

The methodology of the study entitled as “**Physicochemical Characteristics, Safety of Under-Utilized Selected Natural Food Colourants and Development of Food Colour Sensor**” is conducted under the following procedures:

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PHASE – I

3.1 Study on Synthetic and Natural Food Colourants

As an initiation of the study, a detailed survey was conducted on the availability and frequency of usage of synthetic food colourants. In consideration with the frequency in usage of synthetic food colourants, equalized natural colouring sources were selected and analysed.

3.1.1 Market Survey on Synthetic Food Colourants

Industrial dyes are all considered to contain carcinogenic compounds that have been explicitly banned from usage in edible products. However, in view of their strong colouring ability, stable colouring capacity and cheap availability, many manufacturers use them illegally in food products (*Pressman et al., 2017*). This study was conducted on human preferences has obtained the Institutional Human Ethical Clearance from **the Institutional Human Ethics Committee with the proposal No. IHEC/22-23/FSMD-1 issued in the year 2023, with the Approval number for the same as AUW/IHEC/FSMD-22-23/XPD-1** (Appendix-I).

In order to know the usage of synthetic food colours among the consumers, market survey was conducted on these synthetic food colourants in the supermarket of Coimbatore city. One hundred and fifty supermarkets and shops were surveyed to study the commercial brands of synthetic food colourants and powdered colours sold in regard to the preference of the customers in the supermarkets and shops. The questionnaire (Appendix-V) was used to conduct survey in the selected supermarkets. From the 150 supermarkets and shops, there were 32 different brands available, in which three brands were very familiar and were sold in higher quantity. The brands' names are coded for confidentiality purpose and the brands are: BK, BU and GC. The numbers of supermarkets and shops sold the abovementioned brands are listed as 32 supermarkets and shops sold the brand BK out of which 27 supermarkets and shopssold red synthetic colour, 20 supermarkets and shopssold green synthetic colourant, 14 supermarkets and shopssold yellow colour and 11 supermarkets and shopssold blue synthetic food colourants. BUbrand is sold in 23 supermarketsand shops, out of which 18, 11, 14 and one supermarkets and shopssold red, green, yellow and blue synthetic food colourants respectively. The brand GCis sold in 24 supermarkets and shops, where 22 supermarkets and shops sold red food colourants, six supermarkets and shopssold green food colourants, 22 supermarketsand

shops sold yellow synthetic food colour and five supermarkets and shops sold blue synthetic food colourant.






3.1.2 Study on Natural Food Colourants among the Selected Respondents

The plant pigments are present in the cells of any part of a plant that appears to be in certain colour, reflecting different wavelengths of light. Colours produced from the pigments are mostly autotrophic in nature and few are also considered edible (*Owen et al., 2021*). Plants with different coloured pigments were selected and a study was conducted on the plants considering their colours that they produce and their health benefits.

To alternate these commercial food colours, different plants were studied for extracting natural food colours, considering their availability and their coloured pigments. The coloured pigments present in natural form were narrowed down in consideration with their local availability, where the plant based sources were selected for further extraction process after a survey conducted among the selected home makers as respondents.

A survey was conducted among selected participants of home makers (N=100) between the age group of 25 – 45 years in Coimbatore city by random sampling technique using questionnaire shown in Appendix-III. All the selected home makers had knowledge on beetroot, carrot and turmeric as natural food colourants, these food commodities can be used or incorporated as food colourants in different recipes. The natural food colourant samples to six different plant origins namely: annatto seeds (*Bixaorellana*) that produces orange red colour, eucalyptus bark (*Eucalyptus grandis*) that gives brown colour, madder root (*Rubiaccordifolia*) that produces brown red colour, tanner's cassia (*Sennaauriculata*) with yellow orange colour, roselle petals (*Hibiscus sabdariffa*) that gives dark red colour and tamarind seeds (*Tamarindusindica*). Tamarind seeds (*Tamarindusindica*) were colourless and these sources had least recognition rate of below 25 percentage among the selected respondents, home makers. The selected natural food colourants were studied in detail, bringing to light their various medicinal properties and different remedial therapeutic aspects.

Table – I**Selected Natural Food Colourants for Further Analysis**

| Common Name | Botanical Name | Family Name | First Language Name (Hindi) | Regional Name (Tamil) | Plant part used | Colour Extracted | Name of the Pigments Present in the Selected Natural Food Colourants | Images of the selected Samples |
|-------------|----------------------------|-------------|------------------------------|--------------------------|-----------------|-------------------|--|--|
| Madder | <i>Rubia cordifolia</i> | Rubiaceae | Majith | Manditti | Root | Brown Red Colour | Alizarin and Purpurin |  |
| Eucalyptus | <i>Eucalyptus grandis</i> | Myrtaceae | Neelgiri | Karpuram | Barks | Brown Colour | Anthocyanins and Myoglobins |  |
| Annatto | <i>Bixa orellana</i> | Bixaceae | Keshari | Kongaram, Sappira | Seeds | Orange Red Colour | Bixin and Norbixin |  |
| Roselle | <i>Hibiscus sabdariffa</i> | Malvaceae | Ambada bhaji, Meshita | Pilicha keerai | Petals | Dark Red Colour | Anthocyanin |  |
| Tamarind | <i>Tamarindus indica</i> | Fabaceae | Imli | Puli | Seeds | No Colour | No pigment in seeds |  |

3.1.3 Knowledge Attitude and Practice regarding Natural Food Colourants among the Home Makers and Street Food Vendors

A survey was conducted among selected participants of home makers (N=100) and food vendors (N=100) in Coimbatore city, Tamilnadu, India by random sampling technique to know their knowledge, attitude and practice towards the usage of food colourants. Well structured interview schedule (Appendix-III and IV) on knowledge, attitude and practice shows the usage of food colourants along with the preferred brand and frequency of consumption at home was considered. Likewise, the street vendors who use food colourants while preparing their foods were also taken into consideration through the foods that they prepare, the colours they use, the consumer preferred colours along with the frequency of usage of type of foods in which they use were all considered. From the survey, the researcher has come to a conclusion that solid foods like kesari, ladoo, chilli gobi, chilli chicken, tutti frutti, jalebi, bajji, bonda, fruit jam and jelly were highly preferred using synthetic food colourants, whereas liquid foods like juices were only considered in using synthetic food colourants.

PHASE – II

3.2 Processing, Shelf Life Study, Microbial Assay and Testing of Primary Toxicity in the Selected Natural Food Colourants

3.2.1 Processing of Selected Natural Food Colourants

For the process of extraction of colours, the naturally available plant sources that produce colours, which can be partially relevant to that of the synthetic colours commercially available in the market were selected and further analyzed. For the process of isolation, the selected plant parts annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*), madder root (*Rubiaccordifolia*), roselle petals (*Hibiscus sabdariffa*) and tamarind seeds (*Tamarindusindica*) were handpicked from Forest College and Research Institute, Mettupalayam, Tamilnadu and shadow dried to assure that there were no contaminants present in it. Plant sample were then packed in air tight, zip lock bags and stored in dry place at room temperature for further analysis processes.

3.2.1.1 Pulverizing the Selected Natural Food Colourants

The plant sources of madder root (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*), roselle petals (*Hibiscus sabdariffa*) and tamarind seeds (*Tamarindusindica*) were stored and pulverized in a sterilized dry mixer. The mixer grinder with three blades capacity of 500-750 watts with an average motor range of 18,000 to 23,000 rpm (Rotation Per Minute) was used in pulverizing the plant sources. The pulverized natural food colour sample were sieved through white cotton poplin cloth with the radius of 50 nm (nanometer) or 50×10^{-9} meter of porous size to get fine particles of powder. The fine powdered particles were packed in small zip lock bags and stored in dry place for further physical and chemical analysis.

3.2.1.2 Extraction Processes of Selected Natural 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). Thus, the following aqueous extraction processes, also known as 10 percent extraction process was carried out to extract the natural food colourants.

3.2.1.2 (a) Process of Soaking in Aqueous Extraction Method

The plant sources from annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*), madder root (*Rubiacordifolia*), roselle petals (*Hibiscus sabdariffa*) and tamarind seeds (*Tamarindusindica*) were weighed and 25 grams of each sample was measured and soaked in 250 ml of distilled water for 24 hours. On the 24th hour of the next day, the aqueous phase was strained from the samples and stored in sterilized transparent glass bottles. The aqueous extracts were likely to get contaminated compared with the other form of extracts. Thus, the extracts were divided into two equal portions and it was stored in room temperature and the remaining samples were stored in refrigerator for further analysis.

3.2.1.2 (b) Process of Boiling in Aqueous Extraction Method

The selected natural sources of food colourants were extracted by reflux method. The selected natural sources are: madder root (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*), roselle petals (*Hibiscus sabdariffa*) and tamarind seeds (*Tamarindus indica*) were weighed, 25 grams of each of the sample is taken and immersed in 250 ml of distilled water. 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 27°C and the other in the refrigerator at 5°C.



(a)

(b)

(c)

(d)

(e)

Madder root
(*Rubia cordifolia*)

Eucalyptus bark
(*Eucalyptus grandis*)

Annatto seeds
(*Bixa orellana*)

Roselle petals
(*Hibiscus sabdariffa*)

Tamarind seeds
(*Tamarindus indica*)

Plate – 3.2.1.3 (a, b, c, d, e) Boiled Aqueous Extracts of the Selected Natural Food Colourants

3.2.2 Measuring the Intensity of Colours of the Selected Natural Food Colourants using Food Colour Reader

To analyze the physical property, the extracts of annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*), madder roots (*Rubia cordifolia*) and roselle petals (*Hibiscus sabdariffa*) were measured for intensity through Food Colour Reader. Food colour Reader works under the principle that when a beam of incident light travels through a specific solution, the light is reflected, absorbed and transmitted, the concentrate of the solute, i.e., the natural food colourant, in the solution is directly proportional to the amount of light absorbed (*Kakoli Dutta et al., 2023*).

The food colour reader was calibrated using the black and white cavity cover, placing it evenly below the cavity. Then the extracts of food colour samples were placed on a watch glass, with a plain white base. Few drops of the extracts were pipetted out on the watch glass. The cavity of the food colour reader was placed over the extract and the reading was taken with L* (lightness), a* (red/green) and b* (yellow/blue) indicating the colour hue of the food colourants.

