REFERENCES

1. Abhari Khadijeh and Amin Mousavi Khaneghah (2020); Chapter Two - Alternative extraction techniques to obtain, isolate and purify proteins and bioactive from aquaculture and by-products; Advances in Food and Nutrition Research, Academic Press; Volume 92; Pages 35-52; ISSN 1043-4526; ISBN 9780128202166; https://doi.org/10.1016/bs.afnr.2019.12.004
2. Abraham S, Krishnan AT (2017), An analysis of how street food in India can be made safe food, Int J Curr Eng Sci Res, 4(12):57–64.
3. Abuajah CI, Ogbonna AC and Osuji CM (2015), Functional components and medicinal properties of food: a review, J Food Sci Technol; 52: 2522–2529
4. Aceto. M (2016), 8 - The Use of ICP-MS in Food Traceability, Advances in Food Traceability Techniques and Technologies, Improving Quality Throughout the Food Chain, Woodhead Publishing Series in Food Science, Technology and Nutrition (2016), Pages 137-164, https://doi.org/10.1016/B978-0-08-100310-7.00008-9
5. Acts and rules; Drugs and cosmetics act 1940. Website link: http://www.cdsco.nic.in/forms/contentpage1; Accessed on 02/04/2014.
6. Adeel Shahid, Tahsin Gulzar, Muhammad Azeem, Fazal-ur-Rehman, Muhammad Saeed, Iram Hanif and Naeem Iqbal (2017); Appraisal of marigold flower based lutein as natural coloirnat for textile dyeing under the influence of gamma radiations; Radiation Physics and Chemistry; Volume 130; Pg. 35-39; https://doi.org/10.1016/j.radphyschem.2016.07.010
7. Adrija Saha and Humairah Ayman (2022); A Review on the Extraction methods of Food Colours from Natural Sources; Annals: Food Science and Technology; Volume 23 (2); Pg. 274; ISSN: 2065-2828
8. Aguilar-Machado D, Morales-Oyervides L, Contreras-Esquivel JC, Cristobal Aguilar, Alejandro Mendez-Zavala, Javier Raso and Julio Montanes (2017); Effect of ohmic heating processing conditions on color stability of fungal pigments; Food Science and Technology International; Volume 23 (4); Pg. 338-348. doi:10.1177/1082013216689514
9. Ahmad Fauzi, Nurina Titisari, Sutarso amd Venny Mellisa (2020); Getamicin Nephrotoxicity in Animal Model: Study of Kidney Histopathology and Physiological Functions; IOP Conference Series: Earth and Environmental Science; Volume 465; International Conference: Improving Tropical Animal Production for Food Security 22-24 November 2019, South East Sulawesi, Indonesia; 012005

10. Ahmed E. Yousef and Carolyn Carlstrom (2003); Food Microbiology-A Laboratory Manual; ISBN: 0-471-39105-0
11. Ahmed Mohammed Asif, Abdulrahman S. Al-Khalifa, Doha M. Al-Nouri and Mohamed Fekry Serag El-din (2021); Average daily intake of artificially food color additives by school children in Saudi Arabia; Journal of King Saud University – Science; Volume 35 (4), 102596; https://doi.org/10.1016/j.jksus.2023.102596
12. Ahmed Mohammed Asif, Abdulrahman S. Al-Khalifa, Doha M. Al-Nouri and Mohamed Fekry Serag El-din (2021); Dietary intake of artificial food color additives containing food products by school-going children; Saudi Journal of Biological Sciences; Volume 28, Issue 1; Pg. 27-34; https://doi.org/10.1016/j.sjbs.2020.08.025
13. Akhlash P. Singh and Sunil Kumar (2020); Chapter 7 – Applications of Tannins in Industry; Tannins: Structural Properties, Biological Properties and Current Knowledge; ISBN: 978-1-78984-796-3; Online ISBN: 978-1-78984-797-0; eBook (PDF) ISBN: 978-1-83968-358-9; https://dx.doi.org/10.5772/intechopen.80170
14. Alafeef, M., Moitra, P., Dighe, K., Pan D (2021); RNA-extraction-free nano-amplified colorimetric test for point-of-care clinical diagnosis of COVID-19; Nat Protoc.; Volume 16; Pg. 3141–3162; https://doi.org/10.1038/s41596-021-00546-w
15. Albuquerque R. Bianca, M. Beatriz P. P. Oliveira, Lillian Barros and Isabel C. F. R. Ferreira (2020); Could fruits be a reliable source of food colourants? Pros and cons of these natural additives; Critical Reviews in Food Science and Nutrition; Pg. 805-835; https://doi.org/10.1080/10408398.2020.1746904
16. Alena I. Palianskikh, Sergey I. Sychik, Sergey M. Leschev, Yekatsiaryna M. Pliashak, Tatsiana A. Fiodarava, Ludmila L. Belyshava (2022); Development and validation of the HPLC-DAD method for the quantification of 16 synthetic dyes in various foods and the use of liquid anion exchange extraction for qualitative expression determination; Food Chemistry; Volume 369, 130947; ISSN 0308-8146; https://doi.org/10.1016/j.foodchem.2021.130947
17. Alexander Gosslau (2016); Assessment of Food Toxicology, Food Science and Human Wellness; Volume 5 (3); Pg. 103-115; https://doi.org/10.1016/j.fshw.2016.05.003
18. Al-Hilphy Asaad Rehman, Alaa M. Al-Musafer, Mohsen Gavahian (2020); Pilot-scale ohmic heating-assisted extraction of wheat bran bioactive compounds: Effects of the extract on corn oil stability; Food Research International; Volume 137, 109649; ISSN 0963-9969; https://doi.org/10.1016/j.foodres.2020.109649

19. Ali Reza Jahangiri, Payam Rajabi Kalvani, Samaneh Shapouri, Amirhossein Sari, Stefan Talu and Yousef Seyed Jalili (2020); Quantitative SEM characterisation of ceramic target prior and after magnetron sputtering: a case study of aluminium zinc oxide; Journal of Microscopy; Volume 281, Issue 3: Pg. 190-201; https://doi.org/10.1111/jmi.12961
20. Almaguer M. R, R.R. Carpio, T.L.M. Alves, J.P. Bassin (2018); Experimental study and kinetic modelling of the enzymatic degradation of the azo dye Crystal Ponceau 6R by turnip (Brassica rapa) peroxidase; Journal of Environmental Chemical Engineering; Volume 6 (1); Pages 610-615; 2213-3437; https://doi.org/10.1016/j.jece.2017.12.039
21. Amin Arshia, Tehseen Zahra, Hafsa Raja, Mehroze Amin, Erum Dilshad, Muhammad Naveed, Iftikhar Ahmed (2020); Chapter 5 - Major natural sinks for harboring microorganisms with altered antibiotic resistance versus major human contributing sources of antibiotic resistance: a detailed insight; In Advances in Environmental Pollution Research series, Antibiotics and Antimicrobial Resistance Genes in the Environment, Elsevier; Volume 1; Pages 70-98; ISBN 9780128188828; https://doi.org/10.1016/B978-0-12-818882-8.00005-X.
22. Anelise Christ-Ribeiro, Maciel, J.V., Bier, E.M., Pinto J. S. and Dias D. (2022); Application of Electrochemical Sensors in the Determination of Synthetic Dyes in Foods or Beverages and Their Toxicological Effects on Human Health: a Review; Food Anal. Methods; Volume 15; Pg. 2394–2413; https://doi.org/10.1007/s12161-022-02282-7
23. Ankit Jain, Bikash K. Pradhan, Pardesh Mahapatra, Sirsendu S. Ray, Sumit Chakravarty and Kunal Pal (2020); Development of low-cost food color monitoring system; Color Research and Application; Volume 46, Issue 2; Pg. 430-445; https://doi.org/10.1002/col.22577
24. Annamaa Aivar (2015); Introducing Thonny, a Python IDE for learning Programming; Association for Computing Machinery; Koli Calling '15: Proceedings of the 15th Koli Calling Conference on Computing Education Research; Pages 117 – 121; https://doi.org/10.1145/2828959.2828969
25. Anroop B Nair and Shery Jacob (2016); A Simple practice guide for dose conversion between animals and human; J Basic Clin Pharm. Volume 7 (2); Pg. 27-31; doi: 10.4103/0976-0105.177703
26. Anshida Mayeen, Kala M. S., Jayalakshmy M. S., Sabu Thomas, Jacob Philip, Didier Rouxel, R. N. Bhowmik and Nandakumar Kalarikkal (2019); Flexible and self-standing nickel ferrite–PVDF-TrFE cast films: promising candidates for high-end magnetoelectric applications; Dalton Trans; 49, 16961-16973; https://doi.org/10.1039/C9DT02856K

27. Arrizabalaga-Larranaga. A, S. Epigmenio-Chamú, F.J. Santos, E. Moyano (2021); Determination of banned dyes in red spices by ultra-high performance liquid chromatography-atmospheric pressure ionizationtandem mass spectrometry; Analytica Chimica Acta; Volume 1164, 338519; https://doi.org/10.1016/j.aca.2021.338519
28. Arslan, S. (2018). Peanut milk production by the microfluidization, physicochemical, textural and rheological properties of peanut milk products; yoghurt and kefir (Master's thesis, Middle East Technical University).
29. Asher Mullard (2024); FDA approves first peanut allergy drug; Nature Reviews Drug Discovery; Volume 19 (3); Gale OneFile: Health and Medicine, dx.doi.org/10.1038/d41573-020-00025-z. Accessed 13 Dec. 2024.
30. Asif Ahmed Mohammed, Abdulrahman S Al-Khalifa, Doha M Al-Nouri and Mohamed Fekry Serag El-din (2023); Average daily intake of artificially food color additives by school children in Saudi Arabia; Journal of King Saud University-Science; Volume 35, Issue 4: 102596; https://doi.org/10.1016/j.jksus.2023.102596
31. Asif Ahmed Mohammed, Abdulrahman S. Al-Khalifa, Doha M. Al-Nouri and Mohamed Fekry Serag El-din (2021); Dietary intake of artificial food additives containing food products by school-going children; Saudi Journal of Biological Sciences; Volume 28, Issue 1; Pg. 27-34; https://doi.org/10.1016/j.sjbs.2020.08.025
32. Asif Ali, Yi Wai Chiang and Rafael M. Santos (2022); X-ray Diffraction Techniques for Mineral Characterization: A Review for Engineers of the Fundamentals, Applications and Research Directions; Minerals; Volume 12 (2); 205; https://doi.org/10.3390/min12020205
33. Azadeh, Gharibi, and Nazanin, Khakipour, (2021) Evaluation of Lead, Cadmium and Nickel Contamination in Lactuca Sativa Cultivated in Savadkooh Region, Iran. Asian Soil Research Journal; Volume 5 (2); Pg. 25-33. ISSN 2582-3973
34. Azza Dawoud H. Dawoud, Mohamed El-Hassan Shayoub and Sara Mohamed El-Hassan Shayoub (2015); Acute Toxicity Studies of Ethanolic Extract of Eucalyptus Camaldulensis Dehnh Leaves; Journal of Network Communications and Emerging Technologies (JNCET); Volume 2 (2); ISSN: 2395-5317
35. B.P. Harp and J.N. Barrows (2015); 4 - US regulation of color additives in foods, Editor(s): Michael J. Scotter; In Woodhead Publishing Series in Food Science, Technology and Nutrition-Colour Additives for Foods and Beverages; Pg. 75-88; ISBN 9781782420118; https://doi.org/10.1016/B978-1-78242-011-8.00004-0
36. Baiyi Lu and Yajing Zhao (2017); Concise Review, Photo-oxidation of phytochemicals in food and control: a review, Special Issue: J Ann NY Acad Sci, 1398 (1): 72-82

37. Balta, I., Sevastre, B., Mireşan, V. *et al.* Protective effect of blackthorn fruits (*Prunus spinosa*) against tartrazine toxicity development in albino Wistar rats. *BMC Chemistry* **13**, 104 (2019). https://doi.org/10.1186/s13065-019-0610-y
38. Bastaki Maria, Thomas Farrell, Sachin Bhusari, Kamala Pant and Rohan Kulkarani (2017); Lack of genotoxicity in vivo for food colour additive Tartrazine; Food and Chemical Toxicology; Volume 105, Pg. 278-284; https://doi.org/10.1016/j.fct.2017.04.034
39. Beal Ty, Gretchen A Stevens, Mduduzi N N Mbuya, HAnqi Luo, Lynnette M Neufeld (2022); Micronutrient deficiencies among preschool-aged children and women of reproductive age worldwide: a pooled analysis of individual-level data from population-representative surveys; The Lancet Global Health; Volume 10, Issue 11; E1590-1599
40. Becky A. Briesacher, Stephen B. Soumerai, Fang Zhang, Sengwee Toh, Susan E. Andrade, Joann L. Wagner, Azadeh Shoaibi and Jerry H. Gurwitz (2013); A critical review of methods to evaluate the impact of FDA regulatory actions; Pharmacoepidemiology and Drug Safety; Volume 22 (9); Pg. 986-994; https://doi.org/10.1002/pds.3480
41. Benito Soto-Blanco (2022); Chapter 12 – Herbal glycosides in healthcare; Herbal Biomedical in Healthcare Applications; Pg. 239-282; https://doi.org/10.1016/B978-0-323-85852-6.00021-4
42. Benjamin J. Cohen (2019); Advanced Introduction to International Political Economy – Second Edition; Library of Congress Control Number: 2018959457; ISBN: 978-1-78897-155-3
43. Bigwood. T, J.A. Hudson, C. Billington, G.V. Carey-Smith, J.A. Heinemann (2008); Phage inactivation of foodborne pathogens on cooked and raw meat; Food Microbiology; Volume 25, Issue 2; Pages 400-406; ISSN 0740-0020; https://doi.org/10.1016/j.fm.2007.11.003
44. Bonnet M., J.C. Lagier, D. Raoult and S. Khelaifia (2020); Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology; New Microbes and New Infections; Volume 34, 100622; https://doi.org/10.1016/j.nmni.2019.100622
45. Brochier Bethania, Giovana Domeneghini Mercalli, Ligia Damasceno Ferrerirs Marczak (2019); Effect of moderate electric field on peroxidase activity, phenolic compounds and color during ohmic heating of sugarcane juice; Journal of Food Processing and Preservation; Volume 43 (12); e14254; https://doi.org/10.1111/jfpp.14254
46. Burrow T. Alison (2019); Nano-Fed Americans: Novel & Untested Food Additives are Bypassing the Regulatory System into Consumers Mouths; Journal of Science and Technology; Volume 25

