is very advanced by using highly advanced scientific approaches and newly developed technologies. In this respect, spectroscopic techniques like UV-Vis and Near-Infrared spectroscopy have become powerful tools in the accurate assessment of colours, which enables a high level of precision to researchers and professionals in food science. Instrumental tools such as colorimeters and spectrophotometers provide profound measurements of colours based on predefined colour spaces, thus effectively assessing the food products through all phases of production and processing. It was clear that chromatographic techniques, such as HPLC and LC-MS techniques, Involves in meaningful identification and quantification of a specific pigment molecule, much better understanding of their composition on colours and possible differences. Much beyond simple assessment of colour content, they provide molecular-level information for pigment characterization, which can prove invaluable information for research activities as well as quality control. With the expectation of future development, artificial intelligence and machine learning will greatly change the analysis of food colour. New AI algorithms are expected to enhance colour prediction, develop more advanced assessment methodologies, and make real-time quality control measures possible [72-74].

Interlaboratory validation ensures method reproducibility, reference standards with adequate details, and continued development of methods by collaborative studies will continue to be important in scientific merit of novel colour analysis techniques. In the advancement of technology, the ultimate goal is to quantitate colour, but more so to fully appreciate it's scientific and sensory meaning. The future landscape of food colour analysis is going to become an exciting convergence of advanced technologies, molecular science, and sensory perception that will unfold unparalleled opportunities for research, quality control, and innovation in the food industry.

Table 3: Chromatographic methods available for the estimation of food colours chromatographic methods