Table – II
Computation of Intensity Values using Food Colour Reader

| Symbols | Representations | Formula |
|--------------|-----------------|---|
| L* | Black/White | $\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ |
| a* | Red/Green | |
| b* | Yellow/Blue | |
| $\Delta L +$ | White | |
| $\Delta L -$ | Black | |
| $\Delta a +$ | Red | |
| $\Delta a -$ | Green | |
| $\Delta b +$ | Yellow | |
| $\Delta b -$ | Blue | |

L* represents lightness

a* represents either red or green

b* represents either yellow or blue

In *Table-II*, Delta-E is a standard measurement that qualifies the difference between the two colours that appear, where the lower Delta-E figures indicate greater accuracy, while the high Delta-E levels indicate a significant mismatch. The colour intensity analysed by the food colour reader has been recorded and the values were calculated using the formula for Delta-E. The CIE LAB colour

was measured using Food Colour Reader to note the colour hue difference. Using the formula the intensity of colours was calculated using the food colour reader shown below.



Plate – 3.2.2 (a)



Plate – 3.2.2 (b)

Front View of the Food Colour Reader

Rare View of the Food Colour Reader

Plate – 3.2.2 (a, b) – Food Colour Reader used to Measure Intensity of Colours in foods

3.2.3 Testing pH for the Selected Natural Food Colours Extracted using Aqueous Extraction Method

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).

pH values: The pH of the extracts extracted by boiling extraction method was measured using pH meter. The pH values were tabulated along with the quantity of the extracts that was obtained after boiling the raw compounds with distilled water at 60°C for 60 minutes and strained using Whatman filter paper.

3.2.4. Shelf Life Study of the Extracted Natural Food Colours at Different Temperatures

The pulverized substances of madder root (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were stored in zip lock polythene cover and there were no reactions or contaminants found, whereas in the aqueous extracts of natural food colourants were stored in sterilized glass bottles and brown bottles, along with zip lock polythene covers. Quick contamination was found within a period of 10 days. On the 10th day, the natural food colourants extracts stored in the room temperature started forming creamy layers and on the 15th day, the creamy layers became darker in colour forming a slimy layer on the surface of the extracts. Same way on the

15th day, the extracts placed in the refrigerator (5°C), formed a slimy thick layer at the bottom of the bottle and the colour of the extract was too dark than the original colour.

3.2.5. Microbial Analysis for the Selected Natural Food Colours

Analysing food particles for microbial presence and growth is a part of food safety management and conformity tests define microbiological criteria or assess the performance of control strategies based on the Hazard Analysis and Critical Control Point. For testing the presence of microbes in the food, conventional techniques with rapid resulting have come into existence. The conventional methods are used because they were developed many years ago and have been in use 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. 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*).

3.2.5.1. Fungal Assay by Rose Bengal Chloramphenicol Agar Medium

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*).

$$\text{CFU/ml} = \frac{\text{Total number of colonies obtained} \times \text{dilution factor}}{\text{Volume of Specimen used (aliquot)}}$$

3.2.5.1 (a) Procedure Followed

Pour Plate method was adopted and the requirements are: petric plate 10 mm (16 nos.), put on the PPE, sterilize the work surface and the laboratory equipment were set. Sample preparation of the liquid form samples were diluted to make the microbial load to the range of 20 – 300 CFU/ml dilution up to 10⁻¹⁰. Medium preparation of Nutrient Agar for bacteria and Rose Bengal Chloramphenicol

Agar Medium for fungi were prepared and autoclaved. The media was allowed to cool to about 40 – 45°C (maximum up to 55°C), but it must not solidify. The sample in media, prior to pouring it into the petri plate was approximately added to 15 ml of media in a test tube and is autoclaved. Alternatively, a fixed volume of media was prepared in a bottle and sample is added later by calculating the volume which is equivalent to one ml sample per about 15 ml of media. Sterile petri plates were arranged, labeled at the bottom of the plate with the dilution factor, date and sample identification code. Inoculation takes place in a lamellar chamber, using a tube with about 15 ml of molten media was added at room temperature, along with 1ml of sample. Mix the sample properly in the media. Pour the media into a sterile petri plate. The lid of the petri plate was closed and the media was allowed to completely solidify. Incubate the plate in an inverted position under suitable incubation conditions for 24 hours at 27°C (*Sanders et al., 2012*).

Fungal colonies were identified after inoculating it into a sterilized petric plates using standard pour plate culture with Rose Bengal Chloramphenicol Agar Medium. The plates were kept at room temperature (37°C) for the formation of colonies. The number of colonies formed on the third day was counted and recorded.



Plate – 3.2.3.1 (a)

Colonies found in Annatto seeds (Bixa orellana)



Plate –3.2.3.1(b)

Colonies found in Eucalyptus bark (Eucalyptus grandis)

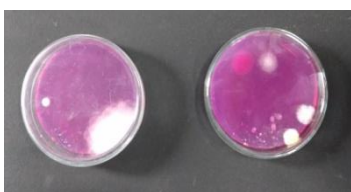


Plate – 3.2.3.1 (c)

Colonies found in Madder root (Rubia cordifolia)



Plate – 3.2.3.1 (d)

Colonies found in Roselle petals (Hibiscus sabdariffa)

Plate –3.2.3.1 (a, b, c, d)– Fungal Identification in Inoculated Petric Plates

3.2.5.2. Bacterial Assay by Nutrient Agar Medium

For detecting the presence of bacteria, Nutrient agar medium was prepared and culture was inoculated for a period of 24 hour in sterilized petric plates, using standard pour plate method, bacterial colonies were identified, counted and recorded based on the procedure (Bonnet, 2020).

Bacterial colonies were identified after inoculating it into a sterilized petric plates using standard pour plate culture with Nutrient agar medium. The plates were kept at room temperature (27°C) for the formation of colonies. The number of colonies formed on the fifth day was counted and recorded.

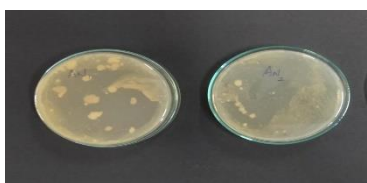


Plate – 3.2.3.2 (a)

Bacterial Colonies in Annatto seeds (Bixa orellana)

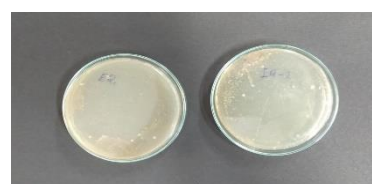


Plate – 3.2.3.2 (b)

Bacterial Colonies in n Eucalyptus bark (Eucalyptus grandis)



Plate – 3.2.3.2 (c)

Bacterial Colonies in Madder root (Rubia cordifolia)



Plate – 3.2.3.2 (d)

Bacterial Colonies in Roselle petals (Hibiscus sabdariffa)

Plate –3.2.3.2(a, b, c, d)–Bacterial Identification in Inoculated Petric Plates

3.2.6. Analysis for Primary Toxicity Level in the Selected Natural Food Colourants using Brine Shrimp Assay

Food toxicology has paved an unique evidential dependency on virtuous human nutrition and heterotrophic metabolism. To equip the evidence with modern studies on biochemistry, molecular and cellular biology, bioinformatics along with computer science have throughout the screening technical technologies to identify advanced effects and to characterise potential toxicants in foods (Alexander Gosslau, 2016).

Analysis of Primary Toxicity Level in Brine Shrimp Assay

For identification the extracts from annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*), madder root (*Rubia cordifolia*) and roselle petals (*Hibiscus sabdariffa*) were coded with the first two alphabets of the source. The extracted natural food colourants sample were taken in different concentration as 100, 250, 500, 1000 and 1500µl in each beaker containing saline

solution. Thirty shrimps were introduced into the solution of the food colourant samples at various concentrations. The movement of the shrimp was monitored at intervals of 1, 2, 4, 6 and 24 hours continuously. There were three groups, in which the shrimp were introduced into blank solution group, control group [Potassium dichromate – $K_2Cr_2O_7$ (1mg/ml)] and prepared samples with different concentrations. The mortality of shrimp was calculated after 24 hour (Manoranjan *et al.*, 2021). For each of the natural colourant sample, 30 shrimp were added to 25ml of the solution prepared as control and extracts of annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*), madder root (*Rubia cordifolia*) and roselle petals (*Hibiscus sabdariffa*). The mortality of the shrimp was monitored as that of blank and control group.

PHASE – III

3.3 Analysis for Chemical, Characterization Properties and Secondary Toxicity Testing of the Natural Food Colourants

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).

3.3.1. Analysis for the Chemical Properties of the Selected Natural Food Colourants

Keeping this in mind, the researcher analyzed for chemical properties like antioxidant assay and phytochemical screening method for the selected natural food colourants like annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*), madder roots (*Rubia cordifolia*) and roselle petals (*Hibiscus sabdariffa*). Phytonutrients help in prevention of diseases and maintain proper functioning of organs, especially in human system (Christine *et al.*, 2022).

3.3.1.1 Analyzing the Antioxidants in the Selected Natural Food Colourants

The protocol used in evaluating the antioxidant capacity of compounds is 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method through the scavenging capacity of free radicals in annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*) and madder roots (*Rubia cordifolia*), reducing the DPPH radical with ascorbic acid as standard. The 2,2-diphenylpicrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. The method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant (Krystyna *et al.*, 2013).

3.3.1.2. Analyzing the Phytonutrients in the Selected Natural Food Colourants

The extraction samples of annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*) and madder roots (*Rubia cordifolia*) were screened for detecting the metabolites like alkaloids, flavonoids, sterols, terpenoids, anthraquinone, anthocyanin, proteins, phenolic compounds, quinones, carbohydrates, tannin, saponines, cardiac glycosides, glycoside's test, lignin, coumarins and volatile oils respectively. Mayer's reagent, Dragendorff's reagent, alkaline test, shinoda test, molisch's test, fehling's test, braymer's test. Bromine water test and other common tests were performed for analyzing the natural food colourant samples for the presence of metabolites are mentioned in the *Annexure (Priyanka et al., 2014)*.

The qualitative analysis for the samples of annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubia cordifolia*) were tested for preliminary phytochemical screening following the guidelines of *Shaikh et al., (2020)* (Appendix-VI).