47. Cameron Faustman, Daniel Aaron, Nicole Negowetti and Emily Broad Leib (2020); Ten years post-GAO assessment, FDA remains uninformed of potentially harmful GRAS substances in foods; Critical Reviews in Food Science and Nutrition; Volume 61(8), Pg. 1260–1268. https://doi.org/10.1080/10408398.2020.1756217
48. Carter, L.; Chase, H.R.; Gieseker, C.M.; Hasbrouck, N.R.; Stine, C.B.; Khan, A.; Ewing-Peeples, L.J.; Tall, B.D (2018).; Gopinath, G.R. Analysis of enterotoxigenic *Bacillus cereus* strains from dried foods using whole genome sequencing, multi-locus sequence analysis and toxin gene prevalence and distribution using endpoint PCR analysis; Int. J. Food Microbiol.; Volume 284; Pg. 31–39.
49. CEPI. Key Statistics 2020 European Pulp and Paper Industry; CEPI: Brussels, Belgium, 2020.
50. Chaitanya Lakshmi G. (2014); Research Journal of Chemical Sciences, International Science Congress Association 87, Review Paper, Food Coloring: The Natural Way; ISSN 2231-606X, Vol. 4(2), 87-96
51. Cheng, Q., Xia, S., Tong, J., and Wu, K. (2015); Highly-sensitive electrochemical sensing platforms for food colourants based on the property-tuning of porous carbon; Analytica Chimica Acta,; Volume 887; Pg. 75-81.
52. Christine Natalia Palis, Anna Safitri and Fatchiyah Fatchiyah (2024); Phytochemical screening of Indonesian brown rice (*Oryza Sativa* L.) extracts compounds; AIP Conference Proceeding; 3055, 020005; https://doi.org/10.1063/5.0193626
53. Ciociola Arthur. A., Karlstadt Robyn G., Pambianco Daniel j., Woods Karen L., Ehrenpreis Eli D., (2014); The Food and Drug Administration Advisory Committee and Panels: How They are Applied to the Drug Regulatory Process; American Journal of Gastroenterology; Volume 109 (10); Pg. 1508-1512; *DOI:* 10.1038/ajg.2014.85
54. Claudia R. Cardarelli, Mart de Toledo Benassi and Adriana Z. Mercadante (2008), Characterization of different annatto extracts based on antioxidant and colour properties: LWT – Food Sci Technol.; 41 (9): 1689-1693
55. Codex Alimentarius Commission (CAC) (2019); General Standard on Food Additives. CODEX STAN 1992-1995. Last updated in 2019, Available online at: https://www.fao.org/gsfaonline
56. Cooksey C. J. (2019); The red insect dyes: carminic, kermesic and laccaic acids and their derivatives; Biotechnic and Histochemistry; Pg. 100-107; https://doi.org/10.1080/10520295.2018.1511065

57. Cuma Zehiroglu and Sevim Beyza Ozturk Sarikaya (2019), The importance of antioxidants and place in today's scientific and technological studies, J Food Sci Technol.; 56(11): 4757–4774.
58. Cuong TV and Chin KB. (2016); Effects of Annatto (Bixa orellana L.) Seeds Powder on Physicochemical Properties; Antioxidant and Antimicrobial Activities of Pork Patties during Refrigerated Storage; Korean J Food Sci Anim Resour.; Volume 36 (4); Pg. 476-86. doi: 10.5851/kosfa.2016.36.4.476. PMID: 27621688; PMCID: PMC5018507.
59. Damergi Eya, Jean-Paul Schwitzguebel, Dominik Refardt, Shivam Sharma, Christof Holliger and Christian Ludwig (2017); Extraction of carotenoids from *Chlorella vulgaris* using green solvents and syngas production from residual biomass; Algal Research; Volume 25; Page: 488-495; https://doi.org/10.1016/j.algal.2017.05.003
60. Daniel Bobo, Kye J. Robinson, Jiaul Islm, Kristofer J. Thurecht and Simon R. Corrie (2016); Nanoparticle-Based Medicines: A Review of FDA-Approved Materials and Clinical Trials to Date; Pharm Res.; Volume 33, Pg. 2373–2387; https://doi.org/10.1007/s11095-016-1958-5
61. Daniel J. Klionsky, Amal Kamal Abdel-Aziz, Sara Abdelfatah, Mahmoud Abdellatif, Asghar Abdoli, Steffrn Abel… Tong, C. K. (2021); Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition); Autophagy; Volume 17 (1); Pg. 1–382. https://doi.org/10.1080/15548627.2020.1797280
62. Darsaut, T. E., Bing, F., Salazkin, I., Gevry, G., and Raymond, J. (2012). Flow diverters failing to occlude experimental bifurcation or curved sidewall aneurysms: an in vivo study in canines: Laboratory investigation; Journal of Neurosurgery JNS; Volume 117 (1); Pg. 37-44. https://doi.org/10.3171/2012.4.JNS111916
63. David R Schoneker (2013); Coloring Agents for Use in Pharmaceuticals; Encyclopedia of Pharmaceutical Science and Technology, Six Volume Set-Fourth Edition; eBook ISBN: 9781351124874
64. Deacon J. Lile (2023); Chapter 79 - FDA overview; In Handbook for Designing and Conducting Clinical and Translational Research, Translational Surgery, Academic Press; Pages 501-507, ISBN 9780323903004, https://doi.org/10.1016/B978-0-323-90300-4.00076-8
65. Delia B. Rodriguez-Amamya (2019); Update on natural food pigments-A mini-review on carotenoids, anthocyanins and betalains; Food Research International; Volume 124, Pg. 200-205; https://doi.org/10.1016/j.foodres.2018.05.028
66. Devi Priya M, E.A. Siril (2014), Traditional and Modern Use of Indian Madder (Rubia cordifolia L.): An Overview; International Journal of Pharmaceutical Sciences Review and

Research; 25(1), Article No. 27, Pages: 154-164 ISSN 0976 – 044X; www.globalresearchonline.net; 154

67. Devi Priya M, Siril EA (2012); Pharmacognostic studies on Indian Madder (Rubia cordifolia L.); J. Pharmacog. Phytochem.; Volume 1; Pg. 113-120
68. Dey Subhashish and Bommu Hema Nagababu (2022); Applications of food color and bio-preservatives in the food and its effect on the human health; Food Chemistry Advances; Volume 1, 100019; https://doi.org/10.1016/j.focha.2022.100019
69. Dharma Rao Tompa, Aruldoss Immanuel, Srimari Srikanth, Saraboji Kadhirvel (2021); Trends and strategies to combat viral infections: A review on FDA approved antiviral drugs; International Journal of Biological Macromolecules; Volume 172; Pages 524-541; ISSN 0141-8130, https://doi.org/10.1016/j.ijbiomac.2021.01.076
70. Dilip Ghosh (2014); NSW Food Authority – Food Safety Regulations in Australia and New Zealand Food Standards; Journal of the Science of Food and Agriculture; Volume 94 (10); Pg. 1970-1973; https://doi.org/10.1002/jsfa.6657
71. Drugs and Cosmetics (Vth Amendment) Rules, 2005. website link: Drugcontrol.org;GSR 285 (E) dated 11.5.2005 Indian FDA.htm. Accessed on 02/04/2014.
72. Dudkina. A. A, T. N. Volgina and N. V. Saranchina (2019); Colorimetric determination of food colourants using solid phase extraction into polymethacrylate matrix; *Talanta,*; Volume **202**; Pg. 186 —189
73. Dun Wu, Jian Wu, Guangqing Hu, Guojun Cai and Xia Gao (2024); Fine Structure Characterization of Representative Coal-Bearing Rocks of the Late Carboniferous Taiyuan Formation in Huainan Coalfield: Combined SEM, FESEM and muCT Techniques; ACS Omega; Volume 9, Issue 2; 2850-2865; https://doi.org/10.1021/acsomega.3c08278
74. Dyanne Medina-Flores, Gabriela Ulloa-Urizar, Rosella Camere-Colarossi, Stefany Caballero-Garcia, Frank Mayta-Tovalino, Juana del Valle-Mendoza (2016); Antibacterial activity of *Bixa orellana* L. (achiote) against *Streptococcus mutans* and *Streptococcus sanguinis*; Asian Pacific Journal of Tropical Biomedicine; Volume 6 (5); https://doi.org/10.1016/j.apjtb.2016.03.005
75. El Golli Narges, Ines Bini-Dhouib, Alcha Jrad, Imene Boudali, Basma Nasri, Nadia Belhadjhmida and Salousa El Fazaa (2016); Toxicity Induced after Subchronic Administration of the Synthetic Food Dye Tartrazine in Adult Rats, Role of Oxidative Stress; Recent Advances in Biology and Medicine; Volume 2; Pg. 20-28; E-ISSN: 2378-654X; dx.doi.org/10.18639/RABM.2016.02.284474

76. EL-Hak Heba N.Gad, Abdel Raouf A. Moustafa and Samira R. Mansour (2018); Toxic effect of Moringa peregrina seeds on histological and biochemical analyses of adult male Albino rats; Toxicology Reports; Volume 5, Pages 38-45; https://doi.org/10.1016/j.toxrep.2017.12.012
77. Elias Blanco, Laura Arias, Luis Vazquez, Maria del Pozo, Lorenzo Sánchez, María Dolores Petit-Domínguez, Carmen Quintana, Elena Casero (2020); Sensor based on diamond nanoparticles and WS_2 for ponceau 4R and tartrazine determination: Influence of green solvents employed for WS_2 exfoliation; FlatChem; Volume 23, 100185; https://doi.org/10.1016/j.flatc.2020.100185
78. Erasmus WS, van Hasselt L, Ebbinge ML, van Ruth MS (2021), Real or fake yellow in the vibrant colour craze: rapid detection of lead chromate in turmeric. Food Control 12:107714. https://doi.org/10.1016/j.foodcont.2020.107714.
79. Erhan Şensoy (2024); Comparison of the effect of Sunset yellow on the stomach and small intestine of developmental period of mice; Science Direct; Volume 10, Issue 11; DOI: 10.1016/j.heliyon.2024.e31998
80. Fabbri A, Chartres N and Bero LA. (2016); Association of industry sponsorship with outcomes of nutrition studies: a systematic review and meta-analysis. JAMA Internal Medicine.; 176(12):1769–77; https://doi.10.1001/jamainternmed.2016.6721
81. Fathi Zahra, Larissa Ribeiro Ramos Tramontin, Gholamhossein Ebrahimipour, Irina Borodina, Farshad Darvishi (2020), Metabolic engineering of *Saccharomyces cerevisiae* for production of B-carotene from hydrophobic substrates; *FEMS Yeast Research*, Volume 21, Issue 1; foaa068, https://doi.org/10.1093/femsyr/foaa068
82. FDA. Federal Registry. 2004. Final Rule Declaring Dietary Supplements Containing Ephedrine Alkaloids Adulterated Because They Present an Unreasonable Risk. *Final rule*. 69(28): 6787–854; https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/DietarySupplements/ucm072997.htm Accessed 2017 August 29.
83. FDA. US. Food and Drug Administration (2017); Color Additives History, Available online at: https://www.fda.gov/industry/color-additives/color-additives-history
84. FDA. US. Food and Drug Administration (2018a); Color Additives Questions and Answer Consumers; Available online at: https://www.fda.gov/food/food-additives-petitions/color-additives-questions-and-answers-consumers