Food matrix	Food colour	Analytical instrument technique	Detection	Stationary phase	Mobile phase	Injection volume	References
Wines and Soft Drinks	Acid blue 9, Acid Yellow 23, Azorubine, Food Yellow 3	TLC with UV detector	UV-Vis	TLC-PET 20×20 cm Silica gel	8 ml of 2-C_3H_8O and 3 ml NH_4OH	5µl (Std) and 30µl (Sample)	[42]
Liquid Foods (Beverage) Dairy Powder, confectionery	Acid Yellow 23, Quinoline, Food Yellow 3, Acid Red14, Azorubine, Acid Red 18, Food Red 17, Patent Bue V, Indigo Carmine, Acid blue 9, Green S	HPLC (Gradient)	DAD	Discovery Reverse Phased C18 Column With measurement of (250 mm× 4.6 mm, 5 µm)	Ammonium acetate 0.13 (pH: 7.5; sodium hydroxide/MeOH: ACN (80:20 v/v)	20µl	[43]
Cool and fizzy drinks	Food red 17, Food yellow 3, Acid yellow 23	HPLC (Gradient)	DAD	-	Methanol and Buffer: pH 7 – 0.1M sodium dihydrogen phosphate	-	[44]
Solid food products and liquid foods	Amaranth, Ponceau 4R Food red 17, Acid Red 103, 101, 26, 41, Acid yellow 23, orange II, Acid yellow 36, Acid blue 1, Indigo carmoisine, acid blue 9.	HPLC with gradient elution	DAD λ_{quant} (620 nm – Blue) (515 nm-Red) (420 and 480 nm – Yellow)	C8 Column with measurement (150 mm× 4.6 mm,3µm)	ACN/Sodium Acetate of pH: 7	20µl	[45]
Beverages and liquid foods	Sunset Yellow	HPLC with gradient elution	-ve potential Electrospray ionization technique coupled with mass spectrometry	C-18 Ether Column with measurement (150 mm×4.6 mm, 5µm	Aq solution: 63%, 20 mmol Ammonim acetate 37% MeOH	20µl	[46]
Multiple range of food	Acid Blue 9, Acid yellow 23, Food red 17, Azorubine, Acid blue 1	HPLC with gradient elution	DAD	Xteravv Reverse-phased C-18 column with measurement of 250 mm× 4.6 mm, 5µm	0.1M CH_3COONH_4 in water	20µl	[47]
Liquid beverages	Acid Red 14, food yellow 3	HPLC with isocratic elution	-ve potential Electrospray ionization technique coupled with mass spectrometry	Reverse phase C-18 Column with measurement of (250 mm×2 mm, 4µm)	MeOH 10 mmol $HCOONH_4$ (45:55 v/v)	20µl	[48]
Beverages	Food red 17	HPLC with gradient elution	-ve potential Electrospray ionization technique coupled with mass spectrometry	High-strength silica – T3 column (2.1× 100 mm, 1.8 µm)	Water: Ammonium acetate.1.0 mmol/MeOH: Ammonium acetate 1.0 mmol	20µl	[49, 50]
Wider range of food and Pharmaceutical Formulation	Acid Yellow 23, Solvent yellow 33, Food yellow 3, Acid red 14,18, Food red 17, Indigo carmine and Acid Blue 9	HPLC Elution: Isocratic	DAD Multiple wavelength	Reverse Phase C18 Column (250 mm× 4.6 mm,5µm)	Triton x-100 (0.25% v/v) 50 mmol/l PBS of pH 7	20µl	[51]
Soft or fizzy drinks	Food Yellow 3, Acid red 14, Azorubine, Acid red 18, Erythrosine Red 2G, Food red 17.	HPLC with gradient elution	Diode array detector with multiple wavelength	Symmetry C18 column with measurement of (150 mm×4.6 mm,5µm)	(1% w/v) (0.13M) (pH: 7.5) Ammonium acetate buffer by adding of 0.1M aq. Ammonia (Solvent A), Ethanol (Solvent B) can (Solvent C)	-	[52]
Proteinaceous Sample	Acid blue 9, indigo carmine, Food red 17, Erythrosine, Acid red18, Food yellow 18, Lemon yellow	HPLC with gradient elution	Diode array detector with various wavelength	Reverse Phased-C 18 column	MeOH 20 mmol of Ammonium acetate	20µl	[53]
Fish egg masses	Acid yellow 23, Food Yellow 3, Amaranth, Cochineal red 2G AC, Food Black Chocolate brown HT, Acid blue 1	HPLC with gradient elution	Diode array detector with multiple wavelength	Xterra Reverse phase C 18 Column with measurement of (250 mm× 4.6 mm, 5µm)	100 mmol/l Sodium acetate buffer with pH of 7.0 ACN	20µl	[54]
Cool drinks and Preserved meats	Acid blue 9, Food red 17, Food yellow 3, Acid yellow 23, Erythrosine, Acid red 18	HPLC with gradient elution	Diode array detector with multiple wavelength	Inerstil ODS-SP Column with measurement of (250 mm× 4.6 mm, 5µm)	Ammonium acetate (0.1M, pH: 7.2) Methanol: can (9:1 v/v)	20µl	[55]
Different foodstuffs	Acid Yellow 23, Azorubine, Leutin, lycopene, carotene	HPLC with gradient elution	Diode array detector with various wavelength	C 18 column with measurement of (250 mm × 4.6 mm, 5µm)	1% ammonium acetate, MeOH and C_3H_6O	20µl	[56]
Sweets and confectioneries	Acid blue 9, Food yellow 3, Acid yellow 23	HPLC with gradient elution	UV-Visible detector with a (λ max: 630 nm,480 nm, 430 nm)	Mod C 18 Column with the measurement of (250 mm× 4.6 mm,5µm) with 0.25% (v/v) Triton X 100 aq. Soln at pH 7	0.25 ml of TritonX-100 (SIGMA) upto100 ml with 50 mmol 1-1 PBS at pH 7	20µl	[57]
Confectioneries and beverages	Acid yellow 3, Azorubine, Food yellow 3, Food red 17, ponceau 4R, Eythrosine	HPLC Elution: Gradient	UV-Visible Detector with λ max: 430 nm, 510 nm.	Column: C18 (250 mm× 4.6 mm. 5µm	0.1 mmol/l Ammonium acetate (pH 7.5) adjusted with 10 mmol/l NaoH, MeOH: AcN(30:70 v/v)	20µl	[58]
Gummies and liquid-based drinks	Variant Class of 34 Water-soluble colours	HPLC with gradient elution	Diode array Detector-IT-MS/λ max: (200 to 700 nm)	Atlantis D C-18 (4.6 mm × 250 mm, 5 µm	20 mmol $HCOONH_4$ Buffer: MeOH: ACN (1:1 v/v)	20 µl	[59]
Animal-based products (Meat)	Acid blue 9, Acid yellow 23, Food yellow 3, Azorubine, Sun red no.1 Acid red, Food red 17.	UPLC with gradient elution	PDA with Multiple Wavelength	C18 Column (4.6 mm× 2.1 mm,1.7 µm)	ACN/Ammonium Acetate	5µl	[60]