3.3.2 Analysis for Secondary Toxicity Level in Selected Natural Food Colourants and Synthetic Food Colourants with Inductively Coupled Plasma Mass Spectrometer

Typical human diet consists of minerals of only five percentage that are essential for normal health and function. For the known essential elements, essentiality and toxicity are unrelated and toxicity is a matter of dose or exposure. In the past years, considerable research has been carried out to better understand the physiological role and the health consequences of trace elements (*Mehri, 2020*).

Inductively Coupled Plasma Mass Spectrometer couples use of an ICP with MS for elemental analysis in madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) along with the synthetic food colourants by generation of ions. This method is carried out to detect the presence of toxic heavy metals that are harmful for consumption. The ICP is involved in generation of a high temperature plasma source at 10,000°C, through which the pre-treated sample is passed. The high temperature attained by this plasma inside the system helps in ionization of elements and thereby in detecting them. Mass spectrometer is used as detector (*Kutralam Muniasamy et al., 2022*).

The natural food colourants samples for dilution were weighed accurately around 0.2 g in a pre-cleaned, dry 50 ml volume microwave digestion vessel. The volume of 6 ml HNO₃ (Nitric acid, 67-70%, for trace metal analysis) was added, vessels were allowed to stand in a fume hood to facilitate pre-digestion at atmospheric conditions for 15 minutes. The microwave digestion vessels

were closed and the microwave digestion process was initiated. The digested sample solutions were quantitatively transferred into 50 ml volumetric flasks (as shown in Appendix-VII) (Mukesh Kumar Singh et al., 2022).

In the primary and secondary toxicity studies, madder roots (*Rubia cordifolia*) and roselle petals (*Hibiscus sabdariffa*) have proved to be toxic in nature, where in the brine shrimp assay, more number of brine were found to be dead in the solutions of roselle petals (*Hibiscus sabdariffa*) and thus, roselle petals (*Hibiscus sabdariffa*) was not taken for further analysis. Likewise in the secondary toxicity study of Inductively Coupled Plasma Mass Spectrometer, the substances of madder roots (*Rubia cordifolia*) had heavy toxic metals in its raw natural form. Thus, madder roots (*Rubia cordifolia*) was ruled out from further analysis.

3.3.3 Analysis for Characterization of the selected Natural Food Colourants

Minerals formed and found in the earth's crust due to thermodynamic, physical and chemical compression are identified through their physical properties. As for the structure of the periodically influence atoms, pressurized as the minerals are modified through temperature and cooled down due to solidification process. In consideration towards the internal arrangement of the atoms, the solid compounds are categorized as amorphous, single crystalline and polycrystalline materials. Amorphous materials are isotropic in nature with similar properties and irregular arrangements of atoms, whereas in crystalline materials, the atoms are arranged in a regular pattern with change in direction of their properties known as anisotropic. Crystals with various physical shapes combine to form polycrystalline materials, where the distributed shapes, arrangements and size of the material affects its properties (Asif Ali et al., 2022).

3.3.3.1. Analyzing the Microstructure of the Selected Natural Food Colourants by Field Emission Scanning Electron Microscope (FE-SEM)

Field Emission Scanning Electron Microscope (FE-SEM) is an advanced technology used in capturing the microstructure image of any material. FE-SEM is conducted with high voltage of 10,000-20,000 kV and with high vacuum, as gas molecules intersects the electron beams which emit secondary and backscattered electrons used for capturing the image of the material. Before exposing the natural food colourants, eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixaorellana*) to the beam of electrons or x-rays, coating the material in vacuum with evaporated carbon is must and this process is known as sputtering, to identify the morphological structure of the materials.

Since the electron probe analyses only to a shallow depth, specimens should be well polished so that surface roughness does not affect the results. Sample preparation is essentially as for reflected light microscopy, with the provision that only vacuum compatible materials must be used. Opaque sample may be embedded in epoxy resin blocks. For transmitted light viewing, polished thin sections on glass slides are prepared. In principle, specimens of any size and shape (within reasonable limits) can be analyzed. Holders are commonly provided for 25mm (1") diameter round specimens and for rectangular glass slides. Standards are either mounted individually in small mounts or in batches in normal sized mounts. Many samples are electrically non-conducting and a conducting surface coat must be applied to provide a path for the incident electrons to flow to ground. The usual coating material is vacuum-evaporated carbon (~10nm thick), which has a minimal influence on X-ray intensities on account of its low atomic number and (unlike gold, which is commonly used for SEM specimens) does not add unwanted peaks to the X-ray spectrum. However, steps should be taken to maintain as constant a thickness as possible (*Hucheng et al., 2016*).

'Characteristic' X-rays result from electron transitions between inner orbits, which are normally full. An electron must first be removed in order to create a vacancy into which another can 'fall' from an orbit further out. In electron probe analysis vacancies are produced by electron bombardment, which also applies to X-ray analysis in the TEM. X-ray lines are identified by a capital Roman letter indicating the shell containing the inner vacancy (K, L or M), a Greek letter specifying the group to which the line belongs in order of decreasing importance α , β , etc.) and a number denoting the intensity of the line within the group in descending order (1, 2, etc.). Thus the most intense K line is $K\alpha_1$ (The less intense $K\alpha_2$ line is usually not resolved and the combined line is designated $K\alpha_{1,2}$ or just $K\alpha$) (*Gao et al., 2016*).

The most intense L line is $L\alpha_1$. Because of the splitting of the L shell into three subshells, the L spectrum is more complicated than the K spectrum and contains at least 12 lines, though many of these are weak. Energies are measured in electron volts (eV), 1 eV being the energy corresponding to a change of 1 V in the potential of an electron (= 1.602×10^{-19} J). This unit is applicable to both X rays and electrons. X-ray energies of interest in electron probe analysis are mostly in the range 1 10 kV (*Anshida Mayeen et al., 2018*).

3.3.3.2. Analyzing the Chemical Composition by X-Ray Diffraction (XRD) for the Selected Natural Food Colourants

To generate x-rays, a source of electrons, an accelerating mode with the electrons at high speeds and a target material to receive the impact of the electrons to interact with them is needed. Crystalline solids consist of regular arrays of atoms, ion, or molecules with inter atomic spacing on the order at 100 pm or 1 Å. The wavelength of the incident light has to be on the same order as the spacing of the atoms. In powder diffraction, the powder of annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) composed of many small and finely ground crystals, known as crystallites. These crystallites are (assumed to be) randomly oriented to one another. If the powder is placed in the path of a monochromatic X-ray beam, diffraction will occur from the planes in those crystallites that are oriented at the correct angle to fulfill the Bragg condition. The diffracted beams make an angle of 2θ with the incident beam. The powder, sealed in a glass capillary tube, diffracts the X-rays (Bragg's law) to produce cones of diffracted beams. These cones intersect a strip of photographic film located in the cylindrical camera to produce a characteristic set of arcs on the film. The film can be removed and examined. Using the radius of the camera and the distance along the film from the center, the Bragg angle 2θ and therefore the d-spacing for each reflection can be calculated (*Dun Wu et al., 2019*).

In modern X-ray Diffractometer, a scintillation or CCD detector is used to record the angles and intensities of the diffracted beams. The resolution obtained by a diffractometer is better than photographic film, since the sample helps refocus the X-ray beam. -intensity is more readily measured and digitally stored. A decrease in the crystallite size causes an increase in the width of the diffraction. On either side of the Bragg angle, the diffracted beam will destructively interfere and result in a sharp peak. The destructive interference is a result of the summation of all the diffracted beams. Close to the Bragg angle it takes diffraction from a large number of planes to produce complete destructive interference. In very small crystallites, there are not enough planes to produce complete destructive interference, so a broadened peak is observed (*Ali et al., 2022*).

3.3.3.3 Thermogravimetric Analysis (TGA) for the Selected Natural Food Colourants

Thermal analysis is a branch which involves the study of the properties of materials which changes with temperature. On heating the material, it may undergo physical and chemical changes. Materials can be studied over a wide range of temperature – Arctic cold to tropical heat. Behavior of materials in various temperature range has to be known for their specific use. It is essential to know about the thermal stability of compounds before moving to its application studies. There are different

types of Thermal Analysis and they are as follows: TGA (Thermo gravimetric analysis), DTA (Differential Thermal Analysis), DSC (Differential Scanning Calorimetry), TMA (Thermo Mechanical Analysis), TT (Thermometric Titration) and DMA (Dynamic Mechanical Analysis) (Vyazovkin *et al.*, 2018).

Thermal gravimetric analysis or thermogravimetric analysis (TGA) is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature with constant heating rate, or as a function of time-with constant temperature and/or constant mass loss. The principles of thermal analysis are: Changes in the mass of a sample due to various thermal events (desorption, absorption, sublimation, vaporization, oxidation, reduction and decomposition) are studied, while the sample is subjected to a program of change in temperature. Therefore, it is used in the analysis of volatile products, gaseous products lost during the reaction in thermoplastics, thermosets, elastomers, composites, films, fibers, coatings, paints, etc. (Gaisford *et al.*, 2016).

Thermogravimetric analysis, the sample is heated in a given environment at controlled rate. The change in the weight of the substance is recorded as a function of temperature or time. The temperature is increased at a constant rate for a known initial weight of the substance and changes in weights are recorded as a function of temperature at different time interval. Isothermal or Static TGA, Quasi-static TGA and Dynamic TGA are three different types of Thermogravimetric Analysis.

Isothermal or Static TGA is the case in which the sample is maintained at a constant temperature for a period of time during which change in weight is recorded. Likewise, quasi-static TGA is a technique, where the sample is heated to a constant weight at each of a series of increasing temperature. Dynamic TGA is a type of analysis in which the sample is subjected to condition of a continuous increase in temperature at a constant heating rate, that is usually linear with time. Differential Thermal Analysis (DTA) is a technique in which the temperature between sample and thermally inert reference substance is continuously recorded as a function of time or temperature (Sravanthi Loganathan *et al.*, 2017).

Adopting the same procedure for the present study, the powder from annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) have been analyzed for thermal gravity, on which heating the material have displayed the degradation of physical and chemical properties, embarking on the loss of organic materials present in its natural form. As the contaminants were found in the aqueous extracts, they were completely ruled out from further processing of the natural food colourants.