85. FDA. US. Food and Drug Administration (2019); Color additive status list of 2019; Available online at: https://www.fda.gov/industry/color-additives-inventories/color-additive-status-list
86. Feng, J., Li, J., Huang, W., Cheng H., Zhanh Z and Li Lijun (2021); Capillary Zone Electrophoresis Determination of Five Trace Food Additives in Beverage Samples Using Counterflow Transient Isotachophoresis; Food Anal. Methods; Volume 14; Pg. 380–388; https://doi.org/10.1007/s12161-020-01894-1
87. Folachodé U. G. Akogou, A. P. Polycarpe Kayodé, Heidy M. W. den Besten and Anita R. Linnemann (2018); Extraction methods and food uses of a natural red colorant from dye sorghum; Journal of the Science of Food and Agriculture; Volume 98 (1); Pg. 361-368; https://doi.org/10.1002/jsfa.8479
88. Food and Drug Adminitration (FDA) – Myanmar (2016)
89. FSSAI (2009), Food Safety and Standards regulations. https://old.fssai.gov.in/Portals/0/FSSAI%20regulations.pdf.
90. FSSAI (2011) Food safety and standards (food products standards and food additives) regulations. https://www.fssai.gov.in/cms/food-safety-and-standards-regulations.php.
91. Gaisford Simon, Vicky Kett and Peter Haines (2016); Principles of Thermal Analysis and Calorimetry, Second Edition; Royal Society of Chemistry; ISSN: 978-1-78262-051-8
92. Gallardo-Cabrera. C and Rojas-barahoma. A (2015); Stability study of an aqueous formulation of the annatto dye; International Food Research Journal; Volume 22 (5); Pg. 2149-2154
93. Gamzenur Ozsin and Ayse Eren Putun (2017); Kinetics and evolved gas analysis for pyrolysis of food processing wastes using TGA/MS/FT-IR; Waste Management; Volume 64; Pg. 315-326; https://doi.org/10.1016/j.wasman.2017.03.020
94. Ganesh. E N. and Stella (2018); Distributed Energy Efficient Zonal Relay Node Based Multi Path Secure Routing Protocol (DEZMSR) for Wireless Sensor Networks; Journal of Computational and Theoretical Nanoscience; Volume 15 (2); Pg. 403-408 (6), American Scientific Publishers DOI: https://doi.org/10.1166/jctn.2018.7102
95. Gao Jie, Hefei Huang, Jizhao Liu, Jianrong Zeng, Ruobing Xie, Yan Li (2016); Coalescence mechanism of helium bubble during tensile deformation revealed by *in situ* small-angle X-ray scattering; Scripta Materialia; Volume 158; Pg. 121-125; https://doi.org/10.1016/j.scriptamat.2018.08.050
96. Garnweidner-Holme L, Lieberg HS, Irgens-Jensen H, Telle-Hansen VH (2021) Facilitators of and barriers to collaboration between universities and the food industry in nutrition research: a qualitative study. Food Nutr Res 27:65. https://doi.org/10.29219/fnr.v65.7874

97. Garver K (2018) Examples of artificial intelligence in the food industry
98. Getasew A, Muluken A, Legesse A (2016) A survey on awareness of consumers about health problems of food additives in packaged foods and their attitude toward consumption of packaged foods: a case study at Jimma University. Int Food Res J 23:375–380
99. Giridhar, P. and Venugopalan, Akshatha and Parimalan, R. (2013); A Review on Annatto Dye Extraction, Analysis and Processing – A Food Technology Perspective*;* Journal of Scientific Research and Reports; Volume 3 (2). Pg. 327-348. ISSN 23200227
100. Gizaw, Z. Public health risks related to food safety issues in the food market: a systematic literature review. Environ Health Prev Med 24, 68 (2019). https://doi.org/10.1186/s12199-019-0825-5
101. Gominho, J., Costa, R.A., Lourenço, A., Quilho, T., and Pereira, H (2021) ; *Eucalyptus globulus* Stumps Bark: Chemical and Anatomical Characterization Under a Valorisation Perspective; Waste Biomass Valor; Volume 12; Pg. 1253–1265; https://doi.org/10.1007/s12649-020-01098-y
102. Gornall J. (2015); Sugar: spinning a web of influence. British Medical Journal.; https://doi.10.1136/bmj.h231
103. Goverdina C.H. Derksen, Frédérique L. van Holthoon, Hendra M. Willemen, Cyrille A.M. Krul, Maurice C.R. Franssen, Teris A. van Beek (2021); Development of a process for obtaining non-mutagenic madder root (Rubia tinctorum) extract for textile dyeing; Industrial Crops and Products; Volume 164, 113344; ISSN 0926-6690; https://doi.org/10.1016/j.indcrop.2021.113344
104. Gray Sacks, Devorah Riesenberg, Melissa Mialon, Sarah Dean, Adrian J. Cameron (2020); The characteristics and extent of food industry involvement in peer-reviewed research articles from 10 leading nutrition-related journals in 2018; Journal of Food Protection; Volume 53, Issue 5; Pages 430-440; PMCID: PMC7743938; PMID: 33326431; 15(12): e0243144., doi: 10.1371/journal.pone.0243144
105. Guilherme Dallarmi Sorita, Simone Palma Favaro, Alan Ambrosi and Marco Di Luccio (2023); Aqueous extraction processing: An innovative and sustainable approach for recovery of unconventional oils; Trends in Food Science and Technology; Volume 133; Pg. 99-113; https://doi.org/10.1016/j.tifs.2023.01.019
106. Gupta V, Sanjnani M, Gupta RK (2020) Street foods: contemporary preference of tourist and its role as a destination attraction in India. Int J Cult Tour Hosp Res 14:136–154. https://doi.org/10.1108/IJCTHR-07-2018-0092

107. Hai Wei Chen, Jiun Haw Lee, Bo Yen Lin, Stanley Chen and Shin Tson Wu (2018); Liquid crystal display and organic light-emitting diode display: present status and future perspectives; Light Science and Applications; Volume 7, 17168; https://doi.org/10.1038/lsa.2017.168
108. Hamed Ahmed Mohamed, Awad A. Awad, Ahmed E. Abdul-Mobdy, Abdulhakeem Alzaharani and Ahmad Mohammad Salamatullah (2021); Buffalo Yogurt Fortified with Eucalyptus (*Eucalyptus camaldulensis)* and Myrrh (*Commiphora Myrrha*) Essential Oils: New Insights into the Functional Properties and Extended Shelf Life; Molecules; Volume 26 (22), 6853; https://doi.org/10.3390/molecules26226853
109. Hanan A. Azoz and Riham M. Raafat (2012); Effect of Lead Toxicity on Cytogenisity, Biochemical Constituents and Tissue Residue with Protective Role of Activated Charcoal and Casein in Male Rats; Australian Journal of Basic and Applied Sciences, Volume 6(7); Pg. 497-509; ISSN 1991-8178
110. Handayani Isti, Aisah Tri Septiana, Pepita Haryanti, Budi Sustriawan and Susanto Budi Sulistyo (2023); Evaluation of colour and physicochemical properties of annatto seed aquadest extract in the variation pH of solvent; AIP Conference Proceedings; Pg. 2586, 060009; https://doi.org/10.1063/5.0108054
111. Handayani, M.; Ganta, M.; Susilo, D.N.A.; Yahya, M.S.; Sunnardianto, G.K.; Darsono, N.; Sulistiyono, E.; Setiawan, I.; Lestari, F.P.; Erryani, (2019); A. Synthesis of graphene oxide from used electrode graphite with controlled oxidation process. IOP Conf. Ser. Mater. Sci. Eng.; Volume 541, 12032.
112. Hayat Khan, Aditya S. Yerramilli, Adrien D'Oliverira, Terry L. Alford, Daria C. Boffito and Gregory S. Patience (2020); Experimental methods un chemical engineering: X-ray diffraction spectroscopy-XRD; The Canadian Journal of Chemical Engineering: Volume 98, Issue 6; Pg. 1255-1266; https://doi.org/10.1002/cjce.23747
113. Hema Kanekar and Anubha Khal (2014); Review Article – Coloring Agents: Current Regulatory Perspective for Coloring Agents Intended for Pharmaceutical Cosmetic Use; International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR); ISSN: 2249-6084
114. Hobbs Cheryl, Mihoko Koyanagi, Carol Swartz, Jeffrey Davis, Robert Maronpot, Leslie Recio and Shim-mo Hayashi (2018); Genetoxicity evaluation of the naturally derived food colourant, gardenia blue, and its precursor, genipin; Food and Chemical Toxicology; Volume 118, Pg. 695-708; https://doi.org/10.1016/j.fct.2018.06.001

115. Huang Hui, Juan Bai, Jiao Li, Lulu Lei, Wenjing Zhang, Shujun Yan and Yongxin Li (2019); Fluorometric and colorimetric analysis of alkaline phosphatase activity based on a nucleotide coordinated copper ion mimicking polyphenol oxidase; Journal of Materials Chemistry B; Volume 7 (42); Pg. 6508-6514; DOI: https://doi.org/10.1039/C9TB01390C

116. Hucheng Deng, Xiaofei Hu, Huazhou Andy Li, Bin Luo and Wei Wang (2016); Improved pore-structure characterization in shale formations with FESEM technique; Journal of Natural Gas Science and Engineering; Volume 35, Part A; Pg. 309-319; https://doi.org/10.1016/j.jngse.2016.08.063

117. Ibáñez Elena, David Villanueva Bermejo, Guillermo Reglero and Tiziana Fornari (2016); Effect of cosolvents (ethyl lactate, ethyl acetate and ethanol) on the supercritical CO2 extraction of caffeine from green tea; The Journal of Supercritical Fluids; Volume 107; Pages 507-512; ISSN 0896-8446; https://doi.org/10.1016/j.supflu.2015.07.008

118. Idham Zuhaili, Ahmad Syahmi Zaini, Nicky Rahmana Putra, Nurfarhain Mohamed Rusli, Noor Sabariah Mahat, Lee Nian Yian and Mohd Azizi Che Yunus (2020); Effect of flow rate. Particle size and modifier ratio on the supercritical fluid extraction of anthocyanins from *Hibiscus sabdariffa* (L).; IOP Conference Series: Materials Science and Engineering, Volume 932, 1st International Conference on Science, Engineering and Technologygy (ICSET) 2020 Penang, Malaysia; Volume 932, 012031; DOI 10.1088/1757-899X/932/1/012031

119. Indhumathy Jagadeeswaran, Nandhini Palani, Ganesh Lakshmanan (2022); FDA-CFR Title 21-Food and Drugs: Parts 800 to 1299. In: Timiri Shanmugam, P.S., Thangaraju, P., Palani, N., Sampath, T. (eds); Medical Device Guidelines and Regulations Handbook; Springer, Cham; Pg. 189-236; https://doi.org/10.1007/978-3-030-91855-2_12

120. India Today (2018) Common food adulterants in India. India Today. https://www.indiatoday.in/education-today/gk-current-affairs/story/common-food-adulterants-in-india-1370601-2018-10-19. Accessed 22 Sept 2022

121. Istif, E., Mirzajani, H., Dağ, Ç., Fariborz m, Elif Yaren O., Cengiz C., Hatice Ceylan K., Iskender Yilgor, Emel Yilgor and Levent Beker (2023); Miniaturized wireless sensor enables real-time monitoring of food spoilage. Nat Food; Volume 4: Pg. 427–436; https://doi.org/10.1038/s43016-023-00750-9

122. J. C. Arthur, A. Monteagudo-Mera, C. Jobin, T. Keku, J. M. Bruno-Barcena, M. A. Azcarate-Peril (2016); High purity galacto-oligosaccharides enhance specific *Bifidobacterium* species and their metabolic activity in the mouse gut microbiome; Beneficial Microbes; Volume 7 (2); Pg. 247-264; https://doi.org/10.3920/BM2015.0114

123. Jailson Rodrigues dos Santos, Larissa de Sousa Soares, Bruno Moreira Soares, Marlene de Gomes Farias, Victor Alves de Oliveira, Natan Antônio Batista de Sousa, Helber Alves Negreiros, Felipe Cavalcanti Carneiro da Silva, Ana Paula Peron, Ana Carolina Landim Pacheco, Márcia Maria Mendes Marques, Juan Carlos Ramos Gonçalves, Raquel Carvalho Montenegro, Muhammad Torequl Islam, Javad Sharifi-Rad, Mohammad S. Mubarak, Ana Amélia Carvalho de Melo Cavalcante and João Marcelo de Castro e Sousa (2024); Cytotoxic and mutagenic effects of the food additive tartrazine on eukaryotic cells. *BMC Pharmacol Toxicol* 23, 95 (2022). https://doi.org/10.1186/s40360-022-00638-7
124. James Harvey Young (2014); Pure food: securing the Federal Food and Drugs Act of 1906; ISBN: 0-691-04763-4
125. Javadi, G., Taleai, M. (2020); Integration of User Generated Geo-contents and Official Data to Assess Quality of Life in Intra-national Level. Soc Indic Res; 152, 205–235; https://doi.org/10.1007/s11205-020-02437-1
126. Jesus Meirielly S., Lina F. Ballesteros, Ricardo N. Pereira, Zlatina Genisheva, Ana C. Carvalho, Cristina Pereira-Wilson, José A Teixeira and Lucília Domingues (2020); Ohmic heating polyphenolic extracts from vine pruning residue with enhanced biological activity; Food Chemistry; Volume 316, 126298; ISSN 0308-8146; https://doi.org/10.1016/j.foodchem.2020.126298
127. Jiao Guangling and Azadeh Kermanshahi pour (2018); Extraction of anthocyanins from haskap berry pulp using supercritical carbon dioxide: Influence of co-solvent composition and pretreatment; LWT; Volume 98; Pages 237-244; ISSN 0023-6438; https://doi.org/10.1016/j.lwt.2018.08.042
128. John E. Lincoln (2012); Overview of the US FDA GMPs: Good Manufacturing Practice (GMP)/Quality System (QS) Regulation (21 CFR Part 820); Journal of Validation Technology; New York; Volume 18 (3); Pg. 17-22
129. Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2016); Guidance document for WHO monographers and reviewers evaluating food additives (excluding enzyme preparations and flavouring agents) World Health Organization, Geneva. Available online at: https;//www.who.int
130. Joosse Sofie (2016); The making and Re-making of a Regional Product: the case of Zeeland Madder; Rural Development; Sociologia Ruralis; Volume 56 (2); Pg. 248-269; https://doi.org/10.1111/soru.12076

