

Foods that attract consumers of all age groups are being added with synthetic food colourants that are stable, cost efficient and harmful for human safety. These food colourants are used in more than the limitations prescribed by Food Safety and Standards Authority of India (FSSAI). Relevant, alternatives for these synthetic food colourants are present in natural form. Thus, in this study the natural form of colourants are explored for bringing in a solution for the greatest uprising demand for food colourants in the food industries.

A driving force of global dietary pattern has been characterised by transition of nutrition that has an increased manufacturing and marketing sectors relatively and collectively referred as food industry. Global food industrial system has a domineering impact over the transnational processors from small to large scale food producers, that continue to provide profit for products and brands that creates and facilitates aggressive marketing strategies to regulate the power of marketing globally to process packaged foods that are believed to aid in nutrition transition for the growing demand in present generations' health and safety (*Gray et al., 2020*).

Food industries involve themselves in the provision of funding an employee as a part of research and there might be various reasons for a food based sector to get involved in the research based on nutrition. Eagerness for creative ideas, transfer of knowledge based research, to versatile in contribution of resources and research can all be different goals of a research based food industry. In consideration towards the health of public welfare, increased opportunities for marketing a product has increased harmful ways of popularizing harmful health benefits in establishment of nurturing relationship between research, credibility of the industry, industrial criticism and encouragement of increased dependence on food industry (*Mozaffarian, 2017*).

Industrial influence on agendas that preferably highlight the research subjects that are likely to benefit the core of an industry, rather than focusing on importance of public health (*Fabbri et al., 2018*); industrial influences on the methodology, results and impact of a food based researches that are favourable for industry and other factors for utilization of research for political purposes (*McCambridge et al., 2018*). Universal food processing sectors have gained their strategical

importance from acknowledged funding, which is documented and funded by academicians, also play an increased role in food industries (*Gornall, 2015*).

To ensure health and wellbeing of the people, commercial food industries have become food laboratories operating to equip with the best analytes that emphasize on the nutrition of the processed products. Research based on nutrition have emerging evidence on the progressive increase of consumer awareness among the present population. Eco friendly products have been systematically explored to identify and extent the involvement of research in food techniques to examine the raising needs for pharmacological benefits that utilize organic compounds to develop viable technologies. In such case, food adulteration and food colourings that are listed as natural food grade colours have attracted the consumers' attention to gain sufficient knowledge on the techniques used either conventionally or through application of technology has gained respect for natural colourings. In contrast, researchers have gained interest in extraction and analysis of food grade pigments from natural sources that are extracted with different techniques, gaining support from large food manufacturers in global markets (*Stich, 2016*).

Legal regulation updates to redefine the rationale of food adulterants in permitted amount have increased the desirable dependency in food industries that have their focus especially of processed fast foods. Scientific reports on the usage of synthetic food colourants have paved requisite research funding to understand the recent scenario in framing a solution in consideration with primary health benefits of the consumers. Global perspective in usage of food colourants in every country varies according to the richness of natural sources in concern with their demographic and socio economic availability. Research conducted based on consumer perception in Switzerland had revealed that people prefer natural food colourants and additives based on their regulation factors obtained from both plant and animal sources. Likewise, people of Germany also prefer natural food colourants added to their foods mostly derived from plant sources, but not from animal sources (*Muller Maatsch et al., 2018*).

Yet another study conducted in regions of Ethiopia had revealed that 64 percentage of the country's population adverse to the consequences of food colourants and nearly 70 percentage of the consumers continue to interest themselves in consuming the food additives even after knowing its discerning effects. A large group of discriminative population the country are unaware of the labelling differentiations that specify the synthetic and natural food colourants to attain food safety and quality (*Getasew et al., 2016*).

According to *Velasco et al. (2016)*, colours are scrutinized as prominent visual cue contributing itself as a major concept of food particles. Researches state that colours in foods psychologically implement the impartation of flavour to a food by generating the human brain with the taste of the food without physically tasting the food. “Consumers’ inclination to a particular food item is primarily visual specific to the colour of the food which aids the consumers to predict the flavour and taste of the food. Interestingly, the perception of colour is deep rooted and comes intuitively to human beings. For ages, the colour of fruits, vegetables and meat has remained a determinant factor to distinguish raw from ripe and fresh from old or spoilt”.