3.3.3.4. Testing the Photosensitivity of the Selected Natural Food Colourants

In accord with *Sharmaine Atencio et al., (2022)*, the illumination condition monitored for degradation process in the solution samples of annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) were conducted according to the market survey conducted on the synthetic food colourants. From the survey of 150 supermarkets and shops, the researcher has come to understand that LED lamps used for the purpose of show casing the food products have degrading effect on the synthetic food colourants. Thus, the minimum voltage used in the supermarkets and the shops for exhibiting the food colourants was five watts, that is 18 lux and the maximum voltage used was 50 watts that is 180 lux, where the luminous efficacy is 90 watts. In a square wooden box, photosensitive chamber was designed, which was 25 centimeters in both length and breadth, and was completely inlaid with aluminum foil to conduct the heat produced by the LED lamp. The natural food colourants annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*), were first exposed to medium illuminous effect of 12 watts, which is approximately of 45 lux in the photosensitivity chamber. The change in degradation of colour was measured using UV spectrophotometer in zero hour, two, four, eight, twelve, eighteen and twenty-four hours, respectively.

PHASE – IV

3.4. *In vivo* Study

Toxicating actions are conglomerate mechanism that are generated to affect the oxidation, causing inflammation, resulting in death of cells, aging and degenerative diseases. Integrated toxicology in food has obtained data through biochemical and cell-based techniques like *in vitro*, *in vivo* models and clinical human testing and for higher level of prediction *in silico* models are being used (*Alexander Gossiau, 2016*).

3.4.1. Testing the Toxicity of the selected Natural Food Colourants in Wistar Albino Rats

While complex cell culture systems can provide unique insights into *in vivo* toxicology, they will never completely model the highest level interactions present in an intact organism. Therefore, the gold standard for toxicity assessment has been *in vivo* toxicology, where a particular molecule or complex food ingredients are given to animals to evaluate acute, subacute and chronic effects. A large body of information about their responses have led to the development of various specific high content animal models that may have the ability to simulate the genetic heterogeneity of the human population and evaluate possible reproductive and developmental toxicity, neurotoxicity,

genotoxicity, carcinogenicity, immunotoxicity, food allergy and endocrine disruption (*El Golli et al., 2016*).

The *in vivo* study carried out to test the toxicity has obtained the permit from **the Institutional Animal Ethical Committee with the approval number of AIW: IAEC.2023:03**(Appendix-II) for the species of Wistar Albino rats for a period of 12 months on 14/03/2023, through which pilot study and the experimental study was carried out from the period of March (2023) to October (2023).

Mice and rats are considered as primary substituents for monitoring the bodily functions of all the organs as that of human beings. Thus, the *in vivo* study was adopted to study the metabolic changes that is taking place in the organism. The appropriate level of the selected natural food colourants annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) to be added in the foods will be verified using animal study that will remain prognostic for human consumption. The *in vivo* study is demonstrated to prove that the selected natural food colourants have various health benefits in enhancing the quality of food. Further analysis procedure was adopted to conduct the biological effects of natural food colourants in animals through histopathological examination by enabling the colourants for further utilization. The permissible level of natural food colourants to be added in foods for human consumption will be determined. The effects of developed natural food colourants will be tested biologically in model Wistar Albino rats to analyze the effects when consumed more frequently.

3.4.1.1 Pilot Study to Optimize the Level of selected Natural Food Colourants Administered to Wistar Albino Rats

For the pilot study, the species of Wistar Albino rats of two adult male rats were allocated and purchased from the Animal House in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India. Wistar albino rats which weighed about 200-250g of 8-12 weeks of age were selected, inhabited to house under the standard environmental condition of $25\pm 2^{\circ}\text{C}$ with sufficient light and dark cycles of 12 hours each. The rats were fed with usual commercial feed of pellets and normal drinking water. The animals were caged in individual boxes to help them get accustomed to the separation, the rats were monitored for a week for some behavioural change. After a week's time, the wistar albino rats were found to be settling down and later, the pilot study and the experimentation processes were progressed.

Adult male rats were selected for the study as their hormonal and behavioural changes will be less compared to that of the adult female rats. Adult female rats undergo hormonal changes at the time of pregnancy and at the time of mothering the young ones. Thus, to confirm that there are no physical and biological changes in the species, adult male rats were chosen for the *in vivo* study.

The animals were divided into three groups with two animals for control and five animals in each group. Group – I was the control group, Group – II was experimental group were the animals were fed with Eucalyptus barks (*Eucalyptus grandis*) for 56 days and Group – III was another set of experimental group were the animals were fed with Annatto seeds (*Bixaorellana*) for 56 days (Najlaa *et al.*, 2020).

According to Organization for Economic Cooperation and Development Guidelines (OECD, 2018), the volume of feed should not exceed 1 ml/100g body weight, except in case of aqueous solution 2ml/100g body weight can be fed. The extracts fed to the rats through oral feed in drinking water were in parts per million/million per liter of solution in kilogram. According to Ramachandran *et al.*, (2012), in 100 ml of water, the dosage in ppm of natural food colourants were prepared using the following formula:

$$\begin{array}{l} V_1 N_1 = V_2 N_2 \\ V_1 = \frac{V_2 N_2}{N_1} \end{array}$$

Formula used for preparing the dosage, where:

- V_1 indicates the ml (milliliter) of water to be calculated and given,
- N_1 measures the number ml present in 1 (liter) of water,
- V_2 represents the weight of the animal and
- N_2 indicates the number of ppm (parts per million) to be calculated.

The parts per million given for 100ml of water to the rats apart from the normal feeding is in case of 2 ppm:

$$2 \text{ ppm} = 100 \times 2 \div 1000 = 0.2 \text{ ml}$$

Likewise, ppm level is increased for every specific dosage fed for five days continuously, if there are no changes found in the animals then the dosage is increased gradually with two days wash-out period.

Table – III
Feed Plan for the Wistar Albino Rats

| PARTICULARS | NO. OF ANIMALS | FOOD AND DOSAGE GIVEN DURING EXPERIMENTATION | PERIOD OF TIME (days) | TYPE OF FEED |
|-------------------------|-----------------------|--|------------------------------|---------------------|
| Control Group | 2 | Commercial Feed (25 g) + Normal Drinking water | 56 | Oral |
| Experimental Group – I | 5 | Commercial Feed (25 g) + Normal Drinking water + Specific Dosage of Food Colourants Eucalyptus barks (<i>Eucalyptus grandis</i>) (calculated according to the weight of the rat) | 56 | Oral |
| Experimental Group – II | 5 | Commercial Feed (25 g) + Drinking water + Specific dosage of Food Colourants Annatto seeds (<i>Bixaorellana</i>) (calculated according to the weight of the rat) | 56 | Oral |

From the scheduled feed plan, the animals are weighed every week and checked for finding any physical changes indicating the weight gain or loss. Level of water consumption and intake of feed will also be checked every week, to verify if the animals are under certain abrupt meal pattern due to the intake of natural food colourants.

A pilot study was carried out with two Wistar Albino rats, where the consumption level of the colourants were finalized by monitoring the animals for seven weeks with two days of wash out period. Around 100 g of weight gain was observed in both the rats. But the sensitivity of thresh hold level of wistar albino rats were less for annatto seeds powder when compared to the eucalyptus bark powder. After determining the maximum threshold level, the experimentation is finalized with the other rats.

Table – IV
Profile of the Wistar Albino Rats used for Pilot Study

| Profile of the Wistra Albino Rats | Eucalyptus bark (<i>Eucalyptus grandis</i>) | | Annatto seeds (<i>Bixa orellana</i>) | |
|--|--|------------------------|---|------------------------|
| Type of Group | Test Group 1 | | Test Group 2 | |
| Sex | Male | | Male | |
| Age (months) | 8-12 months | | 8-12 months | |
| | During Purchase | Seventh Week | During Purchase | Seventh Week |
| Weight (g) | 344 g | 452 g | 341 g | 461 g |
| Water Consumption (ml) | 20-25 ml from 120 ml of water | 10 ml + 8 ml of Sample | 20-25 ml from 120 ml of water | 10 ml + 8 ml of Sample |
| Food Consumption (g) | 25 g | 25 g | 25 g | 25 g |
| PHYSICAL STATUS | | | | |

| Body Parts | Appearance | | | |
|-----------------|-----------------|--------------|-----------------|--------------|
| | During Purchase | Seventh Week | During Purchase | Seventh Week |
| Fur/Skin | Normal White | Normal White | Normal White | Normal White |
| Eyes | Normal Red | Normal Red | Normal Red | Normal Red |
| Ears | Normal Pink | Normal Pink | Normal Pink | Normal Pink |
| Nose and Mouth | Normal Pink | Normal Pink | Normal Pink | Normal Pink |
| Limbs and Nails | Normal | Normal | Normal | Normal |
| Tail | Normal Pink | Normal Pink | Normal Pink | Normal Pink |
| MENTAL STATUS | | | | |
| | During Purchase | Seventh Week | During Purchase | Seventh Week |
| Active | ✓ | ✓ | ✓ | ✓ |
| Sluggish | X | X | X | X |
| Aggressive | X | X | X | X |
| Irritated | X | X | X | ✓ |
| Sleepy | X | X | X | X |



(a)

Feeding the Rat



(b)

Rats caged in individual cages



(c)

Drinking water given for Rats

Plate – 3.4.1.1 (a, b, c) – Feeding of the Wistar Albino Rats

The test groups for both the powdered forms of eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were named as test rat – 1 and test rat – 2. The Wistar Albino rats' level of water to be given was calculated with the body weight of the rats during the purchase. The calculation of aqueous feeding of natural food colourants are as follows for eucalyptus barks (*Eucalyptus grandis*) where the rat was named as test rat -1 and it weighed 376 g. The rat for annatto seeds (*Bixa orellana*) was named as test rat - 2 and it weighed about 373 g.

Table – V
Level of Water Supplemented along with Natural Food Colourants

| Powdered Forms | Name of the Rat | Calculation of Level of Water to be given | Determined Level of Water (ml) |
|--|--------------------|---|--------------------------------|
| Eucalyptus bark (<i>Eucalyptus grandis</i>) | Test rat – 1 (TR1) | $376 \times 2 \div 100 = 7.5$ | 7.5 |
| Annatto seeds (<i>Bixaorellana</i>) | Test rat – 2 (TR2) | $373 \times 2 \div 100 = 7.46$ | 7.5 |

According to the *table – V*, the determined level of water in which the natural food colourants are to be optimized is calculated from the weight of the wistar albino rat multiplied by the two as constant factor and then divided by 100 which is the overall percentage of water to be given to the rats. From the calculation the level of water to be given are 7.5 ml for both the test groups of rats with powdered forms of eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixaorellana*).