131. Joshka Verduin, Mimi J den Uiji, Ruud JB Peters and Maarten R van Bommei (2020); Review article: Photodegradation Products and their Analysis in Food; HSOA Journal of Food Science and Nutrition; Volume 6: 067; DOI: 10.24966/FSN-1076/100067
132. Joy L. Frestedt (2018); Chapter 16 - Foods, Food Additives, and Generally Regarded as Safe (GRAS) Food Assessments, Editor(s): Alina Maria Holban, Alexandru Mihai Grumezescu,: In Handbook of Food Bioengineering, Food Control and Biosecurity, Academic Press; Pages 543-565; ISBN 9780128114452, https://doi.org/10.1016/B978-0-12-811445-2.00016-7
133. Jyoti Mittal (2020); permissible Synthetic Food Dyes in India; Reson. Springer Nature Link; Volume 25; Pg. 567-577; https://doi.org/10.1007/s12045-020-0970-6
134. Kakoli Dutta and Rosalin Nath (2023); Application of Colorimetry in Food Industries; Chapter Metrics Overview; DOI: 10.5772/intechopen.112099
135. Kamal A. Amin and Fawzia S. Al-Shehri (2018); Toxicological and safety assessment of tartrazine as a synthetic food additive on health biomarker: A review; African Journal of Biotechnology; Volume 17 (6); 077C95C55887; Pg. 139-149; https://doi.org/10.5897/AJB2017.16300
136. Kettler S., Kennedy M., McNamara C., Oberdörfer R., O'Mahony C., Schnabel J., Smith B., Sprong C., Faludi R., Tennant D. (2015); Assessing and reporting uncertainties in dietary exposure analysis: mapping of uncertainties in a tiered approach.; Food Chem. Toxicol.; 82:79–95. https://www.sciencedirect.com/science/article/pii/S0278691515001167
137. Khiralla Ghada Mahmoud, Safaa Ahmad Salem and Wagih Ahmad El-Malky (2015); Effect of Natural and Synthetic Food Coloring Agents on the Balance of some Horomones in Rats, Division of Medical Foods, National Organization for Drug Control and Research (NODCAR), Giza, Egypt; International Journal of Food Science and Nutrition Engineering; Volume 5 (2); Pg. 88-95; DOI:10.5923/j.food.20150502.03.
138. Kilic, E. (2018); Non-wood forestry products ın the ottoman empire according to archives; 4th International Non-Wood Forest Products Symposium; 4-6 October Bursa/Tukey, Pg.513–517
139. Kilulya, K.F.; Msagati, T.A.M.; Mamba, B.B.; Catherine Ngila, J.; Bush, T. (2014); Effect of Site, Species and Tree Size on the Quantitative Variation of Lipophilic Extractives in Eucalyptus Woods Used for Pulping in South Africa.; Ind. Crops Prod.; Volume 56; Pg. 166–174; https://doi.org/10.1016/j.indcrop.2014.02.017

140. König. J (2015); 2 – Food Colour Additives of Synthetic Origin; Colour Additives for Foods and Beverages; Woodhead Publishing Series in Food Science, Technology and Nutrition; Pg. 35-60; https://doi.org/10.1016/B978-1-78242-011-8.00002-7

141. Krystyna Pyrzynska and Anna Pekal (2013); Application of Free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples; Analytical Methods; Issue 2013; DOI: https://doi.org/10.1039/C3AY40367J

142. Kumari Rewa and Meghwal Murlidhar (2016); Benefits of food colours and safety in usage; Food-Ingredients South Asia, 1-15 January 2016; Pg. 22-24

143. Kutralam-Muniasamy Gurusamy, V. C. Shruti, Fermin Perez-Guevara and Joel Alba Flores (2024); The emerging field of inductively coupled plasma mass spectrometry for (micro) nanoplastic analysis: "The #As" analysis, advances and applications; TrAC Trends in Analytical Chemistry; Volume 174, 117673; https://doi.org/10.1016/j.trac.2024.117673

144. Leepica K, Siva R (2021) Strategies to meet the global demand for natural food colorant bixin: a multidisciplinary approach. J Biotechnol 338:40–51. https://doi.org/10.1016/j.jbiotec.2021.07.007

145. Lehto, S., Buchweitz, M., Klimm, A., Straßburger, R., Bechtold, C., and Ulberth, F. (2017). Comparison of food colour regulations in the EU and the US: a review of current provisions. Food Additives & Contaminants: Part A; Volume 34 (3); Pg. 335-355.

146. Leonardo M. de Souza Mesquita, Margarida Martins, Luciana P. Pisant, Sonia P M. Ventura, Veridiana V. de Rosso (2020); Insights on the use of alternative solvents and technologies to recover bio-based food pigments; Comprehensive Reviews in Food Science and Food Safety; Volume 20, Issue 1**;** Pg. 787-818; https://doi.org/10.1111/1541-4337.12685

147. Lim, H. S., Choi, E., Lee, J. H., Lee, G., and Kim, M. (2020); Analysis of illegal colourants (citrus red II, diethyl yellow, dimethyl yellow, metanil yellow and rhodamine B) in foods by LC-UV and LC-MS/MS; Food Additives & Contaminants: Part A; Volume 37 (6); Pg. 895–904. https://doi.org/10.1080/19440049.2020.1746840

148. Lima, L., Miranda, I., Knapic, S., Quilho, T., and Pereirah (2018); Chemical and anatomical characterization, and antioxidant properties of barks from 11 *Eucalyptus* species; Eur. J. Wood Prod.; Volume 76; Pg. 783–792; https://doi.org/10.1007/s00107-017-1247-y

149. LipskikhOlgaI, ElenaI. Korotkova, Jiri Barek, Vlastimil Vyskocil, Muhammad Saqib, Ekat erinaP. Khristunova (2020); Simultaneous voltammetric determination of Brilliant Blue

FCF and Tartrazine for food quality control; Talanta; Volume 218, 121136; https://doi.org/10.1016/j.talanta.2020.121136

150. Liu Li, Bing Hu, Shuai Liu, KE Wang and Hanyang Gu (2022); Recognition of gas-liquid flow regimes in helically coiled tube using wire-mesh sensor and KNN algorithm; International Journal of Multiphase Flow; Volume 154, 104144; https://doi.org/10.1016/j.ijmultiphaseflow.2022.104144
151. Lone, N. A., and Yadav, D. K. (2016); Dynamics and Determinants of Food Inflation in India; In International Conference on Changing Paradigm Management Practices for Sustainable Development (ICCPMPSD 2016); Pg. 73-77
152. Loypimai Patiwit, Anuchita Moongngarm, Pheeraya Chottanom, Tanongsak Moontree (2015); Ohmic heating-assisted extraction of anthocyanins from black rice bran to prepare a natural food colourant; Innovative Food Science & Emerging Technologies; Volume 27; Pages 102-110; ISSN 1466-8564; https://doi.org/10.1016/j.ifset.2014.12.009
153. Marchang. N and R. Tripathi (2020); KNN-ST: Exploiting Spatio-Temporal Correlation for Missing Data Inference in Environmental Crowd Sensing; IEEE Sensors Journal, Volume 21, no. 3; Pg. 3429-3436, doi: 10.1109/JSEN.2020.3024976.
154. Marghoob Hasan and Abdelmarouf Mohieldein (2016); In vivo Evaluation of Anti Diabetic, Hypolipidemic, Antioxidative Activities of Saudi Date Seed Extract on Streptozotocin Induced Diabetic Rats; J Clin Diagn Res; Volume 10 (3); FF06-FF12; PMCID: PMC4843279 PMID: 27134893; doi: 10.7860/JCDR/2016/16879.7419
155. Maria Bastaki, Thomas Farrell, Sachin Bhusari, Xiaoyu Bi and Carolyn Scrafford – US FDA (2017); Estimated daily intake and safety of FD & A food colour additives in the US population; Food Additives and Contaminants: Part A; Volume 34 (6); Pg. 891-904; https://doi.org/10.1080/19440049.2017.1308018
156. Maria Manuela Silva, Fernando Henrique Reboredo and Fernando Ceboia Lidon (2022); Food Colour Additives: A Synoptical Overview in their Chemical Properties, Applications in Food Products and Health Side Effects; Foods; Volume 11 (3); https://doi.org/10.3390/foods11030379
157. Marosi Attila Csaba, Attila Farkas, Robert Lovas (2018); An Adaptive Cloud-Based IoT Back-end Architecture and Its Applications; 2018 26th Euromicro International Conference on Parallel, Distributed and Network-based Processing (PDP), Cambridge, UK, Pg. 513-520, doi: 10.1109/PDP2018.2018.00087.

158. Marta Igual and Javier Martinez-Monzo (2022); Physicochemical Properties and Structure Changes of Food Products during Processing; Foods; Volume 11 (15); 2365; https://doi.org/10.3390/foods11152365
159. Marta Oleszek, Iwona Kowalska and Wieslaw Oleszek (2019); Phytochemicals in bioenergy crops; Phytochem Rev; Volume 18; Pg. 893–927; https://doi.org/10.1007/s11101-019-09639-7
160. Martins N., Roriz C.L., Morales P., Barros L., Ferreira I.C. (2016); Food colorants: Challenges, opportunities and current desires of agro-industries to ensure consumer expectations and regulatory practices. Tr. Food Sci. Tech.; Volume 52; Pg. 1–15. https://www.sciencedirect.com/science/article/abs/pii/S0924224416300784
161. Marzieh Alizadeh, Ersin Demir, Nida Aydogdu, Najmeh Zare, Fatemeh Karimi, S. Masoud Kandomal, Hassan Rokni, Younes Ghasemi (2022); Recent advantages in electrochemical monitoring for the analysis of amaranth and carminic acid as food color; Food and Chemical Toxicology; Volume 163, 112929; ISSN 0278-6915; https://doi.org/10.1016/j.fct.2022.112929
162. McCambridge J and Mialon M. (2018); Alcohol industry involvement in science: A systematic review of the perspectives of the alcohol research community. Drug and Alcohol Review.; Volume 37(5); Pg 565–579. https://doi.10.1111/dar.12826
163. Medeiros RA, Lourençao BC, Rocha-Filho RC, Fatibello-Filho O (2012); Simultaneous voltammetric determination of synthetic colorants in food using a cathodically pretreated boron-doped diamond electrode; Talanta; Volume 97; Pg. 291–297. doi:10.1016/j.talanta.2012.04.033
164. Mehri Salari, Alireza Zali, Farzad Ashrafi, Masoud Etemadifar, Soumya Sharma, Nastaran Hajizadeh and Helia Ashourizadeh (2020), Incidence of Anxiety in Parkinson's Disease During the Coronavirus Disease (COVID- 19) Pandemic, 35(7): 1095–1096, PMCID: PMC7273007, PMID: 32395849, doi: 10.1002/mds.28116
165. Michael C. Jelavish (2016); Making the move from synthetic to natural colors; 70th PMCA Production Conference
166. Mingyan Cao, Jiamin Chen, Xiaohong Sun, Feng Xie and Boyan Li (2023); Theoretical predictions and experimental verifications of SERS detection in colorants; Royal Society of Chemistry, RSC ADVANCES; Volume 13 (22), Pg. 15086-15098; DOI: 10.1039/D3RA01584J

167. Mohammad Mehmandoust, Pouran Pourhakkak, Farzaneh Hasannia, Ozgur Ozalp, Mustafa Soylak, Nevin Erk (2022); A reusable and sensitive electrochemical sensor for determination of Allura red in the presence of Tartrazine based on functionalized nanodiamond@SiO_2@TiO_2; an electrochemical and molecular docking investigation; Food and Chemical Toxicology; Volume 164, 113080; https://doi.org/10.1016/j.fct.2022.113080

168. Monica Garcia Silva, Nunes, P., Oliveira, P., Ferreira, R., Fardilha, M., Moreira-Gonçalves, D., Duarte, J. A., Oliveira, M. M., & Peixoto, F. (2022). Long-Term Aerobic Training Improves Mitochondrial and Antioxidant Function in the Liver of Wistar Rats Preventing Hepatic Age-Related Function Decline. *Biology*, *11*(12), 1750. https://doi.org/10.3390/biology11121750

169. Monika Thakur and Modi, V. K. (2022); Biocolorants in food: Sources, extraction, applications and future prospects; Critical Reviews in Food Science and Nutrition; Volume 64 (14); Pg. 4674–4713. https://doi.org/10.1080/10408398.2022.2144997

170. Motwadie Mina E., Mohamed M. Hashem, Khaled Abo-EL-Sooud, Yasmina M. Abd-Elhakim, Abeer E. El-Metwally, Haytham A. Ali (2021); Modulation of immune functions, inflammatory response, and cytokine production following long-term oral exposure to three food additives; thiabendazole, monosodium glutamate, and brilliant blue in rats; International Immunopharmacology; Volume 98, 107902; ISSN 1567-5769; https://doi.org/10.1016/j.intimp.2021.107902

171. Mounika Addanki, Bhaargavi Ilangavan, Sushmita Mandal, Waghaye Shraddha Yashwant, Swetha Priya Gali and Akalya Shanmugam (2022); Prospects of ultrasonically extracted food bioactives in the field of non-invasive biomedical applications-A review; Ultrasonics Sonochemistry; Volume 89, 106121; https://doi.org/10.1016/j.ultsonch.2022.106121

172. Mozaffarian D. (2017); Conflict of interest and the role of the food industry in nutrition research. JAMA. 2017;317(17):1755–6; https://doi.10.1001/jama.2017.3456

173. MRF (2018) Global food colorants market: information by type (natural and synthetic), application (bakery and confectionery, dairy and frozen desserts, beverages, sweet and savory snacks, meat, poultry and seafood products and others) and region (North America, Europe, Asia-Pacific and Rest of the World)—forecast till 2027. Market Research Future. https://www.marketresearchfuture.com/reports/food-colorants-market-8812. Accessed 22 Sept 2022