The colour to the foods have high impact on the decisions of global food processors to incorporate foods with a varieties of colour additives. The bio food colourants obtained from natural sources like plants and microbes were used to include colour to food which therewith was replaced by synthetic food colourants. Natural colouring agents are of two categories: organic (derived from living sources) and inorganic (derived from metals like gold, silver, etc). Synthetic food colourants are chemically processed from and through coal tar, its compounds and thus, maximum of the particles contain dyes from the azo group (*Dey et al., 2022*).

Health hazards have been repeatedly reported among children influenced by the synthetic food colourants needs to be addressed in this situation, where the colossal usage of permitted and non-permitted food colourants have been detected with toxicities associated with adulterations that are global concerns with varying gravity in different countries. Moreover, naturally available, yet identical to man made colours like riboflavin, also cause demands of food colourants in the global markets (*Sezgin et al., 2018*).

To create health consciousness among people who act negligently concerning their health in order to pace with the criteria of fast-forwarding health eating, the Global organizations that empires the International standard of foods which include Codex Alimentarius Commission (CAC), an Inter-Governmental body founded by FAO and WHO that orient the global food standards of developing countries such as India. Since the industrial revolution, the usage of synthetic food colorants hit the markets globally. In 1906, United States’ first published list of approved food colours to curb the usage of harmful chemical dyes, whereas in 1957, the United Kingdom published legally approved colorants and in the early 1950s, a combined committee under the leadership of Food and Agricultural Organization (FAO) and World Health Organization (WHO) was formed to assess the safety of food additives which also had an inclusion of food colorants (*Lehto et al., 2017*).

On a larger look out for culture based heritage foods, Indian cuisine is well known for its versatility in flavours and uniqueness that depends on the heritage blending combination of spices and herbs. Wherein, the increased demand for processed foods and a competitive food market has paved way for a promotion in the usage of synthetic food colourants in native Indian recipes. The Indian food colour market would achieve a CAGR of 5.3 percentage by 2027, which is highly impacted by synthetic colourants, mostly used in foods. Herewith, India's exports of food colours to the United States, China, Indonesia, Brazil, Mexico and Italy are being increased. The export market sizes have raised from over \$ 203 M to \$ 263 M with a difference of \$ 60 M from 2014 to 2019. The market for natural food colourants is also increasing rapidly and is expected to exceed up to \$ 92.96 M by 2027, growing at a CAGR of nearly four percentage (*EI, 2018*).

Colourants like tartrazine, sunset yellow, quinoline yellow, indigo carmine and amaranth are highly used to richen the visual appeal of Indian food. In India, there are over hundreds of industries manufacturing chemicals as food colours. On the other hand, the natural food colourant market is on the rise due to an increased awareness in health among Indian consumers and reports on the toxic effects of synthetic colorants (*Satyanarayana, 2022*).

Amin et al., (2018) has reported that “several synthetic food colorants banned in developed countries are regularly used as food ingredient in India, example azo dyes. Azo dyes possess one or more azo groups ($-N=N-$) in their chemical structures and are toxic. Tartrazine is one of the widely used azo dyes which is reported to have toxic impacts on the liver, renal function, lipid profiles and behaviour”. The increase in the natural food colour industries is due to an increasing consciousness of serious health effects caused by the colossal use of synthetic colours. Eventhough, the factor restraining the growth of the industry is costlier in natural colorants when compared to synthetic counterparts. Red is the majorly used food colourant under demand, followed by green in the Indian market. In addition to this increasing demand, the COVID-19 pandemic has highly influenced the Indian organic market exhibiting around 40 percentage increased growth in demand. Eventually, consumers asserting natural colourants never understood the differenciation between neither natural nor chemical colourants (*EI, 2018*).

Adulteration is known as addition of non-permitted food colour additives to a food product. Compression of permitted food colours in exceeding levels is also known an adulteration that results in severe health hazards (*Gizaw, 2019*). A worldwide sensation reported by *India Today (2018)* is that “adulterated milk with chalk or diluted water, coffee seeds spiked with tamarind or mustard seeds), ice cream with pepperoni or washing powder etc. are examples of food fraud in order to make

more profit. Synthetic colorants emerged in order to deceive consumers regarding food freshness, and to increase the visual attractiveness of food, e.g. malachite is green in vegetables, metanil yellow in dal, sudan red in red chili powder. Nevertheless, recent strategies are trying to promote the ‘naturalistic fallacy’.