Table – VI

Optimizing the Level of Eucalyptus bark (*Eucalyptus grandis*) Fed Orally to the Rats (Pilot Study)

| Number of Weeks | Water (ml) | Ppm | Calculation of ppm | ppm of Natural Food Colourants added in Water(mg) |
|-----------------|------------|-----|---------------------------|---|
| 1 | 7.5 | 2 | $7.5 \times 2 \div 1000$ | 0.01 |
| | 7.5 | 12 | $7.5 \times 12 \div 1000$ | 0.08 |
| 2 | 7.5 | 14 | $7.5 \times 14 \div 1000$ | 0.10 |
| 3 | 7.5 | 28 | $7.5 \times 28 \div 1000$ | 0.20 |
| 4 | 7.5 | 41 | $7.5 \times 41 \div 1000$ | 0.30 |
| 5 | 7.5 | 54 | $7.5 \times 54 \div 1000$ | 0.40 |
| 6 | 7.5 | 67 | $7.5 \times 67 \div 1000$ | 0.50 |
| 7 | 7.5 | 80 | $7.5 \times 80 \div 1000$ | 0.60 |
| 8 | 7.5 | 94 | $7.5 \times 94 \div 1000$ | 0.70 |

Table – VII

Optimizing the Level of Annatto seeds (*Bixa orellana*) Fed Orally to the Rats (Pilot Study)

| Number of Weeks | Water (ml) | ppm | Calculation of ppm | ppm of Natural Food Colourants added in Water(mg) |
|-----------------|------------|-----|---------------------------|---|
| 1 | 7.5 | 2 | $7.5 \times 2 \div 1000$ | 0.01 |
| | 7.5 | 12 | $7.5 \times 12 \div 1000$ | 0.08 |
| 2 | 7.5 | 14 | $7.5 \times 14 \div 1000$ | 0.10 |
| 3 | 7.5 | 28 | $7.5 \times 28 \div 1000$ | 0.20 |
| 4 | 7.5 | 41 | $7.5 \times 41 \div 1000$ | 0.30 |
| 5 | 7.5 | 54 | $7.5 \times 54 \div 1000$ | 0.40 |
| 6 | 7.5 | 54 | $7.5 \times 54 \div 1000$ | 0.40 |
| 7 | 7.5 | 50 | $7.5 \times 50 \div 1000$ | 0.37 |
| 8 | 7.5 | 47 | $7.5 \times 47 \div 1000$ | 0.35 |

From the *tables VI and VII*, standardization of feeding level formula is used to calculate the optimization of natural food colourants. It is interpreted that 7.5 ml of water mixed with the natural food colourant from week one to week seven, where the ppm was increased every week and the maximum threshold level of the rats were analysed and the ppm to be given was optimized. For eucalyptus bark (*Eucalyptus grandis*) it is 6 mg in 7.5 ml of water, whereas for annatto seeds (*Bixa orellana*) it is 3 mg in 7.5 ml of water.

3.4.1.2. Experimentation for Toxicity of the Selected Natural Food Colourants in the Wistar Albino Rats

Rest of the animals were purchased and divided into three groups with five animals in experimental groups and two animals in control group. Group – I is the control group, Group – II is experimental group fed with eucalyptus bark (*E. grandis*) and Group – III is experimental group fed with annatto seeds (*Bixa Orellana*) for 56 days. From the scheduled feed plan *table -III*, the animals

are weighed every week and checked for physical changes indicating the weight gain or loss. Level of water consumption and intake of feed is also checked every week, to verify if the animals are under certain abrupt meal pattern due to the intake of natural food colourants. After 56 days, the animals were euthanasia was carried out for histopathology study in all the animals, to identify the effects of the natural food colourant in the experimental group. The animals were harvested and organs (the heart, liver, kidney, intestine, stomach and spleen) were collected in 1:10 formalin solutio



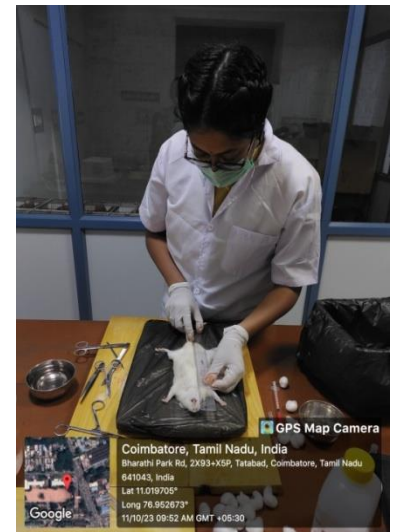
(a)

Measuring the weight of the rat



(b)

Pinning the rat to the dissection Board after anaesthetization



(c)

Measuring the height of the rat



(d)



(e)

Dissection and Harvesting of the Organs for further Analysis

Plate – 3.4.1.2 (a, b, c, d, e) – *In vivo* Models Harvested for Analyzing Histology and Hematology

3.4.2 Histopathological Analysis

The procedure that was followed for the pilot study was carried out for experimenting the rats for 56 days (eight weeks). Eight weeks study was carried out because on prolonged feeding of the rats with the natural food colourants of both eucalyptus barks (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) had shown behavioural changes during the eighth week of administering the oral feed. Thus, to evaluate the administered substances in the organs of the rats, eight weeks study was carried out (*Marghoob Hasan et al., 2016*).

The control group and the experimental group of rats were sacrificed on the 57th day. The rats were anesthetized using chloroform for 10-15 minutes. After dosing off of the rat, they were put on the dissection board and the fur was removed. Later the inner skin of the rat was cut and the rat was opened for harvesting of organs. As the rat was only anesthetized, the heart was punctured and perforated using chloroform. After forcefully stopping the heart, the organs were harvested and immersed in 1:10 formalin for histopathological analysis of the organs. Later histopathology study is performed in all the animals, in control group as well as in experimental group to identify the effects of the selected natural food colourants in the experimental groups.

Microscopic Examination using Paraffin and Staining Techniques

According to *Suvarna et al., (2018)*, the specimens were fixed in neutral-buffered formalin solution of 10% for two days at room temperature. After fixation process, the tissues of the samples of the vital organs (the heart, liver, kidney, intestine, stomach and spleen) were dehydrated in alcoholic, concentrated and cleared in xylene and embedded in paraffin wax that was cut by a microtome. 5 µm sections were mounted on glass slides to be stained. The sliced sections of the organs were mounted on glass slides, deparaffinized and stained with hematoxylin and eosin. They were placed in hematoxylin stain for seven minutes, washed well in running tap water and then put in eosin for three minutes. The excess stain was washed away with water. The sections were dehydrated by alcohol, cleared by xylene and then mounted on glass slides to be viewed by the light microscopy for histological analysis.

3.4.2.1 Analysis for Haematology and Blood Parameters for the Wistar Albino Rats Fed with Selected Natural Food Colourants

The haematology and biochemical parameters are tested to identify the permissible level of natural food colourants to be added to food for human consumption, as animals and especially rats are miniature models for humans (*Yadav et al., 2016*). The blood was drawn out during the dissection

and was filled in two vacutainers with two colours lids of purple and red. The red vacutainer was slightly whirled to make it anti-coagulant, whereas the purple tubes were centrifuged at 3000 rpm for 10 minutes and the serum was separated. The separated serum and the blood samples were stored in refrigerator at 10°C for hametological and biochemical parameter analysis.

Conversion of Animal Study Dosage to Human Equivalent Dosage (HED)

According to *Anroop B. Nair, et al., (2016)*, the dosage factor method applied an exponent for body surface area (0.67), which account for difference in metabolic rate, to convert doses between animals and humans. Thus, HED is determined by the equation (No Observed Adverse Effect Levels – NOAEL):

$$\text{HED (mg/kg)} = \text{Animal NOAEL (mg/kg)} \times (\text{Weight of the animal (kg)} \div \text{Weight of the human (kg)})^{(1-0.67)}$$

Where, i. **HED (mg/kg) = Human Equivalent Dose in mg/kg of body weight**

ii. Animal dose (mg/kg) = Dose used in the animal study in mg/kg of animal body weight

iii. Km = Conversion factor based on the body surface area-to-weight ratio for different species

Km Calculation: For Human: Average Human Weight = 60 kg

$$\text{Average Human Body Surface Area} = 1.8 \text{ m}^2$$

Formula: Km = Average Body Weight ÷ Body Surface Area (m²)

PHASE – V

3.5. Nutrient and Organoleptic Analysis of the Selected Recipes Incorporated with Natural Food Colourants

Nutrients play a vital role in composition of living organisms found in foods. The necessity of a material to be utilized in foods or to be used as foods will be determined only if there are essential nutrients present in it that can be metabolized by human organs that begins with physiological and biochemical processes that involve nourishment through substances in food. The essential nutrients found in food are chemical components that at times cannot be synthesized to sufficient quantity in the body, which is necessary for growth and repairing of tissues, that are important for survival (*Stewart Truswell et al., 2023*).

3.5.1 Nutrient Analysis for the Selected Natural Food Colourants

To improve the quality of food intake, reduce malnutrition and micronutrients deficiencies especially among the population with increased nutrients requirements, the density of micro and

macronutrients present (Beal et al., 2022) in the natural food colourants were analyzed, to prescribe better alternative for synthetic food colourants available in the local markets. The major nutrients like carbohydrates, protein, fat, fiber along with the minor nutrients like the vitamins and minerals were analysed for 100 g of the natural food colourants of eucalyptus barks (*Eucalyptus grandis*) and annatto seeds (*Bixa Orellana*) using AOAC method (2023) (Appendix VIII).

3.5.2 Incorporation of the selected Natural Food Colourants in the Selected Recipes and Measuring the Intensity of Colour in Incorporated Selected Recipes

Extensive use of non-permitted carcinogenic, neurotoxic colorants is a common practice observed in different parts of India. Melanil yellow, a potent carcinogenic dye banned by the Government of India was found in turmeric, laddoo and besan at high levels in unorganized food sectors of West Bengal corroborating the lack of quality control and ignorance of food regulations. An analytical study performed to investigate synthetic food colorant usage in different states of India revealed that candyfloss, sugar toys, beverages, mouth fresheners, ice candy and bakery product samples contain exceeded the limit of colourants (Nandakumar, 2015). Thus, traditional and major consumed recipes such as kesari, motichoor laddoo, jalebi, chilli gobi, chilli chicken, aloo bajji, aloo bonda, tutti frutti, apple jam and jelly are selected for incorporation of natural colourants. The procedures are given in Appendix-IX.