174. Muhammad Shafur Rahman (2020); Handbook of Food Preservatives, Third Edition; ISBN-13: 978-1-4987-4048-7

175. Mukesh Kumar Mahato, Abhay Kumar Singh and Soma Giri (2022); Evaluation of metal contamination, flux and the associated human health risk from atmospheric dustfall in metal mining areas of Southern Jharkhand, India; Environmental Science and Pollution Research; Volume 29; Pg. 30348-30362; https://doi.org/10.1007/s11356-021-18463-6
176. Muller-Maatsch J, Schweiggert MR, Carle R (2016) Adulteration of anthocyanin- and betalain-based coloring foodstuffs with the textile dye 'Reactive Red 195' and its detection by spectrophotometric, chromatic and HPLC-PDA-MS/MS analyses. Food Control 70:333–338. https://doi.org/10.1016/j.foodcont.2016.06.012
177. Mustafa Kuntoğlu and Hacı Sağlam (2021); Investigation of signal behaviors for sensor fusion with tool condition monitoring system in turning; Measurement; Volume 173, 108582; ISSN 0263-2241; https://doi.org/10.1016/j.measurement.2020.108582
178. Muthusamy Ramesh and Arunachalam Muthuraman (2018); Chapter 1 – Flavouring and Colouring Agents; Health Risks and Potential Problems; Natural and Artificial Flavouring Agents and Food Dyes: Handbook of Food Engineering; Pg. 1-28; https://doi.org/10.1016/B978-0-12-811518-3.00001-6
179. Najlaa Bint Saud Al-Saud (2020); Impact of curcumin treatment on diabetic albino rats; Saudi Journal of Biological Sciences; Volume 27, Issue 2, February 2020, Pages 689-694
180. Nandakumar T (2015) Synthetic food colours pose health risk. The Hindu. https://www.thehindu.com/news/cities/Thiruvananthapuram/synthetic-food-colours-pose-health-risk/article7825708.ece. Accessed 21 Jan 2023
181. Narges El Golli, Ines Bini-Dhouib, Alcha Jrad, Imene Boudali, Basma Nasri, Nadia Belhadjhmida and Salousa El Fazaa (2016); Toxicity Induced after Subchronic Administration of the Synthetic Food Dye Tartrazine in Adult Rats, Role of Oxidative Stress; Recent Advances in Biology and Medicine; Volume 2; Pg. 20-28; E-ISSN: 2378-654X; dx.doi.org/10.18639/RABM.2016.02.284474
182. Naseer. S, Afzal. M.S, Nisa A, Hussain. S, Ahmad M. A, Parveen. S, Anjum. F, Riaz. M, (2019), Research Article, Extraction of brown dye from Eucalyptus bark and its applications in food storage, Quality Assurance and Safety of Crops & Foods: 11 (8)-Pages: 769 – 780, https://doi.org/10.3920/QAS2019.1569, ISSN: 1757-8361, Online ISSN: 1757-837X.
183. Nath PP, Sarkar K, Tarafder P, Mondal M, Das K, Paul G (2015) Practice of using metanil yellow as food colour to process food in unorganized sector of West Bengal—a case study. Int Food Res J 22(4):1424–1428

184. Nawaz Rimsha (2019); Natural food grade dye extraction techniques, Review Paper, Research Journal of Chemical Vol. 9(2), 24-27, ISSN 2231-606X

185. Neiva Duarte M., Solange Araújo, Jorge Gominho, Angélica de Cássia Carneiro, Helena Pereira (2018); Potential of Eucalyptus globulus industrial bark as a biorefinery feedstock: Chemical and fuel characterization; Industrial Crops and Products; Volume 123; Pages 262-270; ISSN 0926-6690; https://doi.org/10.1016/j.indcrop.2018.06.070.

186. Neiva, D.M.; Rencoret, J.; Marques, G.; Gutiérrez, A.; Gominho, J.; Pereira, H.; Río, J.C. del (2020); Lignin from Tree Barks: Chemical Structure and Valorization; Chem Sus Chem; Volume 13; Pg. 4537–4547. https://doi.org/10.1002/cssc.202000431

187. New Oo, Yu Mon Saw, Hnin New Ni Aye, ZAW ZAW Aung, Huin Nandar Kyaw, Ae Mon Tun, Tetsuyoshi Kariya, Eiko Yamamoto and Nobuyuki Hamajima (2019); Consumption of foods containing prohibited artificial colors among middle-school children in Nay Pyi Taw union territory, Myanmar ; *BMC Public Health;* Volume 19, Article number: 344

188. Nidhi Shukla M N., Deo K N Uttam and Rajiv Dutta (2021); Biochemical evaluation of the Bottle gourd (*Lagenaria siceraria*) fruit by nondestructive fourier transform Raman and Attenuated total reflectance fourier transform Infrared Spectroscopy; Plant Cell Biotechnology and Molecular Biology; Volume 22 (45 & 46); Pg. 95-102; ISSN: 0972-2025

189. Novais Claudia, Adriana K. Molina, Rui M. V. Abreu, Celestino Santo-Buelga, Isabel C. F. R. Ferreira, Carla Pereira and Lillian Barros (2022); Natural Food Colorants and Preservatives: A Review, a Demand and a Challenge; Journal of Agricultural and Food Chemistry, Volume 70 (9); Pg. 2789-2805; https://doi.org/10.1021/acs.jafc.1c07533

190. Novais Claudia, Adriana K. Molina, Rui M. V. Abreu, Celestino Santo-Buelga, Isabel C. F. R. Ferreira, Carla Pereira and Lillian Barros (2022); Natural Food Colorants and Preservatives: A Review, a Demand and a Challenge; Journal of Agricultural and Food Chemistry, Volume 70 (9); Pg. 2789-2805; https://doi.org/10.1021/acs.jafc.1c07533

191. Novelli ELS, Diniz YS, Galhardi CM, Ebaid GMX, Rodrigues HG, Mani F, Fernandes AAH, Cicogna AC, Novelli Filho JLVB (2007); Anthropometrical parameters and markers of obesity in rats; Lab Anim.; Volume 41(1); Pg. 111-119. http://dx.doi.org/10.1258/002367707779399518

192. NSW Food Authority. Artificial colors in local and imported food products. Newington NSW; 2014

193. Nwoba, E.G., Ogbonna, C.N., Ishika, T., Vadiveloo, A. (2020); Microalgal Pigments: A Source of Natural Food Colors. In: Alam, M., Xu, JL., Wang, Z. (eds); Microalgae

Biotechnology for Food, Health and High Value Products; Springer, Singapore; Pg. 81-123; ISBN: 978-981-15-0168-5; Online ISBN: 978-981-15-0169-2; https://doi.org/10.1007/978-981-15-0169-2_3

194. Ogundare O. S., M. Sirajuddin, K. O. Iyevhobu and E. I. Ogundare (2022); Histopathological Effects of Potash on the Hearts of Adult Wistar Rats; Asian Journal of Cardiology Research 6(3): 23-30, 2022; Article no.AJCR.82759; : https://www.sdiarticle5.com/review-history/82759
195. Olugbenga Awolu and Oluwatoyin Oladeji (2021); Natural plant Pigments and Derivatives in Functional Foods Developments; Eurasian Journal of Food Science and Technology; Volume 5 (1); Pg. 25-40
196. Organization for Economic Cooperation and Development Guidelines (OECD, 2018)
197. Owen P. Jennifer, Christian A. Yates and Robert N. Kelsh (2021); Differential growth is a critical determinant of zebrafish pigment pattern formation; bioRXiv, The Preprint Server for Biology-Cold Spring Harbor Laboratory; https://doi.org/10.1101/2021.06.11.448058
198. Pan Y. M., P. F. Hu, K. W. Leung and X. Y. Zhang (2018); Compact Single-/Dual-Polarized Filtering Dielectric Resonator Antennas; IEEE Transactions on Antennas and Propagation; Volume 66 (9); Pg. 4474-4484, doi: 10.1109/TAP.2018.2845457.
199. Pariza W. Michael, Kevin O. Gillies, Sarah F. Kraak-Ripple, Gregory Leyer and Amy B. Smith (2015); Determining the safety of microbial cultures for consumption by humans and animals; Regulatory Toxicology an Pharmacology; Volume 73, Issue 1; Pg. 164-171; https://doi.org/10.1016/j.yrtph.2015.07.003
200. Patra Santanu, Ekta Roy, Rashmi Madhuri, Prashant K. Sharma (2017); 17 - A technique comes to life for security of life: the food contaminant sensors; Nanobiosensors, Academic Press; Pages 713-772; ISBN 9780128043011; https://doi.org/10.1016/B978-0-12-804301-1.00017-5
201. Peng Xiong-Xin, Guang-Ming Bao, Yu-Fei Zhong, Li Zhang, Kang-Bo Zeng, Jia-Xin He, Wei Xiao, Yi-Fan Xia, Qing Fan, Hou-Qun Yuan (2021); Highly sensitive and rapid detection of thiabendazole residues in oranges based on a luminescent Tb3+-functionalized MOF; Food Chemistry; Volume 343, 128504; ISSN 0308-8146; https://doi.org/10.1016/j.foodchem.2020.128504
202. Pereira Renata Nunes, Débora Pez Jaeschke, Rosane Rech, Giovana Domeneghini Mercali, Ligia Damasceno Ferreira Marczak, Javier Raso Pueyo (2024); Pulsed electric field-assisted extraction of carotenoids from Chlorella zofingiensis; Algal Research; Volume 79, 103472; ISSN 2211-9264; https://doi.org/10.1016/j.algal.2024.103472

203. Petrakis AE, Caglini RL, Tarantilis PA, Polissiou GM, Consonni R (2017), Sudan dyes in adulterated saffron (Crocus sativus L.): identification and quantification by ^{1}H NMR., Food Chem 217:418–424. https://doi.org/10.1016/j.foodchem.2016.08.078.

204. Ponlakhet. K, K. Phooplub and N. Phongsanam (2022); Smartphone-based portable fluorescence sensor with gold nanoparticle mediation for selective detection of nitrite ions, *Food Chem.*; Volume **384,** 132478

205. Pradeshi S (2019), Food adulteration: injurious adulterants and contaminants in foods and their health effects and its safety measures in India., Int J Sci Res, 4:229–236.

206. Pressman. P, R. Clemens , W. Hayes and C. Reddy (2017); Food additive safety: A review of toxicologic and regulatory issues, *Toxicol. Res. Appl.*, https://doi.10.1177/2397847317723572

207. Priyadeep Bhutani, Gaurav Joshi, Nivethitha Raja, Namrata Bachhav, Prabhakar K. Rajanna, Hemant Bhutani, Atish T. Paul and Raj Kumar (2021); U.S. FDA Approved Drugs from 2015-June 2020: A Perspective; Journal of Medical Chemistry; Volume 64 (5); Pg. 2339-2381; https://doi.org/10.1021/acs.jmedchem.0c01786

208. Priyanka Singh, Ramesh H. Jayaramaiah, Priya Sarate, Hirekodathakallu V. Thulasiram, Mahesh J. Kulkarni and Ashok P. Giri (2014); Insecticidal Potential of Defense Metabolites from *Ocimum kilimandscharicum* against *Helicoverpa armigera*; PLOS One; https://doi.org/10.1371/journal.pone.0104377

209. Qian Liu, Zhiyong He, Maomao Zeng, Fang Qin, Zhaojun Wang Guoping Liu and Jie Chen (2020); Effects of different food ingredients on the color and absorption spectrum carminic acid and carminic aluminium lake; Food Science and Nutrition; Volume 9 (1); Pg. 36-43; https://doi.org/10.1002/fsn3.1628

210. Qin Cheng, Shanhong Xia, Jianhua Tong and Kangbing Wu (2015); Highly-sensitive electrochemical sensing platforms for food colourants based on the property-tuning of porous carbon; Analytica Chimica Acta; Volume 887; Pages 75-81; https://doi.org/10.1016/j.aca.2015.06.013

211. Ramachandran Shakila (2013); Review on *Sphaeranthus indicus* Linn. (Kottaikkarantai); Pharmacogn Rev; Volume 7 (14); Pg. 157-169; doi: 10.4103/0973-7847.120517

212. Ramachandran Subramaniam, Aiyalu Rajasekaran, KT Manisenthilkumar (2012); Investigation of hypoglycemic, hypolipidemic and antioxidant activities of aqueous extract of Terminalia paniculata bark in diabetic rats; Asian Pacific Journal of Tropical Biomedicine; Volume 2, Issue 4; Pages 262-268; ISSN 2221-1691; https://doi.org/10.1016/S2221-1691(12)60020-3