Food matrix	Food colour	Analytical instrument technique	Detection	Stationary phase	Mobile phase	Injection volume	References
Animal based products (Meat)	Tartrazine, New red, Azorubine, Acid Red 18, Food Yellow 3, Acid Blue 9, Erythrosine, Acid Orange, Basic Flavin O, Siperse blue 106, Basic Violet 3, leucine.	UPLC Elution: Gradient	Diode Array Detector (λ max: 200-800 nm)	Column: C18 (2.1 mm × 50 mm, 1.7 µm	20 mmol CH_3COONH_4-0.02% Acetic acid (pH: 5). ACN	2µl	[61]
Spices	53 Variant Classes of food colorants	UPLC	Quadrapole-Time of flight hypernation	AQUITY UPLC BEH C18 Column (2.1 mm×150 mm, 5 µm)	ACN and 10 mmol CH_3COONH_4: pH: 7	-	[62]
Animal fodder and meat	Acid Red 18, Food yellow 3, Food red 17, Azophloxine, Ponceuxylidine, erythrosine, orange II	LC with gradient elution	Electrospray ionization – Mass spectrometry	C18 Column (2.1 mm× 150 mm, 5µm	20 mmol/l CH_3COONH_4: ACN	-	[63]
Chilli powders, frozen and pereseved foods, syrups	New cocaine, Indigo carmine, Erythrosine, Acid yellow 3, Food yellow 3, Food green 3, acid blue 9, Food red 17, Azorubine, Dimethyl yellow, Solvent yellow 3, Para red, Sudan I, II, III, IV, Orange G, Sudan Red, Sudan Red B and G	LC with gradient elution	-Ve and-Ve potential electrospray ionization-triple quadrapole mass spectropmetry	Acclaim C16 Column (3 mm,4.6×150 mm)	ACN and 20 mmol CH_3COOH	-	[64]

Table 4: Other analytical techniques available for the estimation of food colours

Other analytical techniques							
Source	**Food color**	**Analytical technique**	**Detection**	**Stationary phase**	**Mobile phase**	**Injection volume**	**References**
Milk Powders syrups	Acid Blue 9, Indigo Carmine, Food red 17, Sun Red No 1 Acid red 18, Food yellow 3, Acid 23	Capillary electrophoresis	UV detection (200 nm) with 1M sodium Hydroxide, H20 electrode polarity (25 kV)	-	Continuous running buffer with pH: 10 (20 mmol Sodium Hydroxide to 14 mmol Disodium Tetraborate (borax) to 20 mmol NaoH Until the Desired pH occurs	Large volume injection	[65]
Liquid food products	Erioglucine disodium salt	Capillary Electrophoresis	Ultraviolet-Visible (λ max 200 nm, 36 cm capillary; separation voltage (8kv,	Capillary O. D: 375 µm and I. D: 75 µm	10 mmol PBS With pH: 6 with 0.9 mg/ml DSANP's and 2 mmol β-cyclodextrin	Electro kinetic Injection	[66]
Syrup	Erythrosine, Carmoisine, Azorubine, Acid red 18, Red 3G	Capillary Electrophoresis	LIF detection Various λ Excitation/λ emission: (Nd: YAG Laser with λmax of 532 nm and Power: 5mW)	Capillary with fused Silica with I. D. 50 µm O. D. 360µm, length 30 cm	V= 17 kV (intensity of electrical field V cm^{-1}	-	[67]
Liquid Beverages	Acid yellow 23, food yellow 3, Amaranth, Bordeaux S Acid red 18, Erythrosine, Red No. 40, acid blue 1, Indigo carmine, acid blue 9	Capillary Electrophoresis	UV-Vis PBS with SDS 10 mmol, pH: 11 and+25 kV voltage	Capillary with fused silica	PBS of 10 mmol with SDS 10 mmol, pH 11 and+25 voltage	-	[68]
Milk powder and confectioneries	Acid Blue 9, food yellow 3, Acid yellow 23	FIA	Amperometry detection (boron-doped diamond electrode)	-	$E_{det:}$ 450mv 100ms Duration vs Ag/AgCl (3.0M Kcl)	-	[69]
Starch-based confectioneries	Food yellow 3	Enzyme-linked immunosorbent Assay	-	-	-	-	[70]
Beverages and liquid syrups	Acid Blue 9, Acid yellow 23, Azorubine, Food yellow 3	Differential pulse voltammetry	Cathode pretreated boron-doped diamond electrode	-	30Hz; amplitude(a), 40mV; for DPV scan rate (v), 0mVs	-	[71]