The selected natural food colourants of annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) are added in the selected recipes with different variations. The variations of the natural food colourants are listed below.

Table – VIII

Variations of Annatto seeds (*Bixa orellana*) and Eucalyptus bark (*Eucalyptus grandis*) as Natural Food Colourants added in Selected Recipes

| Food Colourants used in Preparation of Recipes | Standard (mg) | Variation – I (mg) | Variation – II (mg) | Variation – III (mg) |
|---|----------------------|---------------------------|----------------------------|-----------------------------|
| Synthetic Food Colourant Bush | 1 (a pinch) | - | - | - |
| Annatto seeds (<i>Bixa orellana</i>) | - | 1 | 2 | 3 |
| Eucalyptus bark (<i>Eucalyptus grandis</i>) | - | 4 | 5 | 6 |

Based on the intensity of colour variations observed by the Food Colour Reader, selected natural food colourants were incorporated into selected recipes in different proportions. Annatto

seeds (*Bixa orellana*) was incorporated in variations I, II and III in the proportions of 1 mg, 2 mg and 3 mg. Eucalyptus bark (*Eucalyptus grandis*) is taken in the quantity of 4, 5 and 6 mg, as 1, 2 and 3 mg of the powdered substance of bark did not give any colour to the food. Thus, using the measures denoted in the table, standard preparation method of the selected recipes were prepared using Gold Camel as synthetic food colourant along with the variations of I, II and III for both annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) powders were incorporated in the selected recipes.

The recipes incorporated with annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) were measured for intensity through Food Colour Reader. The food colour reader was calibrated using the black and white cavity cover, placing it evenly below the cavity. Then the extracts of food colour samples were placed on a watch glass, with a plain white base. Few drops of the extracts were pipetted out on the watch glass. The cavity of the food colour reader was placed over the extract and the reading was taken with L* (lightness), a* (red/green) and b* (yellow/blue) indicating the colour hue of the food colourants were noted.

3.5.3 Organoleptic Evaluation of the Selected Recipes Incorporated with Natural Food Colourants

The properties of foods and their components observed through human senses are organoleptic properties, which include appearance, taste, flavour and texture. But in terms of scientific measures, organoleptic evaluation or sensory properties can be discriminative, descriptive and hedonic. When randomly selected panelists are instructed to use five hedonic scale test, determination of the preferences depends directly upon the acceptability and preference range of the panelists. Thus, after testing the selected recipes for the intensity of colour, they were organoleptically evaluated through semi-trained panel members to analyze the odour, taste, texture, flavor and consistency of the selected recipes using five hedonic scale rating. With five as the maximum score of excellent, four as very good, three as good, two as poor and one as fair, the selected and prepared recipes were evaluated accordingly (Azadeh *et al.*, 2021).

Ten trained panel members between the age group of 20 to 30 years old, which include adolescents and young adults were the organoleptic evaluators. Each panelist was given an individual score card shown as in Appendix-X and the panelists were asked to evaluate the products from the standard to the variations of annatto seeds (*Bixa orellana*) and eucalyptus bark (*Eucalyptus grandis*) incorporated into the recipes.

PHASE – VI

3.6 Development of Food Colour Sensor

In current scenario most of the food processing industries are majorly focusing on quality of food, nutritional value and method of processing as the consumers are demanding for foods lined up with qualities, sensory attributes and shelf life of the products. Emerging technology in Artificial Intelligence (AI) and Machine Learning (ML) helps to measure the drifting issues in food processing technology. AI is an interdisciplinary promising approach for promoting performances in different areas of food sectors. Tremendous changes are being carried out to solve problems and to grow food industries. To advance the technology multiple strategies were used in different food sectors (*Mounika et al., 2022*).

3.6.1 Developing a Food Colour Sensor to Identify the Level of Food Colours in Common Recipes

Artificial food colourings have been in the controversy for many years and scrutinized for being possibly linked to cancer, allergies and hyperactivity. Globally, natural as well as synthetic food colourants are being cautiously researched and regulated by the food safety authorities. In India, the Prevention of Food Adulteration Act, 1954 (now called the Food Safety and Standards Act, 2006), has been implemented for the quality assurance of various types of foods and food products and only eight food colourants have been suggested edible, but within prescribed limits. To explore the history and journey of the captivating colourant materials which have been brightening the world of foods for more than 3500 years, along with a detailed overview of their physical, chemical properties and the usage of eight permissible food colourants with toxicity in India (*Jyoti, 2020*).

Compliance with administrative necessities is basic to guarantee the security of nourishment items and ensure customer health. Synthetic nourishment colorants have been examined for their potential genotoxic and carcinogenic impacts. In spite of the fact that numerous colorants have been considered secure for utilization at allowed levels, long-term introduction to tall dosages or certain combinations of colorants may posture wellbeing risks. Certain manufactured nourishment colorants, especially those inferred from azo colors such as Tartrazine (E102), Nightfall Yellow (E110) and Allura Ruddy (E129), have been related with hyperactivity, consideration shortage hyperactivity clutter (ADHD) and behavioral changes in children. Be that as it may, the prove is blended, and not all considers have appeared a clear interface between nourishment colorants and

behavioral issues (*Asif Ahmed et al., 2023*).

The safety of food colorants is of paramount importance. Food regulatory agencies, such as the FDA in the United States and the European Food Safety Authority (EFSA) in Europe, regulate the use of food colorants and set maximum allowable levels to ensure consumer safety. Colorants must undergo rigorous testing to ensure they are safe for consumption. While most food colorants are considered safe for consumption, some consumers may have concerns about their health effects.

3.6.1.1 Fabrication of Food Colour Sensor

Select reasonable sensors, such as optical, biosensors, or electrochemical sensors, depending on the target colorants and location necessities. Calibrate the sensors to guarantee precise discovery inside the required concentration extend. Approve sensor execution through testing with known measures or reference materials. Create computer program to interface with the sensors, procure information, and handle crude sensor yields into important concentration values. Apply calibration bends or calculations to change over sensor signals into colorant concentrations.

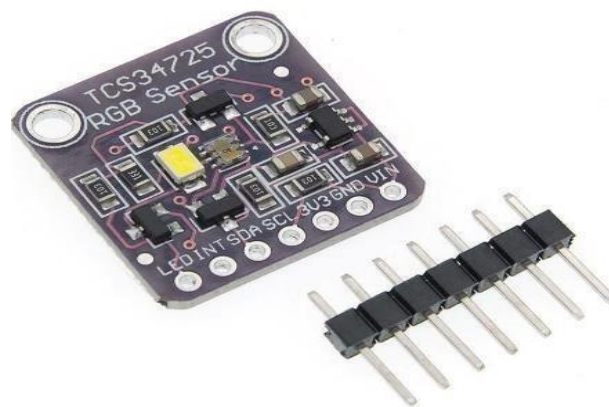
Plan and create a user-friendly webpage interface where clients can be associated with the colourant discovery framework. The webpage ought to empower the users to input test data, start location tests and see or download comes about. Coordinated sensor information handling program with the webpage to empower real time or bunch examination of colorant tests. Permit users to transfer sensor information records or input information straightforwardly through the webpage interface.

The device consists of a hardware and a software (Color Magic) combination, which may allow the hardware to be used either for quality testing or process monitoring applications. In the quality-testing mode, the software captures the image of the food products through the imaging system. Subsequently, the software processes the acquired image and computes color parameters in RGB (red, green, blue), CIELAB and HSI (hue, saturation, intensity) color spaces. The software also synthesizes and displays the perceived color information in the display panel. The datalog of the sample color information can be sent to the user. Further, a separate software (Color Process), which is installed in a central server, was developed to implement a wireless star network topology for multi-node process monitoring. The “Color Process” software allows users to acquire color information from multiple hardware. he implementation of the wireless sensor network (WSN) in designing the multi-node process monitoring makes the device a unique system (*Ankit Jain et al., 2020*).

3.6.1.2 System Specification in Description of Hardware

3.6.1.2 (a) TCS34725 SENSOR

The color sensor TCS34725 is used for the development of AI based sensor in detecting colours. According to (Siyuan *et al.*, 2023) the TCS34725 is a color light-to-digital converter that provides red, green, blue (RGB) and clear light sensing data digitally via the use of an IR filter and an I2C interface. The surface is illuminated with a white light source. In order to measure the RGB color wavelengths, the sensor then activates three filters, each of which has a wavelength sensitivity of



three. These three hues are used to determine the color of the material.

Plate - 3.6.1.2 (a)

RGB Color Sensor used to Determine the Colour in the selected Recipes

RGB Color Sensing: Using red (R), green (G), and blue (B) components as a reference, the TCS34725 sensor can precisely identify and quantify an object's color. A computed color temperature and lux value can also be obtained from it.

Programmable Integration Time: To better adapt to varying lighting conditions and increase accuracy, you can modify the integration time—the length of time the sensor gathers light data—using this module.

Features of the RGB Sensor: Digital return values for read, green, blue (RGB) and strong light detection are provided by the TCS34725 device. To achieve more accurate color management, a color-sensitive photodiode with integrated cut and targeted infrared shading filter reduces the incident infrared spectrum components. The TCS34725 is perfect for color-sensitive component solutions in a variety of lighting scenarios and through materials that attenuate light because of its IR shading filters (Sivachandran *et al.*, 2021).

3.6.1.2 (b) NODE MCU

The Node MCU ESP8266 is a well-liked development board for Internet of Things applications that provides an adaptable and reasonably priced means of connecting items to the internet. Its programming and Wi-Fi features make it possible to design and deploy IoT solutions quickly. Thirty two general purpose working registers and a robust instruction set are combined in the Atmel AVR core. The Arithmetic Logic Unit (ALU) is directly coupled to all 32 registers, enabling the access of two independent registers with a single instruction that is completed in a single clock cycle. This enables reduced power usage and extremely quick startup. The primary oscillator and the asynchronous oscillator in the prolonged standby mode (*Yogendra et al., 2019*).