213. Raul Jesus Martinez Sandoval, Miguel Enrique Martinez Rosas, Ernesto Martinez Sandoval, Manuel Moises Miranda Velasco and Humberto Cervantes De Avila (2018); Chapter 4-Color Analysis and Image Processing Applied in Agriculture; Colorimetry and Image Processing; Pg, 61-78; ISBN: 978-953-51-3744-3; Online ISBN: 978-953-51-3745-0; https://dx.doi.org/10.5772/intechopen.71539
214. Ravikiran B. Humbare, Joyita Sarkar, Anjali A. Kulkarni, Mugdha G. Juwale, Sushil H. Deshmukh, Dinesh Amlnerkar, Manohar Chaskar, Maria C. Albertini, Marco B. L. Rocchi, Swapnil C. Kamble and Seeram Ramakrishna (2022), Phytochemical characterization, antioxidant and anti-proliferative properties of Rubia cordifolia L. extracts prepared with improved extraction conditions; J antiox; 11 (5): 1006
215. Ressin Varghese and Siva Ramamoorthy (2023); Status of food colorants in India: conflicts and prospects; Journal of Consumer Protection and Food Safety; Volume 18; Pg. 107-118; https://doi.org/10.1007/s00003-023-01427-y
216. Rimsha Nawaz (2019); Natural food grade dye extraction techniques, Review Paper, Research Journal of Chemical Vol. 9(2), 24-27, ISSN 2231-606X
217. Rimsha Nawaz (2019); Natural food grade dye extraction techniques, Review Paper, Research Journal of Chemical Vol. 9(2), 24-27, ISSN 2231-606X
218. Rodriguez-Amamya(2019); Update on natural food pigments-A mini-review on carotenoids, anthocyanins and betalains; Food Research International; Volume 124, Pg. 200-205; https://doi.org/10.1016/j.foodres.2018.05.028
219. Roman, K.; Barwicki, J.; Rzodkiewicz, W.; Dawidowski, M. (2021); Evaluation of Mechanical and Energetic Properties of the Forest Residues Shredded Chips during Briquetting Process; Energies; Volume 14; Pg. 3270; https://doi.org/10.3390/en14113270
220. Roxana, Lorena, Anamaria, Daniela and Doina (2024); Yellow and Red Synthetic Food Dyes and Potential Health Hazards: A Mini Review; Vol. 81 No. 1 (2024): Bulletin Of University Of Agricultural Sciences And Veterinary Medicine Cluj-Napoca. Food Science and Technology; https://doi.org/10.15835/buasvmcn-fst:2024.0005
221. Saini Ramesh Kumar, Young Soo Keum, Maria Daglia, Kannan RR Rengasamy (2020); Dietary carotenoids in cancer chemoprevention and chemotherapy: A review of emerging evidence; Pharmacological Research; Volume 157; 104830; https://doi.org/10.1016/j.phrs.2020.104830
222. Sakr Mohamed and Shuli Liu (2014); A comprehensive review on application of ohmic heating (OH); Renewable and Sustainable Energy Reviews; Volume 39; Pg. 262-269; https://doi.org/10.1016/j.rser.2014.07.061

223. Sameh M. Farouk, Fatma A. Gad, Rafa Almeer, Mohamed M. Abdel-Daim, Mahmoud A. Emam (2021); Exploring the possible neuroprotective and antioxidant potency of lycopene against acrylamide-induced neurotoxicity in rats' brain; Biomedicine and Pharmacotherapy; Volume 138, 111458; ISSN 0753-3322; https://doi.org/10.1016/j.biopha.2021.111458
224. Sanders R. Erin (2012); Aseptic Laboratory Techniques: Plating Methods; Journal of Visual Experiments; Volume 11 (63); 3064; doi: 10.3791/3064
225. Sandra Magina, Susana Marques, Francisco Girio, Ana Lourenco, Ana Barros-Timmons and Dmitry V. Evtuguin (2024); Chemical composition and structural features of cellolignin from steam explosion followed by enzymatic hydrolysis of *Eucalyptus globulus* bark; Industrial Crops and Products; Volume 211, 118217; https://doi.org/10.1016/j.indcrop.2024.118217
226. Sanli H S., Catalkaya Gok E. (2017); Importance of Ruby Dye (*Rubia tinctorum L.*) in Herb Dyeing; Journal of International Social Research; Volume 10 (48); Pg. 772-778; ISSN: 1307-9581; DOI: 10.17719/jisr.2017.1549
227. Sari Lehto, Maria Buchweitz, Alexandra Klimm, Raphaela Straßburger, Cato Bechtold, and Franz Ulberth (2017); Comparison of food colour regulations in the EU and the US: a review of current provisions; Food Additives & Contaminants: Part A; Volume 34 (3); Pg. 335–355. https://doi.org/10.1080/19440049.2016.1274431
228. Satyanarayana KV (2022) Why natural food colours are way forward. Foods Spectrum. https://nuffoodsspectrum.in/2022/02/20/why-natural-food-colours-are-way-forward.html. Accessed 12 Dec 2022
229. Satyanarayana. L, K. Madhusudan Reddy, Sunkara V. Manorama (2003); Synthesis of nanocrystalline Ni1−xCoxMnxFe2−xO4: a material for liquefied petroleum gas sensing; Sensors and Actuators B: Chemical; Volume 89, Issues 1–2; Pages 62-67; ISSN 0925-4005; https://doi.org/10.1016/S0925-4005(02)00429-X
230. Scientific Opinion (2016); The safety of annatto extracts (E 160b) as a food additive; EFSA Journal; Volume 14 (8); e04544; https://doi.org/10.2903/j.efsa.2016.4544
231. Séïde Marilyne, Michel Marion, Mircea Alexandru Mateescu and Diana A. Averill-Bates (2016); The fungicide thiabendazole causes apoptosis in rat hepatocytes; Toxicology in Vitro; Volume 32; Pages 232-239; ISSN 0887-2333; https://doi.org/10.1016/j.tiv.2015.12.018
232. Sekeroglu Zulal Atli, Vedat Sekeroglu, Seval Kontas Yedier, Emre Ilkun and Louis S. Liou (2017); Increased DNA strabd breaks and neoplastic transformation in human bladded

cells treated with pioglitazone; Environmental and Molecular Mutagenesis; Volume 62, Issue 2; Pg. 143-154; https://doi.org/10.1002/em.22424

233. Sezgin CA, Ayyildiz S (2018) Food additives: colorants
234. Shahid Mohammad and Arvind Misra (2017); Photoenolization via excited state proton transfer and ion sensing studies of hydroxy imidazole derivatives; Journal of Photochemistry and Photobiology A: Chemistry; Volume 335; Pages 190-199; ISSN 1010-6030; https://doi.org/10.1016/j.jphotochem.2016.12.003
235. Shahid-ul-Islam, Rather LJ, Mohammad F. (2016); Phytochemistry, biological activities and potential of annatto in natural colorant production for industrial applications - a review; J Adv Res.; Volume 7; Pg. 499-514; doi.10.1016/j.jare.2015.11.002.
236. Shaikh R. Junaid and MK Patil (2020); Qualitative tests for preliminary phytochemical screening: An overview; International Journal of Chemical Studies; Volume ; 8(2): 603-608; P-ISSN: 2349–8528 E-ISSN: 2321–4902; : https://doi.org/10.22271/chemi.2020.v8.i2i.8834
237. Shakoor Sadaf, Ismail Amin and Sabran Mohd Redzwan (2022); In-vivo study of synthetic and natural food colours effect on biochemical and immunity parameters; Food Sci. Technol (Campinas); 42; https://doi.org/10.1590/fst.41420
238. Sharma, P., Panghal A., Gaiwad V., Jadhav S., Bagal A., Jadhav A. and Chhikara N (2019); Nanotechnology: A Boon for Food Safety and Food Defense; Nanobiotechnology in Bioformulations. Nanotechnology in the Life Sciences, Springer, Cham; Pg. 225-242; https://doi.org/10.1007/978-3-030-17061-5_9
239. Sharmaine Atencio, Sarah H. E. Verkempinck, Kai Reineke, Marc Hendrickx and Ann Van Loey (2022); Heat and Light Stability of Pumpkin-Based Carotenoid in a Photosensitive Food: A Carotenoid-Coloure Beverage; Foods; Volume 11 (3); Pg. 485; https://doi.org/10.3390/foods11030485
240. Shuangshou Wang, Yuwen Ding, Lu Zhang, Yingle Cheng, Ying Deng, Qin Jiang, Hongrui Gao, Jing Gu, Ganggang Yang, Lei Zhu, Tingxuan Yan, Qi Zhang and Jin Ye (2023); Combination of colorimetry, inner filter effect-induced fluorometry and smartphone-based digital image analysis: A versatile and reliable strategy for multi-mode visualization of food dyes; Journal of Hazardous Materials; Volume 445; 130563; https://doi.org/10.1016/j.jhazmat.2022.130563
241. Silva da P.F.F. Rui, Teresa A. P. Rocha-Santos and Armando C. Duarte (2016); Supercritical fluid extraction of bioactive compounds; TrAC Trends in Analytical

Chemistry; Volume 76; Pages 40-51; ISSN 0165-9936; https://doi.org/10.1016/j.trac.2015.11.013

242. Simon. E. James, Eric A. Decker, Mario G. Ferruzzi. M. Monica Giusti, Carla D. Mejia, Mark Goldschmidt and Stephen T. Talcott (2017); Establishing Standardson Colours from Natural Sources; Journal of Food Science; Volume 82 (11); Pg. 2539-2553; https://doi.org/10.1111/1750-3841.13927
243. Singh P, Gandhi N (2015) Milk preservatives and adulterants: processing, regulatory and safety issues. Food Rev Int 31:236–261
244. Singh, R. P., Singh, P. K., & Singh, R. L. (2017b); Present status of biodegradation of textile dyes; Current Trends in Biomedical Engineering & Biosciences; Volume 3(4), 555618.
245. Singh, R. P., Singh, P. K., and Singh, R. L. (2017a); Role of azoreductases in bacterial decolorization of azo dyes; Current Trends in Biomedical Engineering & Biosciences; Volume 9(3), 555764.
246. Sivachandran R. Perumal and Faizal Baharum (2021); Measurement, Simulation and Quantificatopn of Lighting-Space Flicker Risk Levels using Low-cost TCS34725 colour Sensor and IEEE 1789-2015 Standard; Journal of Daylighting; Volume 8, Issue 2; Pg. 239-254; doi: 10.15627/jd.2021.19
247. Sohaib, M., Anjum, F. M., Arshad, M. S., and Rahman, U. U. (2016); **Postharvest intervention technologies for safety enhancement of meat and meat based products; a critical review;** Journal of Food Science and Technology, Volume 53; Pg. 19-30, https//doi.10.1007/s13197-015-1985-y
248. Solymosi. K, N. Latruffe A. Morant-Manceau and B.Schoefs (2015); Colour Additives for Foods and Beverages; Woodhead Publishing Series in Food Science, Technology and Nutrition, 1 - Food colour additives of natural origin; Pages 3-34; https://doi.org/10.1016/B978-1-78242-011-8.00001-5
249. Sowbhagya, H. B., and Chitra, V. N. (2010); Enzyme-Assisted Extraction of Flavorings and Colorants from Plant Materials; Critical Reviews in Food Science and Nutrition; Volume 50 (2). Pg. 146–161; https://doi.org/10.1080/10408390802248775
250. SridharAdithya, Muthamilselvi Ponnuchamy, Ashish Kapoor, Sivaraman Prabhakar (2022); Valorization of food waste as adsorbents for toxic dye removal from contaminated waters: a review; Journal of Hazardous Materials; Volume 424, Part B, 127432; https://doi.org/10.1016/j.jhazmat.2021.127432

251. Srivastava, J. and Vankar, P.S. (2012), Principal phenolic phytochemicals and antioxidant property in Eucalyptus bark; Nutrition & Food Science; 42 (6); 412-421
252. Stich. E (2016); Handbook on Natural Pigments in Food and Beverages-Industrial Applications for Improving Food Color; Food Color and Coloring Food: Quality, Differentiation and Regulatory Requirements in the European Union and the United States, Woodhead Publishing Series in Food Science, Technology and Nutrition; Pages 3-27, https://doi.org/10.1016/B978-0-08-100371-8.00001-4
253. Stoica Florina, Roxana Nicoleta Ratu, Petru Marian Carlescu, Ionut Dumitru Velescu, Sandu Talpa, Vlad Nicolae Arsenoaia and Florin Daniel Lipsa (2023), Assessment of utilizing annatto seeds powder as a natural food ingredient for cheddar cheese; Lucrari Stiintifice – Seria Agronomie; 66 (1): 181-186
254. SuarezTorres D Jose, Camilo A. Orozco, Carlos E. Ciangherotti (2021); Applying Bayesian forecasting to predictive toxicology: The probability of innate carcinogenicity to humans of colorants synthesized from benzidine; Toxicology Letters; Volume 351; Pages 111-134; ISSN 0378-4274; https://doi.org/10.1016/j.toxlet.2021.08.004
255. Subhashish Dey and Bommu Hema Nagababu (2022); Applications of food color and bio-preservatives in the food and its effect on the human health; Food Chemistry Advances; Volume 1, 100019; https://doi.org/10.1016/j.focha.2022.100019
256. Sujithra Vijayakumar and Siva Ramamoorthy (2022); Endophytism of *Fusarium Solani* in Meristems of Delonix Regia (Bojer ex Hook.) Raf.; Indian Journal of Microbiology; Volume 62; Pg. 142-145; https://doi.org/10.1007/s12088-021-00968-w
257. Sulieman Ibraheem Shelash Al-Hawary, Ahmed omar Bali, Shavan Askar, Holya A. Lafta, Zainab Jawad Kadhim, Bakhodir Kholdorov, Yassine Riadi, Reena Solanki, Qais Ismaeel Kadhem, Yasser Fakri Mustafa (2023); Review Article-Recent advances in nanomaterials-based electrochemical and optical sensing approaches for detection of food dyes in food samples: A comprehensive overview; Microchemical Journal; Volume 189, 108540; https://doi.org/10.1016/j.microc.2023.108540
258. Sunday N. Okafor, Wilfred Obonga, Mercy A. Ezeokonkwo, Jamiu Nurudeen, Ufoma Orovwigho, & Joshua Ahiabuike. (2016); Assessment of the Health implications of Synthetic and Natural Food Colourants – A Critical Review. Pharmaceutical and Biosciences Journal, 01-11. https://doi.org/10.20510/ukjpb/4/i4/110639
259. Surbhi, Kumar, A., Singh, S., Pooja Kumari and Prasad Rasane (2021); Eucalyptus: phytochemical composition, extraction methods and food and medicinal applications;