Blending of food colourants with non edible colours have become highly prevalent, exceeding the permissible limitation of the synthetic food colourants that are proved to be used in food products like crushed ice with about eight to 20 percentage higher than their permitted level. Non-permitted colorants are detected to be used in a varieties of food. An exclusive and extensive survey conducted on products available from bakeries, supermarkets, street food shops and fast food both in urban and rural areas of Hyderabad among different age groups of pre-schoolers of 1–5 years and school children of 6–18 years, early adults from 19–44 years and greater than 45 years of age from high, middle and low income groups showed an excessive intake of tartrazine, erythrosine and sunset yellow higher than the permitted limits of 100 ppm (*Nath et al. 2015*).

Extreme usage of non-permitted carcinogenic, neurotoxic colourants are a common practices observed in various parts of India. Most potent carcinogenic dye, melanil yellow is banned by the Government of India, which was found in turmeric, also laddoo and besan used at extensive level in unorganized food sectors of West Bengal lack quality and it is due to ignorance towards food regulations. An analytical study performed to investigate synthetic food colourant usage in different states of India revealed that candyfloss, sugar toys, beverages, mouth fresheners, ice candy and bakery product samples contain colourants exceeding the limitations (*Nandakumar, 2015*).

To analyse the presence of toxic adultere chemical synthetic colourants in foods, nearly 500 samples of food and 62 samples of natural, herbal colours were collected by the Society of Pollution and Environmental Conservation Scientists (SPECS) from places like Dehradun, Vikas Nagar, Sahaspur, Doiwala, Rishikesh, Haridwar, Rajpur, Mussoorie (*Tribune, 2016*). Saffran, also known as the globally demanded spice and food colorant was found to be adulterated with sudan dyes (*Petrakis et al. 2017*) and the food colours anthocyanin and betalain were also adulterated with the textile dye known as ‘Reactive 95’ (*Müller-Maatsch et al., 2016*). Most commonly used spice, turmeric labelled as natural and organic was highly adulterated with lead chromate (*Erasmus et al. 2021*). *Pradeshi, (2019)* also quotes that “many organic labelled foodstuffs like tea powder, jaggery and edible oils were also reported to contain non-permitted colorants”.

Usage of natural food colorants in India is considered healthy within the permitted limits and in permitted foods. Unfortunate cases of adding natural food colorants, in unrestrained manner have been reported in India, for example, to imitate buffalo milk extensive addition of annatto powder is added to cow milk for a yellowish appearance. Labels specifying the product as “natural” may not mean that the pigment used for colouring the food has undergone clinical trials which does not report in being contagious with allergens and carcinogenic substances. Reports of urticaria, angioedema, hypotension, anaphylaxis in hypersensitive individuals during the usage of plant pigment, pressures for broad systematic clinical research on natural pigments. Extended studies are still necessitated to regulate the permissible levels of pigments although it is natural (*Singh et al., 2015*).

“The unrestrained use of colorants in Indian street food markets urgently needs to be appraised. Analysis studies on street food vendors in Chennai, Tamilnadu underpin that 94 percentage are unaware of even the hygiene practices and 74 percentage believe that applying for a license is an arduous task” (*Abraham et al., 2017*). The scenario with other metropolitan and rural areas of India, which attracts tourists in larger groups for their street foods. Therefore, to avoid addition of harmful substances in foods by street vendors, awareness programs must be conducted, which should be initiatives from the regional level itself to curtail the sources of banned pigments in foods. With the collective effort strengthened by officials at the regional and national level, street food vendors will undoubtedly alter the fortune of Indian cuisines and magnetize more foreigners (*Gupta et al. 2020*).

FSSAI (*2009 and 2011*) had published the list of natural and synthetic colorants with permitted limits in 2 regulations. The non-permitted colours are Fast red, Rhodamine B, Metanil yellow, Bromo-cresol purple, Green S, Sudan 1, Sudan 2, Sudan 3 and Sudan 4, as well as the overuse of permitted colours. However, the regulations do not provide a proper definition to define artificial colouring substances. There is no distinction between natural colours and the synthetically produced natural colours. As per FSSAI, food products are sold under the label ‘contains permitted natural colours/permitted synthetic colours/contains permitted natural and synthetic colours’ without any depiction of the used colorant (*FSSAI, 2011*).