Plate - 3.6.1.2 (b)
Node MCU used in Determining the Colour in the selected Recipes

3.6.1.2 (c) LCD Display

Information is frequently displayed in a variety of electronic devices using flat panel display technologies, such as LCD (Liquid Crystal Display). Performance of the LCD display as information display is that, an LCD display's main purpose is to provide the user with information graphically. Depending on the application and display capabilities, this information may consist of text, numbers, graphics, photos or video. Visual Representation in the liquid crystal displays use light-controlling techniques to manipulate light in order to produce visual representations of data. LCDs produce visible text and images by varying the amount of light allowed or blocked in different regions of the display (*Hai Wei Chen et al., 2018*).

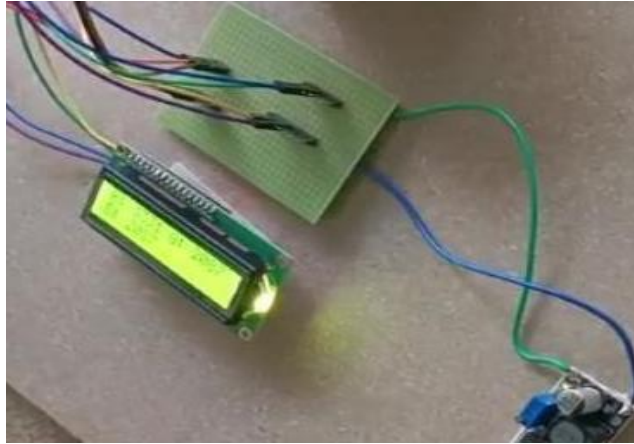


Plate – 3.6.1.2 (c)

LCD Display connected to the Sensor Board

3.6.1.2 (d) Bread Board

A solderless prototype incorporating electronics and test circuit designs is called a breadboard. The majority of electronic components in circuits can be connected by simply putting their leads or terminals into the holes and, if necessary, connecting them with wires. The breadboard is connected to the holes on top via metal strips that run underneath the board. The arrangement of the metal strips is seen below. Keeping this in mind that the remaining holes are connected vertically, while the holes in the top and bottom rows are connected horizontally and divide in the middle (*Sukanto et al., 2021*).

3.6.2 Specific Application of Software in the Developed Food Colour Sensor to find out the Level of Toxicity in the Formulated Recipes

Thonny: Thonny is a Python programming language integrated development environment (IDE). An outline of the Thonny platform that offers an easy-to-use interface along with a number of capabilities that make creating, debugging, and executing Python code easier. Among the essential elements are: code completion and syntax highlighting in a code editor. Python applications can be executed step-by-step and debugged using an integrated debugger. Variable explorer: a tool for viewing variable values while a program is running. An interactive Python shell for idea testing and code snippet experimentation. Supports the use of integrated package manager to install and manage Python packages (*Annamaa et al., 2015*).

3.6.2.1 Backend of the Sensor

3.6.2.1 (a) Cloud: Cloud describes the software and databases that operate on servers that may be accessed via the Internet. Around the globe, data centers house cloud servers. Users and

businesses can avoid managing physical servers or running software on their own computers by utilizing cloud computing. Upon getting the information, the sensor information stage stores it in a adaptable and versatile cloud-based capacity foundation. This capacity foundation is ordinarily based on advances such as databases (SQL or NoSQL), information lakes, or disseminated record frameworks, which can handle expansive volumes of information effectively. Once the information comes to the cloud, it is put away in a cloud-based capacity arrangement. This capacity may well be in a database, information lake, protest capacity, or a combination of these, depending on variables such as the volume of data, the require for real-time get to, and the specified information preparing capabilities (*Marosi et al., 2018*).

3.6.2.1 (b) Algorithm: KNN Algorithm (k-nearest neighbor) is used for high accurate predictions. The difficulties with the classification models are resolved by this algorithm. In order to categorize the data, the K-nearest neighbor, or K-NN, method essentially draws an imaginary border. The program will attempt to anticipate additional data points to the closest boundary line when they become available. Larger k values, then, correspond to softer separation curves and fewer complex models. Conversely, models with a smaller k number tend to overfit the data and become more complex (*Liu et al., 2022*).

Characteristics of the compiled algorithm is that, it is among the simplest machine learning algorithms and is suitable for a wide range of applications. The primary basis for it is feature similarity. KNN groups data points into the most similar class based on how similar they are to each other and their neighbor. Within the preparing stage, the calculation basically stores all the accessible information focuses and their comparing names or results. This step includes no express preparing, as the show memorizes the whole preparing dataset. The calculation at that point chooses the k closest information focuses (neighbors) to the unused information point based on the calculated separations. These closest neighbors serve as the foremost comparative occurrences to the modern information point. As the size of the dataset develops, the computational taken a toll of k-NN can ended up critical, because it requires calculating separations to all information focuses. Surmised closest neighbor calculations and information structures like KD-trees can offer assistance relieve this issue (*Marchang et al., 2020*).

3.6.2.2 Frontend of the Sensor

3.6.2.2 (a) HTML: HTML stands for Hyper Text Markup Language. Web pages are created using this common markup language for both their structure and content. Since HTML is supported by every web browser, HTML measures advance over time to suit unused innovations, highlights,

and best hones. Standard upgrades and modifications guarantee that HTML remains pertinent and versatile to the changing scene of the web. While HTML basically centers on characterizing report structure and substance, it can too encourage interactivity through components with shapes and input controls (), as well as integration with JavaScript for energetic behavior. HTML archives are various leveled in nature, organized as a tree-like structure known as the Report Protest Show (DOM). Components are settled inside other components, shaping parent-child connections (*Javadi et al., 2020*).

3.6.2.2 (b) CSS: CSS advances the division of substance (HTML) from introduction (styling). This division permits designers to overhaul the visual appearance of a webpage without modifying its structure or substance. It too encourages superior organization and viability of code. Selectors are designs utilized to choose and fashion components inside an HTML archive. CSS selectors can target components based on different criteria, such as component sort, lesson, ID, property, or relationship with other elements. Specificity could be a degree of how particular a selector is in focusing on components. It decides which fashion rules take priority when numerous rules strife. Specificity is calculated based on the combination of selectors utilized and their arrange of importance. Media questions permit designers to apply diverse styles based on different characteristics of the user's gadget, such as screen measure, determination, introduction, or color capabilities. Media inquiries are commonly utilized for making responsive plans that adjust to distinctive gadgets and screen sizes. To guarantee compatibility with diverse web browsers, designers may have to be utilize seller prefixes for certain CSS properties. Seller prefixes are prefixes included to CSS property names to demonstrate exploratory or browser-specific usage. In any case, as browser bolster progresses, the require for seller prefixes reduces (*Istif et al., 2023*).

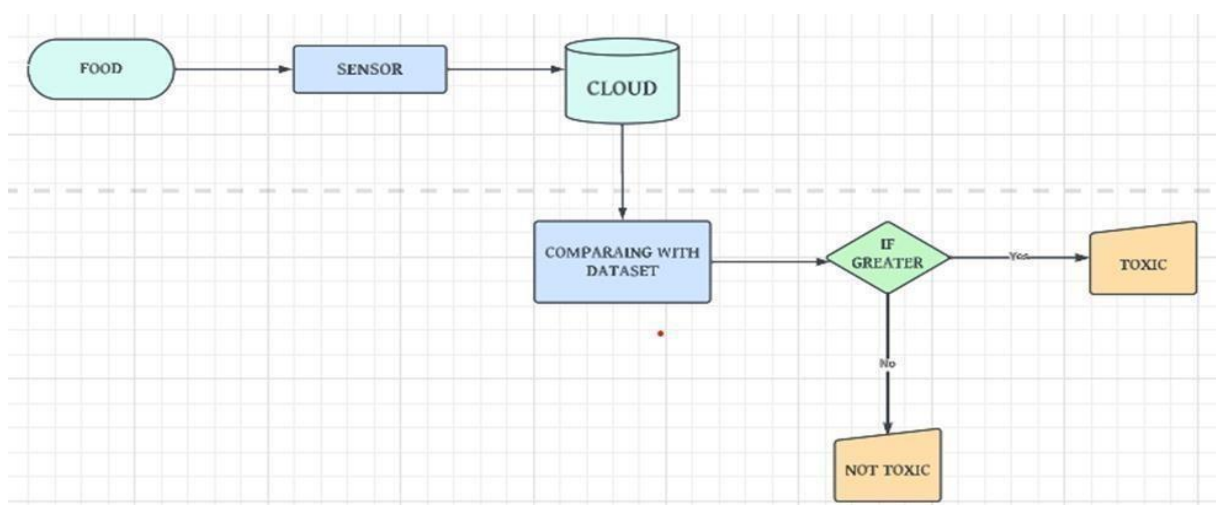


Figure – 3.6.2

Block Diagram of the Principle Mechanism of the Developed Food Colour Sensor

Sensors are data-based equipment used to provide prognosis help in early detecting system using intelligent signal processing technology. The efficient focus of improvising a set of chain based data that determines the performance of the tool is broadly classified as machine learning process, that analyze the encountered state of processing signals. In precise, with numerous advantages in terms of efficiency, cost and time saving hacks, the sensors used are not in sufficient level of increasing investment cost in acquisition of hardware and software that contributes to the purpose of the sensor (Mustafa Kuntoglu et al., 2021).

As for the regulations of Food Safety and Standards Authority of India (FSSAI), the toxicity level and the permissible level of usage of synthetic food colourants were added to the data set of the sensor and trained. According to FSSAI (2011), the food colourants must fulfill requirements that are listed below and the other colours mentioned were tartrazine, sunset yellow, indigo carmine and annatto. The limitations of annatto as food colourants were prescribed only for milk and milk products, as annatto is widely used in providing the food substances with shades of yellow and orange.