ADV TRADIT MED (ADTM); Volume 23, Pg. 369–380; https://doi.org/10.1007/s13596-021-00582-7

260. Susmita Ghosh, Tanmay Sarkar, Arpita Das and Runu Chakraborty (2022); Natural Colourants from plant pigments and their encapsulation: An emerging window for the food industry; LWT; Volume 153, 112527; https://doi.org/10.1016/j.lwt.2021.112527

261. Svetlana V. Smirnova and Vladimir V. Apyari (2023); Aqueous Two-Phase Systems Based on Cationic and Anionic Surfactants Mixture for Rapid Extraction and Colorimetric Determination of Synthetic Food Dyes; IMPI JOURNAL of sensors; *Sensors;* Volume *23* (7), Pg. 3519; https://doi.org/10.3390/s23073519

262. Taham Thiago, Fernando A. Cabral, Marcos A.S. Barrozo (2015); Extraction of bixin from annatto seeds using combined technologies; The Journal of Supercritical Fluids; Volume 100; Pages 175-183; ISSN 0896-8446; https://doi.org/10.1016/j.supflu.2015.02.006

263. The Association of Official Analytical Chemists-AOAC (2021); Measurement of Dietary Fiber; Journal of AOAC International-A Journal of Analytical Chemistry and Microbiology; Volume 106, Issue 4; Pg. 917-930; https://doi.org/10.1093/jaoacint/qsad051

264. The Tribune (2016) Adulteration in Holicolours, fooditems, https://www.tribuneindia.com/news/archive/features/adulteration-in-holicolours-food-items-212822

265. Tom Coultate and Richard S. Blackburn (2018); Food Colorants; their past, present and future; Coloration Technology; Wiley Online Library; Volume 134, Issue 3; Pg. 163-239; https://doi.org/10.1111/cote.12334

266. Tousley, R. D. (1941); The Federal Food, Drug, and Cosmetic Act of 1938; Journal of Marketing; Volume *5* (3); Pg. 259-269. https://doi.org/10.1177/002224294100500312

267. Trouvé, G., Michelin, L., Kehrli, D., Josien, L., Rigolet, S., Lebeau, B., and Gieré, R. (2023); The Multi-Analytical Characterization of Calcium Oxalate Phytolith Crystals from Grapevine after Treatment with Calcination; Crystals; Volume 13(6), Pg. 967; https://doi.org/10.3390/cryst13060967

268. Varghese R, George Priya Doss C, Kumar RS, Almansour AI, Arumugam N, Efferth T, Ramamoorthy S (2022) Cardioprotective effects of phytopigments via multiple signaling pathways. Phytomedicine 95:153859. https://doi.org/10.1016/j.phymed.2021.153859

269. Vasudha DR., Raveesha HR., Nayana S., Shabaaz Begum JP., Pratibha S., Ravikumara CR. and Dhananjaya N. (2019); The electrochemical behaviour, antifungal and cytotoxic activities of phytofabricated MgO nanoparticles using *Withania somifera* leaf extract;

Journal of Science: Advanced Materials and Devices; Volume 4, Issue 1; Pg. 57-65; https://doi.org/10.1016/j.jsamd.2019.01.003

270. Vecchio Maria Gabriella, Claudia Loganes and Clara Minto (2016); Beneficial and Healthy Properties of Eucalyptus Plants: A Great Potential Use; The Open Agriculture Journal; DOI: 10.2174/1874331501610010052
271. Velasco Carlos, Charles Michel, Jozef Youssef, Xavier Gamez, Adrian David Cheok and Charles Spence (2016); Colour-Taste correspondences: Designing food experiences to meet expectations or to surprise; International Journal of Food Design; Volume 1, Issue 2; Pg: 83-102; https://doi.org/10.1386/ijfd.1.2.83_1
272. Verma Anuradha, Babita Kumar, Perwaiz Alam, Vijendra Singh and Shiv Kumar Gupta (2016), Rubia Cordifolia – A review on pharmaconosy and phytochemistry; IJPSR; 7 (7): 2720-2731
273. Vidal Maider, Rosa Gracia-Arrona, Ane Bordagaray, Miren Ostra, Gorka Albizu (2018); Simultaneous determination of color additives tartrazine and allura red in food products by digital image analysis; Talanta; Volume 184, Pages 58-64; https://doi.org/10.1016/j.talanta.2018.02.111
274. Vigneshwar R., Arivuchelvan A., Mekala P., Imayarasi K (2021); Sex-specific reference intervals for Wistar albino rats: hematology and clinical biochemistry; Indian Journal of Animal Health; Volume 60 (1); Pg. 58-65; DOI:10.36062/ijah.60.1.2021.58-65
275. Wang D, Shi Y, Chang C, Stewart D, Ji Y, Wang Y (2014); Knowledge, attitude and behavior regarding nutrition and dietary intake of seventh-grade students in rural areas of mi Yun County, Beijing, China. Environ Health Prev Med.; 19; 179–86
276. Wang Dong-Geng, Wen-Ying Liu, Guang-Tong Chen (2013); A simple method for the isolation and purification of resveratrol from Polygonum cuspidatum; Journal of Pharmaceutical Analysis; Volume 3, Issue 4; Pages 241-247; ISSN 2095-1779; https://doi.org/10.1016/j.jpha.2012.12.001
277. Wang. S, H. Wang and Z. Yuan (2022); Colorimetry Combined with Inner Filter Effect-Based Fluorometry: A Versatile and Robust Strategy for Multimode Visualization of Food Dyes, *ACS Appl. Mater. Interfaces*; Volume **14**; Pg. 57251 —57264
278. Williams. N L., Juranek L. W., Johnson K. S., Feely R. A., Riser S. C., Talley L. D., Russel J. L., Sarmiento J. L., Wanninkhof R., (2016); Empirical algorithms to estimate water column pH in the Southern Ocean; Geophysical Research Letters; Volume 43, Issue 7; Pg. 3415-3422; https://doi.org/10.1002/2016GL068539

279. Xaq Frohlich (2022); Making Food Standard: the U.S. Food and Drug Administration's Food Standards of IDentiti, 1930s-1960s; Business History Review; Volume 96 (1); Pg. 145-176. doi:10.1017/S0007680521000726
280. Xu Keyulu, Weihua Hu, Jure Leskovec and Stefanie Jegelka (2018); How Powerful are Graph Neural Networks?; arXiv:1810.00826 ; https://doi.org/10.48550/arXiv.1810.00826
281. Yamjala Karthik, Meyyanathan Subramania Nainar and Nageswara Rao Ramisetti (2016); Ultra high performance liquid chromatography with tandem mass spectrometry screening and mutagenicity evaluation of photodegradation products of Carmoisine (E122) dye in a beverage; Journal of Separation Science; Volume 39 (12); Pg. 2246-2251; https://doi.org/10.1002/jssc.201600209
282. Yanjun Feng, Min Zhang, Arun S. Mujumdar and Zhongxue Gao (2017); Recent research process of fermented plant extract: A review; Trends in Food Science & Technology; Volume 65; Pages 40-48; ISSN 0924-2244; https://doi.org/10.1016/j.tifs.2017.04.006
283. Yolmeh Mahmoud, Mohammad B. Habibi Najafi, Reza Farhoosh (2014); Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM); Food Chemistry; Volume 155, Pages 319-324; ISSN 0308-8146; https://doi.org/10.1016/j.foodchem.2014.01.059
284. Yongming Guo, Junyan Shi, Chengwei Wei, Tiancheng Fang, Tao Tao (2023); One-pot synthesis of fluorescent aminoclay and the ratiometric fluorescence detection of sunset yellow; Dyes and Pigments; Volume 212, 111102; ISSN 0143-7208; https://doi.org/10.1016/j.dyepig.2023.111102
285. Zaw T (2015); Food safety in Myanmar; Japan Med Assoc J.; Volume 58; Pg. 185–188.
286. Zhang Yanyun, Shahid-ul-Islam, Luqman Jameel Rather, Qing Li (2022); Recent advances in the surface modification strategies to improve functional finishing of cotton with natural colourants - A review; of Cleaner Production; Volume 335, 130313; ISSN 0959-6526; https://doi.org/10.1016/j.jclepro.2021.130313
287. Zhou. J , L. J. Liang and B. Zeng (2022); Separation and determination of basic orange II, acid orange II and auramine O in soybean products based on ionic liquid reverse micelle microextraction and ultra-high-performance liquid chromatography, *J. Chromatogr.;* **1673,** Pg. 4630-42

APPENDIX – I

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be university under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A⁺⁺' Grade by NAAC.
Recognised by UGC Under Section 12 B
Coimbatore- 641043, Tamil Nadu, India

05.01.2023

To
Ms. Yoshia Leela, J.
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore- 641043

Dear Yoshia Leela,

Ref: Your proposal No. IHEC/22-23/FSMD-1 entitled "Physiochemical Analysis, In vivo Study on Natural Food Colours and Development of AI Based Food Colour Detecting Device" submitted for approval of IHEC 21.11.2022

The Institutional Human ethics Committee of our University hereby grants approval to your research proposal No. IHEC/22-23/ FSMD-1 entitled "Physiochemical Analysis, In vivo Study on Natural Food Colours and Development of AI Based Food Colour Detecting Device" submitted by you. The Approval number for the same is AUW/IHEC/FSMD- 22-23/XPD-1.

We wish you all the best in your research endeavours.

Regards

5.1.23

Dr. A Thirumani Devi
Member Secretary

Institutional Human Ethics Committee

APPENDIX – II

INSTITUTIONAL ANIMAL ETHICAL CLEARANCE

Avinashilingam Institute for Home Science and Higher Education for Women

(Deemed to be University under Category 'A' by MHRD, Estd. u/s 3 of UGC Act, 1956)
Re-accredited with 'A++' grade by NAAC, Recognised by UGC under Section 12 B
Coimbatore – 641 043, Tamil Nadu, India
(Reg. No. 623/PO/ReBi/S/02/CCSEA)

Certificate

This is to certify that the project proposal no **AIW:IAEC.2023:03** entitled **Physiochemical analysis, *in vivo* study of natural food colours and development of AI based food colour sensor testing of its efficacy** submitted by **Ms. J. Yoshia Leela** has been approved/recommended by the IAEC of **Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore** (Organization) in its meeting held on **14/03/2023** (date) and **12 Albino rats (Wistar)** (Number and Species if animals) have been sanctioned under this proposal for a duration of next **12** months.

Authorized by	Name	Signature	Date
Chairman:	Dr. Anitha Subash	.....	14/3/2023
Member Secretary:	Dr. R. Nirmaladevi	.....	14/03/2023
Main Nominee of CCSEA:	Dr. V. M. Berlin Grace	.....	14-03-2023

APPENDIX – III

INTERVIEW SCHEDULED USED TO COLLECT INFORMATION FROM HOME MAKERS ON KNOWLEDGE, ATTITUDE AND PRACTICE ON FOOD COLOURANTS

DEMOGRAPHIC PROFILE								
Name			**Age (years)**	16 – 20	21 – 30	31 – 40	41 – 50	>50
Type of Family	Joint Family	Nuclear Family	**No. of Family Members**	2	3	4	5	>5
Educational Qualification		High School	Higher Secondary	Graduate		**Illiterate**		
Occupation	Home Maker	Employed	**If, Employed: What do you do?**					

KNOWLEDGE BASED QUESTIONS		
Kindly answer All the questions with the given options "Yes/No"		
Do you know there are two types of Food Colourants as Synthetic and Natural Food Colourants?	Yes	No
Do you know that the colours are used mainly to give an appetizing look to the foods?	Yes	No
Do you know that the synthetic food colourants are harmful to health?	Yes	No
Have you added any Natural Food Colourants to the recipes that you prepare at home?	Yes	No
Have you found any difference in the food that you have prepared using synthetic colour and natural colour?	Yes	No
Do you know any synthetic colour by name?	Yes	No
If Yes, Kindly specify:		
Do you know that there are some limitations in using the Food colourants?	Yes	No
If Yes, Kindly specify the Source of your Knowledge:		
Do you know that Food Safety and Standards Authority of India have coined certain terms and conditions/regulations in using different synthetic and natural food colourants?	Yes	No
Have you got any idea of prohibited food colourants?	Yes	No
Do you know that in commercially available foods, the Food Colourants used in it are specified in the label (as Synthetic or Natural Food Colourants)?	Yes	No
ATTITUDE BASED QUESTIONS		
I know that excess consumption of synthetic food colourants cause food poisoning/allergies/cancer/hyperactivity in children/other ailments.	Yes	No
I use synthetic food colourants because they are cheap and easily available.	Yes	No
I do not use synthetic food colourants as I know the ill effects caused by it.	Yes	No
I think synthetic food colourants are considered safe while cooked and consumed.	Yes	No
I feel natural food colourants are difficult to purchase.	Yes	No