The International identification number of colours needs to be listed on the label. The presence of annatto colour in oils is labelled as ‘annatto colour in oil’. But there is no specification about other colorants. The readability of these terms for consumers is also questionable. The manufacturing and sale of synthetic colours should be under license and their packaging labelled as

'food colours'. There are certain specifications regarding the diluents, filler materials for the preparation of synthetic colours (FSSAI, 2009).

In consideration towards the regulatory and societal aspects of food colourants in India, a regularly update on the list of permissible and non-permissible food colourants is very much in need of the hour. An unbiased, unambiguous standard regulated on the toxicity levels for permitting any colourant for usage. An obligatory source on the production of natural colourants is delegated apprehensible with description of the norms in production. In overall production, proper labelling defining the colourant should be provided esteeming the rights of consumers to know the ingredients of the food that is being consumed (Xu et al. 2018).

Knowledge on natural pigments help with the progression and expansion of healthier, safety-specific functional food products and also the consumption of natural colourants incorporated in food provides health benefits like cardioprotective activity (Varghese et al. 2022). Garnweidner Holme (et al. 2021), described that "there needs to be a focus on scientific innovations and upgradation in the processing and storing conditions of the food such that it would reduce or eliminate the inclusion of food additives and also assist in the contribution of risk free, secure food products to consumers without compromising on their catch factors and demands. Due to this demand and change in consumer expectations, food industries have been collaborating with top food research institutions to apply scientific knowledge to produce high valued nutrient-enriched food products".

The upcoming collaborations of food industries with research in food science has aided to stay updated on the latest progress that makes possible the commercialization of safe, natural, healthy and functional ingredients in food products. Recent updates on foods emphasizes on microparticles that enhance the solubility of pigments by a century's fold, encapsulating an emulsion system and polymers used for stabilisation of pigments for longer storage periods, where gamma irradiations have also remained as a reliable technique to extend shelf life and stability (Martins et al. 2016).

On reaching out to the increasing demands of natural colourants has become a challenging tedious extraction procedure, with expensive and less availability of raw materials. Excellent strategies such as gene editing technologies like CRISPR/CAS 9, techniques of optimization of tissue culture, utilization of stress factors like sonication, ultrasound and magnetic field and recognition of molecular markers for breeding should be employed for augmenting colorant production. In case of exemplification, to induce the production of β -carotene, lipases and carotenogenic genes were expressed in *Saccharomyces cerevisiae* (Fathi et al. 2021).

Production of natural colourant annatto has been enhanced through application of abiotic elicitors like methyl jasmonate, salicylic acid and paclobutrazol. However, researchers are exploring replacements for natural colourants with synthetic colourants from studies substantiating their level of usage in their original forms that are uncertain. Thereby, researchers focus on elucidating promising natural sources for pigments. For example, beta-carotenes extracts from microalgae such as *Chlorella vulgaris* and *Dunaliella salina* (Damergi et al. 2017). Also, additional research in exploring the likelihood to produce pigments from endophytic microbial sources and large scale production in bioreactors are providing possibilities for safer food colorant production (Sujithra et al., 2022).

Extraction of natural pigments from both edible and non-edible parts of plants such as flowers, seeds, leaves, fruits, roots, etc. and other sources include marine fungi, insects and microalgae. Natural pigments like anthocyanins, betalains and beta carotenes are extracted from edible substances like grapes, blue berries (Albuquerque et al. 2021) and carrots. Also the pigments like crocin, bixia, lutein and carminic acid from *Crocus sativus*, *Bixa orellana* (Rodriguez-Amaya, 2015), marigold flower (Adeel et al. 2017) and cochineal insect (Cooksey, 2019) are derived from non-edible matter. Natural pigments are often combined with carriers, emulsifiers and antioxidants to maintain colour stability since they are highly sensitive to air, light and temperature. Besides, the impeccable curative properties of natural colorants make them apt candidates for chemo preventive therapy through diets (Saini et al. 2020).