Table – IX

Synthetic Food Colourants and the Quantity to be used by FSSAI (2011).

| Name of the Synthetic Colourant | Colour Produced | Quantity of Colour to be used (NMT* % by Mass of Food) | |
|---------------------------------|-------------------------|--|---------------------------------|
| | | | |
| Erythrosine | Red | On drying | 13% |
| | | Water-insoluble | 0.2% |
| | | Inorganic iodine | 0.1% |
| | | Organic compound | 0.2% |
| β-carotene | Natural Yellow 26 | Spectrophotometric (aqueous solution) | 456 to 484µm |
| | | Colour reaction (purity) | NLT **96% |
| Chlorophyll | Natural Green 3 | Magbesium complexes | 10% |
| | | Residual solvents | 10% |
| Riboflavin | Yellow or orange-yellow | Spectrophotometry (aqueous solution) | 220 to 225, 266, 371 and 444 µm |
| | | Colour reaction (purity) | 97% |

NMT* - Not More Than

NLT** - Not Less Than

Along with these standard limitations of the synthetic food colourants, the RGB colour value of the common recipes like kesari, laddoo, chilli gobi and chilli chicken were collected as data set. As the sensor sensing the colourants in the food works under the principle of absorption and

reflection of light from the foods, the primary colours red, green and blue principle is used to set the data values. The nourishment colorant location gadget gives the taking after perceptions and comes about after the testing of the executed model. It too appears the prepared dataset.

Table – X

Data Set Constructed using the FSSAI Standard and RGB Value of Selected Foods to Measure the Toxicity

| Recipes Codes | R* | G* | B* | Target |
|----------------------|-----------|-----------|-----------|---------------|
| 1 | 856 | 145 | 145 | 0 |
| 1 | 675 | 130 | 130 | 0 |
| 1 | 702 | 125 | 125 | 0 |
| 1 | 578 | 125 | 125 | 0 |
| 1 | 718 | 136 | 136 | 0 |
| 2 | 172 | 36 | 36 | 1 |
| 2 | 125 | 33 | 33 | 1 |
| 2 | 166 | 34 | 34 | 1 |
| 2 | 254 | 30 | 30 | 1 |
| 2 | 110 | 36 | 36 | 1 |
| 3 | 870 | 160 | 160 | 2 |
| 3 | 296 | 145 | 145 | 2 |
| 3 | 960 | 172 | 172 | 2 |
| 3 | 293 | 57 | 57 | 2 |
| 3 | 439 | 89 | 89 | 2 |
| 2 | 407 | 68 | 68 | 3 |
| 2 | 418 | 69 | 69 | 3 |
| 2 | 509 | 101 | 101 | 3 |
| 2 | 188 | 43 | 43 | 3 |
| 2 | 194 | 38 | 38 | 3 |
| 1 | 222 | 42 | 42 | 4 |
| 1 | 223 | 43 | 43 | 4 |
| 1 | 622 | 90 | 90 | 4 |
| 1 | 924 | 131 | 131 | 4 |
| 1 | 496 | 72 | 72 | 4 |

R* - Red G* - Green B*- Blue

In the table, the selected recipes are coded with item numbers as 1, 2 and 3, where their target is the toxicity of colourants present in the foods. From the mentioned data set the coding (Appendix-XI) is done with KNN algorithm, then the food is concluded with the RGB value, when the values exceeds the given set criteria of colourants, then the food is classified into mild, medium and highly toxic.

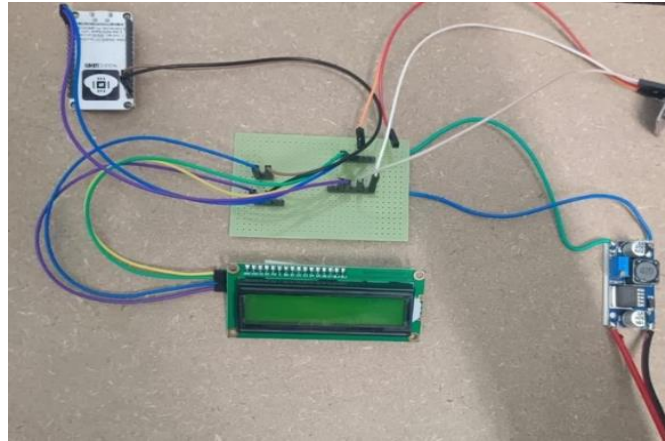


Plate – 3.6.2 (a)

System Prototype Implemented as Sensor Board

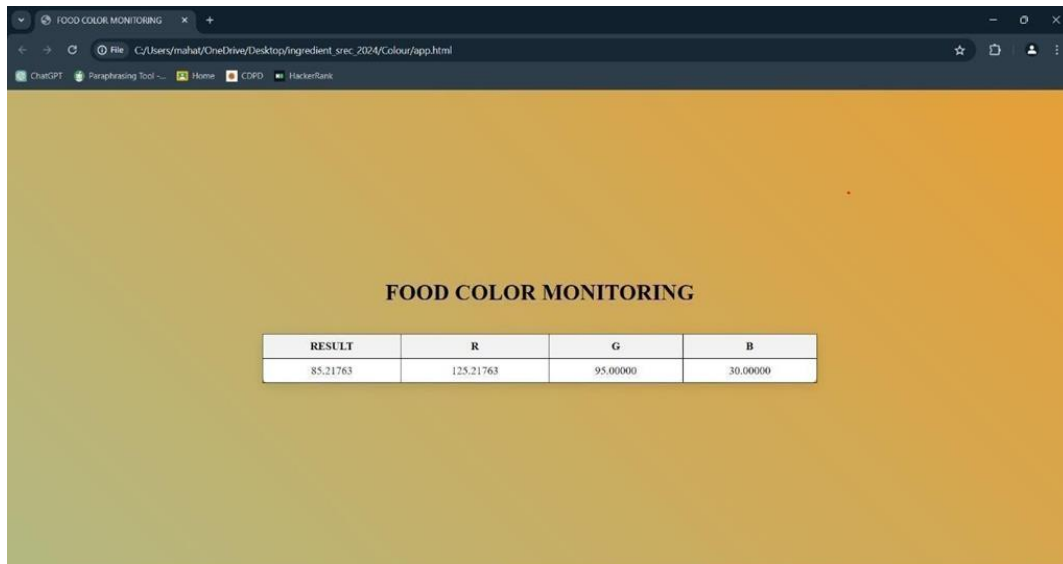


Plate – 3.6.2 (b)

Implemented Webpage Designed to Monitor the Toxicity of Foods Connected to the Sensor Board

Productive utilization of the developed signal processing system can aid the community in analyzing the toxicity in the food colourants that are being used in selected foods. This system is efficient for usage among both home makers and food vendors, who are conscious about serving their family and the community with healthy food through acquiring sufficient knowledge through their practice in handling the signal processing system called sensor.

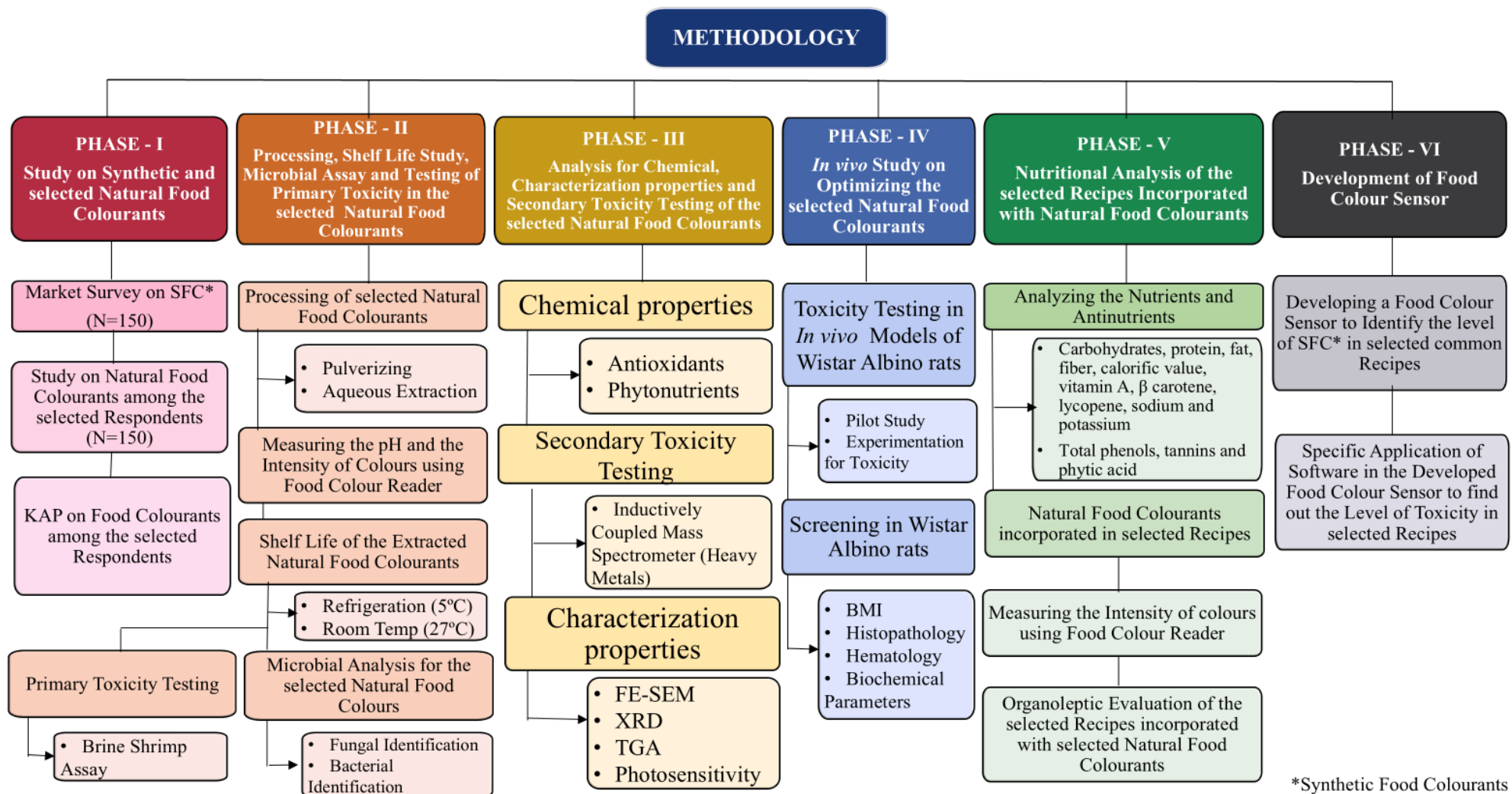


Figure – 3

Methodology of the Study on Physicochemical Analysis, *In vivo* Study and Development of Food Colour Sensor