CONCLUSION

This review article covers the discipline of food science, which is essentially concerned with the integration of nutrition, health, and technological innovation on various food additives such as: acidity regulators, emulsifying and stabilizing agents, antioxidants, colorants, flavouring agents, leavening agents, bulking agents, and preservatives. Particular emphasis is placed on the colorants in food and divided it into three main categories: natural, nature-identical, and synthetic. Despite the safety that emanates from the FDA and EFSA, food additives are currently faced with more and significant critique concerning their long-term impact on human health. FSSAI, EFSA, and USFDA strictly controls food colour regulation. The main regulatory frameworks continued implementing specific regulations concerning food safety and consumer protection. It follows Food Safety and Standards Act 2006 by FSSAI wherein all colours have been categorized in natural, nature-identical, and synthetic forms with definite maximum permitted levels. EFSA follows E-number system, E100 to E199, under Regulation (EC) No 1333/2008. USFDA, considering the Federal Food, Drug, and Cosmetic Act, classifies colour as certified (synthetic) or exempt (naturally occurring), with lot certification for synthetic colours. Scientific evidence shows the food colorants have advantages and disadvantages. The advantages include that the additives enhance the sensory attraction of products, hence improving consumer acceptance and satisfaction. Natural colorants pose no threat when compared to synthetic colorants. Food dyes, such as Citrus Red 2, FD and C Red 40, FD and C Yellow 5 (Tartrazine), Yellow 6 (Sunset Yellow) can cause human health issues, potential harmful effects on human body and acceptable daily intake is limited. Most synthetic food colorants were banned in developed countries. Various Analytical techniques are developed, such techniques include spectrophotometry and other chromatographic methods such as thin-layer chromatography, high-performance liquid chromatography, Enzyme-Linked Immunosorbent Assay, and capillary electrophoresis techniques. Hyphenated techniques, for instance, LC-MS/MS are examples of the intricacy involved in food safety analysis. Each technique has its strength and weakness. The spectrophotometric methods are simple to carry out and inexpensive while being unspecific for very complex food matrices. These methods have a lot of potential regarding the sensitivity and separations. Despite lower sensitivity compared to others, the possible achievement of quick screening makes field testing through TLC methods practical. More sophisticated hyphenated technology incorporates LC-MS/MS in high sensitivity specificity, while this role might be played by no one else at trace analysis besides it in the confirmation test. Non-destructive quick techniques such as Surface enhanced Raman spectroscopy(SERS) was demonstrated to have the ability to identify wide range of synthetic and natural food colouring agents and it was environment-friendly friendly fast and efficient and has the versatility where it can detect natural carotenoids and synthetic dyes. The recent researches has focused on both approved and prohibited colorants, making it valuable for the regulatory compliance. (AI) Artificial intelligence plays a major role in enhanced detection accuracy for detecting and reducing food contamination and precise analysis, predicting contamination patterns enabling proactive mitigation, which enables immediate detection of harmful and unauthorized food colourants in production lines. (ML) Machine learnings algorithms were widely used to optimize supply chains, which reduces wastes and improves the quality monitoring, where computerized visions are used for colorant detection through image analysis.

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All authors made substantial contributions to this manuscript. Sharan Raj Rajmohan conceptualized and drafted the manuscript. Krishnaveni Nagappan supervised and validated the manuscript. Mohit Ananda contributed to data analysis and manuscript drafting. Aiswarya Mahendran, Balagee Muthukumar and Padma Priya V assisted in data collection and reviewing the collected data.

CONFLICT OF INTERESTS

Declared none

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