Carotenoids are organic and water-insoluble natural pigments used for the extraction of pigments that needs only separation from the plant without other components like minerals, carbohydrates and proteins from the overall source. Eventhough the process of extraction does not assist in changing of either physical, that is structural or chemical properties in the pigment, extensively purified pigments are subjected to investigation. Where ascorbyl palmitate, an antioxidant, is used to prevent degradation of oxides in the pigments. Plants extracts are converted from water insoluble to water soluble matter through polysorbates, fatty acid sucrose esters and additional encapsulation of polysaccharides. Thus, food safety and regulations are stringently monitored in India (Leepica et al., 2021).

To analyse the quality of food colourants, widely used analytical techniques like spectrometry, thin layer chromatography, ion chromatography, coupled plasma mass spectrometry, gravimetric analysis, the most sensitive technique, high performance liquid chromatography (HPLC)

and quadrupole time of flight mass spectrometry are considered more accurate and precise. According to food safety and regulations, a keen inspection and certification of food colourants regulated for health safety, yet non-permissible additives are somehow bought into consumption. Thus, there is a need for more stricter regulations to be endorsed on the usage of synthetic food colourants and also the products of natural food colourants (*Martins et al. 2016*).

Modernized and emerging techniques to detect food additives have become more portable, convenient and visualized. For instance, colourimetry, fluorometry, combination of colorimetry and fluorometry, where the technology is known as Surface Enhanced Raman spectroscopy (SERS), in which the derivatives combine the usage of filter paper and/or smartphone for visual reading have made an excellent progress in enhancement of portability and practical experiences. These techniques are useful and promising for food colourant detection due to their sensitivity, repeatability and stability (*Ponlakh et al., 2022*).

To design a desired food product incorporated with food colourants, challenging and futuristic technologies like microencapsulation and nanoencapsulation have been developed to enhance the intensity and stability of the colours used in foods. Encapsulation technique in capsulating colourants have a varied applications in coloured based products of textiles, pharmaceuticals, as well as in food industries. Researchers have studied a broader view displaying diverse colourants in foods, taking advanced steps in substituting natural colourants with synthetic colours, where food sectors have taken control of an innovative method, encapsulation technique, where pigments are encapsulated to impersonate unflavourable tastes, unstable food compounds along with the addition of functional, nutritional, visulling and restrained release of pigments equally on the entire food stuff.

Microencapsulation and nanoencapsulation of natural pigments have wider benefits of protecting the pigments from harmful environmental conditions and emitting a constrained target release on foods. These micro and nano applications vary on different aspects of food and digestive system, which has to be investigated to explore the mechanism and efficacy of the application. Futuristic studies on natural colourants should emphasis and have a detailed insight in expansion of range of colours, without altering their health benefits and stabilizing the colour, where the stabilization property is managed through techniques like molecular complexation and microencapsulation (*Susmita Ghosh et al., 2022*).

Researched on plant-based pigments suitable for foods like carotenoids, anthocyanins and betalains have exhibited an exclusive clarified structure that is being investigated for their pigmented composition, stability, influencing factors and effects on processing. During thermal processing, carotenoids undergo isomerization and oxidization, whereas anthocyanins undergo hydrolysis, hydrogenic nucleophilic attack and polymerization. Likewise, betacyanins undergo deglycosylation, isomerization, dehydrogenation, hydrolysis and decarboxylation. Researches have dominated the search on carotenoids in terms of nutritional effects, in vitro, in vivo and human intervention studies where persist results are obtained. Whereas researches are still being continued on anthocyanins and betalains, which requires clinical studies on human than cell and animal studies. Also research on certain aspects like stabilization which requires microencapsulation, nanoencapsulation, but in the case of anthocyanina, co-pigmentation process is being undertaken by researchers (Delia, 2019).

Muthusamy Ramesh (et al., 2018) has quoted in one of his research paper emphasising that 'Food safety is the major concern for human life. The Food and Drug Administration (FDA) has approved over 3000 ingredients as safe food additives to preserve, colour, flavour, or thicken food items. Many of these ingredients are restricted due to the observation of unexpected adverse effects. In some cases, adulteration of food and additives causes potential health risks, such as learning disabilities, hyperkinesis, kidney damage, tumours, skin rashes, migraine, asthma, sleep disturbance and gastric upset. Modern food products often include several flavouring and colouring agents from natural or artificial sources. The consumption of these agents is also increased in recent days due to lack of health awareness. To create awareness of the health risks due to the presence of flavouring agents and dyes in food-related products. Knowledge of these food additives may help manufacturing industries to develop standard foods, pharmaceuticals and cosmetic preparations'.

In association with food safety lies the risk of lack of nutrition in children. Thus, a research has been carried out to monitor the food consumption pattern among school going children between the age group of 6 to 17 years in Saudi Arabia, where 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), two non-permitted ones, Erythrosine (E127) and Red 2G (E128) were determined and confirmed of usage in the 24 hours dietary recall questionnaires. Artificial colour added in 839 food products were of 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 color additives, Brilliant Blue (E133) (54 percentage) and Tartrazine (E102) (42 percentage) 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. 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 (*Asif Ahmed et al., 2021*).

Synthetic dyes are widely used in the food industry to maintain or restore the colour of food during processing or storage, the azo ones occupying the first place in terms of the extent of use. Compared to dyes obtained from natural sources, synthetic ones present relevant advantages, such as higher stability and lower cost. Despite these advantages, their use has been linked to side effects, such as allergic reactions, carcinogenic effects, behavioural and neurocognitive effects, but also medium and long-term toxicity. The most used yellow and red synthetic dyes, namely Tartrazine, Quinoline Yellow, Sunset Yellow FCF, Carmoisine/Azorubine, Amaranth, Ponceau 4R, Allura Red AC, as well as their applications in food products. Moreover, it aims to provide current data on the toxicity issues and the possible negative effects on children's behaviour of these dyes, based on the evaluations previously carried out by European Food Safety Authority (EFSA) (*Roxana et al., 2024*).

'Cysts of *Artemia salina*', acquired in the central market of Teresina (Piaui), Brazil, were used to evaluate the toxicity of tartrazine. The *A. salina* cysts were incubated in a beaker containing a 50:50 mixture of saline (artificial sea water: 23.0 g NaCl, 11.0 g MgCl₂.6H₂O, 4 g Na₂SO₄, 1.3 g CaCl₂.2H₂O, 0.7 g KCl, in 1 L distilled water at pH 8.5 (adjusted by using 1 N Na₂CO₃) and mineral water under constant aeration for 48 hours at 27 ± 3 °C. After incubation, the live nauplii free from microcrustacean shells were collected from the lighter portion of the incubation chamber and are used for this assay. Ten (10) nauplii were placed into each test tube containing 4.5 mL of the saline solution. The experiment was performed by serial dilutions of TRZ at 31.25 to 1000 µg/mL. In each experiment, 0.5 mL of the test sample was added to 4.5 mL of saline solution, and the mortality of *A. salina* was recorded after 48 h of exposure time. According to the scale, lethal concentration (LC₅₀) values >1000, 500 to 1000, within 100-500, and <100 µg/mL were considered non-toxic, low toxic, moderately toxic, and highly toxic, respectively (*Jailson et al., 2022*).

In the *A. salina* toxicity assay, yellow colourant was moderately toxic, although they were evaluated the main TRZ metabolite, sulfanilic acid, where they found mild toxicity. The metabolites of the colourant demonstrated genotoxic activity in human lymphocyte cells. Although there are controversial results, it showed that the yellow colourants' toxic effect in the *A. cepa* test with root growth reduction. This shows the toxicity has been demonstrated in different organisms (animals and plants). More sensitive *in vitro* and *in vivo* studies using different models are needed to evaluate genotoxic and mutagenic effects. DNA damage, which is one of the major causes of cancer in animals. Therefore, the successive accumulation of damage caused by regular intake of food containing the yellow colourant can lead to DNA mutations and ultimately, the onset of diseases such as cancer (Sekeroglu *et al.*, 2017).

The MTT test showed significant cytotoxicity on both human stomach and rat fibroblast cell lines. Previous studies have evaluated tartrazine cytotoxic effects *in vitro* and *in vivo* models. Tartrazine cytotoxicity on the liver, kidney, spleen and brain of albino Wistar rats, demonstrating that tartrazine significantly increased kidney and liver weight while reducing spleen weight in comparison to the group control. Moreover, a histopathological assay showed that tartrazine produced lesions in the kidney, spleen and liver of all rodents. Tartrazine promoted histopathological changes, causing significant liver tissue damage and changes in blood parameters (Balta *et al.*, 2019).

Recently assessed yellow food colourant administered in 25, 500 or 2000 mg/kg in *in vivo* models for genotoxicity testing has showed no genotoxic activity through the comet assay to identify the DNA damage of various organs caused by the consumption of food additives in mice. The dye is found to have higher genotoxic effects, causing damage to the colon cells, even at low doses of 10 mg/kg weight, which is close enough to Acceptable Daily Intake of 7.5 mg/kg body weight (Bastaki *et al.*, 2017).

According to Kamal amin (*et al.*, 2018) research which concludes that 'the dosage levels of 125 to 500 mg/kg of tartrazine given for 30 days induced a rare adverse effects on memory and learning in animals model, this is might be because of its promotion of lipid peroxidation metabolites and ROS, preventing endogenous enzymes of antioxidant protection and the brain tissue injury. Taken together, because of the current evidence presented, the daily consumption of Tartrazine as agreed by the Acceptable Daily Intake rate seems to be reasonably harmless; however, exposure is unlikely to be reached after ingestion of food. Tartrazine induced hyperactivity, antisocial behavior and anxiety in male Wistar rats at 0, 1 and 2.5 percentage doses in drinking water as recorded for different animal models of raised plus-maze, open ground and the dark-light transition experiments.

Moreover, 0.05, 0.15 and 0.45 percentage tartrazine doses induced a few antagonistic effects on neurobehavioral markers all over generations in mice’.

Genotoxicity study conducted on blue dye to be used as food colourant from gardenia and genipin, induced in male and female mice of 74, 222 mg/kg body weight/day and 2000 mg/kg body weight/day has neither induced any of the micronuclei blood cells, nor did any damage to the tissues of liver, duodenum and stomach. Whereas, in the in vitro micronuclei and chromosomal assays, gardenia blue tested negative, but genipin tested positive (*Hobbs et al., 2018*).

Shakoor Sadaf et al., (2022) has reported that “incorporation of the colouring agent, tartrazine and curcumin affects healthy physiological system leading to changes in hematology, biochemistry and enzymatic activity. Consequences of oral administration of tartrazine and curcumin were explored for 15, 30 and 45 days in female adult rat. Two doses were based on the admissible daily intake (ADI) of 9.6 and 96 (high) mg/kg/body weight for tartrazine, 3.85 and 38.5 mg/kg/body weight for curcumin. Phytochemicals such as saponins, glycosides, alkaloids, flavonoids, tannin, carbohydrates and phenolics were present in coloring agents. The renal function tests and lipid profile showed alterations in the values of uric acid, urea, total protein, albumin and cholesterol in all treatment groups. Additionally, the levels of liver enzymes fluctuated after 45 days of treatment with curcumin and tartrazine. Histopathological changes were also recorded in liver and kidney. In contrast, blood biochemistry depicted the significant reduction of RBCs, Hb, MCH, glucose, WBCs, MCHC and LDH at 15, 30 and 45 days. Besides, IgG, IgM and overall antibody GMT at 45 days were significantly increased. Study indicated that ADI and doses up to 10 times of the ADI of food colors intake exerted adverse effects on immune response and altered the biochemical parameters”.

RESEARCH GAP

1. In consideration towards the growing demands in food safety, this study is carried out to ease the need for the hour by developing alternative natural food colourants.
2. Enhancement of traditional knowledge on plant based products and few research studies on the analysis of natural food colourants was investigated by the researcher.

NEED FOR THE STUDY

1. Common ill-effects in consuming synthetic colourants have negative impact on cognitive development, functioning of the liver and other vital organs.
2. Optimizing the natural food colourants as alternative for synthetic food colourants for safe human consumption.

OBJECTIVES OF THE STUDY

Primary Objective

To alternate synthetic and artificial food colours as the non-natural colours cause illness to human beings.

Secondary Objectives

- To select natural food colourants from plant sources and to analyse their physical, chemical and characterization properties.
- To measure the shelf-life, analyse the microbial growth, to test the toxicity of the natural food colourants using primary, secondary and *in vivo* model through histopathological and haematological parameters.
- To incorporate and evaluate the developed natural food colourants in selected recipes through organoleptic evaluation.
- To develop a sensor detecting the toxicity in the foods colourants in selected recipes incorporated with commercially available synthetic food colourants in consideration with the guidelines of FSSAI.