I often prefer using food colourants in foods just to make my children eat the food.	Yes	No
I prefer eating natural food colourants, but I find them expensive than the synthetic food colourants.	Yes	No
My family enjoys eating food that looks attractive and appetizing.	Yes	No
I prefer using the following colours: Red, Yellow, Green, Orange and Blue (Tick the colours that you use)		
I use food colourants in foods to have more recipes	Yes	No
PRACTICE BASED QUESTIONS		
I use natural food colourants.	Yes	No
I use natural colours to provide my family with healthy foods.	Yes	No
I use synthetic colourants because I do not know their ill effects	Yes	No
Before adding the food colourants to the food, I read the limitations of it printed on the label.	Yes	No
I often feel adding colours to the foods are essential.	Yes	No
I think it is very difficult to find a natural food colourant equivalently colourful like the commercially available synthetic food colourants.	Yes	No
I think bright colours are harmful for health.	Yes	No
If you are a Home Maker/a mother, do your child/children like food colours added to their food?	Yes	No
In which all foods do they like added colours, please specify?		
Which colour does your child/children like to be added in their food? Red, Yellow, Green, Orange and Blue (Tick the colours that you use)		

APPENDIX – IV

INTERVIEW SCHEDULED USED TO COLLECT INFORMATION FROM FOOD VENDORS ON KNOWLEDGE, ATTITUDE AND PRACTICE ON FOOD COLOURANTS

DEMOGRAPHIC PROFILE								
Name			**Age (years)**	16 – 20	21 – 30	31 – 40	41 – 50	>50
Type of Food Sold	Fast Food Center	Restaurant	**No. of Varieties Sold**	2	3	4	5	>5
Educational Qualification of the Vendor		High School	Higher Secondary	Graduate		**Illiterate**		
Ownership	Sole Proprietor	Partnered	**If Partnered, is it Franchised?**			If Yes, Name your company :		

KNOWLEDGE BASED QUESTIONS		
Kindly answer All the questions with the given options "Yes/No"		
Do you know there are two types of Food Colourants as Synthetic and Natural Food Colourants?	Yes	No
Do you know that the colours are used mainly to give an appetizing look to the foods?	Yes	No
Do you know that the synthetic food colourants are harmful to health?	Yes	No
Have you added any Natural Food Colourants to the recipes that you prepare?	Yes	No
Have you found any difference in the food that you have prepared using synthetic colour and natural colour?	Yes	No
Do you know any synthetic colour by name?	Yes	No
If Yes, Kindly specify:		
Do you know that there are some limitations in using the Food colourants?	Yes	No
If Yes, Kindly specify the Source of your Knowledge on food colours :		
Do you know that Food Safety and Standards Authority of India have coined certain terms and conditions/regulations in using different synthetic and natural food colourants?	Yes	No
Have you got any idea of prohibited artificial food colourants?	Yes	No
Do you know that in commercially available foods, the Food Colourants used in it are specified in the label (as Synthetic or Natural Food Colourants)?	Yes	No
ATTITUDE BASED QUESTIONS		
I know that excess addition of synthetic food colourants in foods cause food poisoning/allergies/cancer/hyperactivity in children/other ailments.	Yes	No
I use synthetic food colourants because they are cheap and easily available.	Yes	No
I do not use synthetic food colourants as I know the ill effects caused by it.	Yes	No
I think synthetic food colourants are considered safe in cooked foods and consumed.	Yes	No

I feel natural food colourants are difficult to purchase.	Yes	No
I often prefer using food colourants in foods just to make the consumers feel satisfied.	Yes	No
I prefer using natural food colourants, but I find them expensive than the synthetic food colourants.	Yes	No
My consumers enjoy eating foods that look attractive and appetizing	Yes	No
I have a particular target of customers, who visit my shop daily.	Yes	No
I want the prepared food to be visually appetizing.	Yes	No
I prefer using the following colours: Red, Yellow, Green, Orange and Blue (Tick the colours that you use)		
I use food colourants in foods to have more recipes	Yes	No
PRACTICE BASED QUESTIONS		
I use natural food colourants.	Yes	No
I use natural colours to provide my customers with healthy foods.	Yes	No
I use synthetic food colourants because I do not know their ill effects	Yes	No
Before adding the food colourants to the food, I read the limitations of it printed on the label.	Yes	No
I often feel adding colours to the foods are essential.	Yes	No
I think it is very difficult to find a natural food colourant equivalently colourful like the commercially available synthetic food colourants.	Yes	No
I think bright colours are harmful for the health.	Yes	No
I use _____ number of synthetic food colour packets per day to make the prepared food look delicious.		
_____ grams of synthetic food colourants are available in the packet.		
The most frequently used synthetic food colourant in the shop is:		
The most common brand that I use for colouring the foods is:		

	Food Colour	**Brand Used**	**Used in the Food**
The colour that I prefer to be added to the food is:	Red		
	Orange		
	Red Orange		
	Yellow		
	Pink		
	Green		
	Blue		
	Brown		

APPENDIX – V

INTERVIEW SCHEDULED USED FOR MARKET SURVEY ON SYNTHETIC FOOD COLOURS

S. No.	Area of the Supermarket	Brand Name of the Synthetic Food colours	Type of Colour				Stored in which form?				Mention the types of Light used in the Shop	Watts of light used	Frequency of the food colour sold
			Red	Green	Yellow	Blue	Glass Bottles		Packets				
							Transparent	Brown	Polythene	Paper Bags			
1.													
2.													
3.													
4.													
5.													
6.													
7.													
8.													
9.													
10.													

APPENDIX – VI

PROCEDURES ADOPTED FOR PHYTONUTRIENT ANALYSIS

(a) Test for Alkaloids:

There are different tests to be carried out for ensuring the presence of alkaloids in food substances. In this study to detect the presence of alkaloids tests with mayer's reagent and dragendorff's reagent were carried out using the following procedure, where the dried form of the natural food colourants of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) were added to dilute HCl dropwise and filtered. The resulting filtrates were screened for the preliminary test of alkaloids with the reagents.

i) Test with Mayer's reagent:

Few drops of Mayer's reagent was added with the filtrates of one ml of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) on different watch glasses to observe the presence or absence of yellowish or white coloured precipitates that revealed the alkaloid occurrence.

ii) Test with Dragendorff's reagent:

Added one ml of Dragendroff's reagent with the filtrates of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) on different watch glasses to observe the presence or absence of orange red coloured precipitates that indicates the presence of alkaloids.

(b) Test for Flavonoids:

i) Alkaline reagent test:

Two or three drops of sodium hydroxide were added to two ml of the substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*). Development of intense yellow colour initially on addition of a few drops of sodium hydroxide solution, which turns to colourless after addition of few drops of dilute hydrochloric acid indicates the presence of flavonoids.

ii) Test with H_2SO_4:

Concentrated sulphuric acid of one ml added to the substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*), where yellow colouration starts to disappear on standing the acid indicates the presence of flavonoids.

iii) Test with lead acetate:

A small portion of the substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) heated with 10 ml of ethyl acetate each over a steam bath for three minutes. Then the mixture is filtered and four ml of the filtrate substance were shaken with one ml of dilute ammonia solution. Yellow colouration of the substance ensures the presence of flavonoids.

iv) **Shinoda test**:

Ten drops of dilute hydrochloric acid and a piece of magnesium when added with one ml of the substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) resulting in development of a deep pink, scarlet, crimson red or occasionally green to blue colour appearance indicates the presence of flavonoids.

(c) Test for Sterols:

i) Libermann test:

Added one ml of the substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) to a dry test tube with constant addition of ten drops of acetic anhydride along with two drops of concentrated sulphuric acid and shake. The mixture in the test tube will change colour from red to rapid violet, deep blue and on standing it will finally change to deep bluish green colour, indicating the presence of sterols.

(d) Test for Terpenoids:

i) Libermann test:

Extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added to the side of test tube, shows brown ring at the junction of two layer and the upper layer turns green which shows the presence of sterols and formation of deep red colour indicate the triterpenoids.

(e) Test for Anthraquinone:

i) Borntrager's test:

To the three ml of extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*), dilute H_2SO_4 was added separately. The solutions were then boiled and filtered. The filtrate were cooled and to it equal volume of benzene was added. The solutions were shaken well and the organic layer was separated. Equal volume of

dilute ammonia solution was added to the organic layer. The ammonia layer if turned pink shows the presence of Anthraquinone glycosides.

(f) Test for Anthocyanin:

Two milliliter of substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) added with two ml of 2N HCl and a few drops of ammonia appears to be pink red solution, which turns blue-violet after addition of ammonia shows the presence of anthocyanin.

(g) Test for Protein:

i) Ninhydrine reagent:

Two drops of 0.2 percentage of freshly prepared ninhydrine solution added to one ml of the substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) holding it on the flame until it boils and if the solution produces purple colour then it shows the presence of proteins.

ii) Biuret test:

Two drops of two percentage of copper sulphate and few drops of ten percentage sodium hydroxide added with one ml of extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)shows the development of violet or red colour formation, then it indicates the presence of proteins.

iii) Xanthoproteic test:

In a test tube few drops of concentrated sulfuric acid is added and shaken with substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*). The solution is then gently heated on flame in which the appearance of yellow colour shows the presence of proteins.

(h) Test for Phenolic Compounds:

i) Ferric chloride test:

Two milliliters of five percentage of neutral ferric chloride solution added with one ml of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*), the dark blue colouring indicates the presence of phenols.

ii) Gelatin test:

The extracts and substances of annatto seeds (*Bixa orellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubia cordifolia*)dissolved in five ml of distilled water and two ml of one

percentage of gelatin containing 10 percentage of sodium chloride is added and the presence of white precipitate indicates the presence of phenolic compounds.

iii) Ellagic Acid test:

Aqueous solution of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)is added with 5% of glacial acetic acid and 5% of sodium nitrite solution. If the solution turns muddy or niger brown precipitate, it indicates the presence of phenolic compounds.

(i) Test for Quinones:

When the extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)are treated with hydrochloric acid and sodium hydroxide, the appearance of red or blue colour shows the presence of quinones.

(j) Test for Carbohydrates:

i) Molisch's test:

Few drops of alcoholic a-naphthol solution added to two ml of extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)along with few drops of concentrated H_2SO_4 added along the walls of test tube. At the junction of two liquids, if a violet colour ring appears, then it indicates that carbohydrates are present.

ii) Fehling's test:

To two ml of extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)an equal volume of Fehling's solution added and heated for five minutes, the resulting red or dark red precipitate indicating the presence of carbohydrates.

(k) Test for Tannin:

i) Braymer's test:

To one ml of extracts and substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)add three ml of distilled water followed by three drops of 5 % ferric chloride solution, formation of blue-green colour indicates the presence of tannins.

ii) Gelatin test:

The extract of 50 mg of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) dissolved in 5 ml of distilled water and 2 ml of 1% solution of gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of tannins.

iii) Hydrolysable Tannins test (10% NaOH test):

An aqueous solution of the extract ofannatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)of 0.4 ml is treated with 4 ml of 10% NaOH and shaken well. Formation of emulsion indicates the presence of tannins

(l) Test for saponins:

The extracts of 50 mgofannatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) is diluted with distilled water and made up to 20 ml. The suspension is shaken in a graduated cylinder for 15 minutes. A layer of foam (2 cm) indicates the presence of saponins.

(m) Test for Cardiac Glycosides:

i) Baljet Reagent test:

The substances of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)is added with sodium oicrate solution, the appearance of yellow to orange colour indicates the presence of cardiac glycosides.

ii) Bromine water test:

Each extract of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)is added with bromine water, formation of yellow coloured precipitate indicates the positive testing of cardiac glycosides.

iii) **Keller-Killiani Test:**

To two ml of test solution of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*), Three milliliter of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. 0.5 ml of concentrated sulphuric acid is added by the side of the test tube. Formation of blue colour in the acetic acid layer indicates the presence of cardiac glycosides.

(n) Glycoside's Test:

i) Borntrager's Test:

To three ml of test solution of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*), dilute sulphuric acid is added, boiled for 5 minutes and

filtered. To the cold filtrate, equal volume of benzene was added and shake it welled. The organic solvent layer is separated and ammonia is added to it. Formation of pink to red colour in ammonical layer indicates presence of anthraquinone glycoside.

ii) Aqueous NaOH test:

A small amount of alcoholic solution of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) taken in one ml of water in a test tube and a few drops of aqueous NaOH is added to it. Yellow colouration in the test tube shows the presence of glycosides.

(o) Test for Lignin:

i) Labat test:

A small quantity of extractof annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) with gallic acid appears olive green colour, then it shows the presence of lignin.

(p) Test for Coumarins:

0.5 g of the moistened extracts of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*) is taken in a test tube. The mouth of the tube is covered with filter paper treated with 1N NaOH solution. Test tube is placed for few minutes in boiling water and then the filter paper is removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

(q) Test for Volatile Oil:

i) Fluorescence Test:

For volatile oil estimation 10 ml of powdered material of annatto seeds (*Bixaorellana*), eucalyptus bark (*Eucalyptus grandis*) and madder root (*Rubiacordifolia*)is taken and filtered till saturation point, then exposed to UV light. The presence of bright pinkish fluorescence indicates the presence of volatile oils.

APPENDIX – VII

PROCEDURE ADOPTED FOR INDUCTIVELY COUPLED PLASMA MASS SPECTROMETER

Inductively Coupled Plasma Mass Spectrometer couples use of an ICP with MS for elemental analysis by generation of ions. The ICP is involved in generation of a high temperature plasma source at 10,000 degree Celsius, 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.

Specifications:

Model	Thermo Scientific™ iCAP™ RQ
Type	Single Quadrupole ICP-MS
Dynamic Range	> 9 orders of magnitude (< 1 - > $1 \cdot 10^9$ cps)
Hertz	2 mHz
Description	iCAP RQ ICP-MS
Nebulizer	Borosilicate glass
Spray chamber	Quartz, cyclonic

Experimental Chemicalsand reagents

High pure Millipore Water
Nitric acid, 67-70%, for trace metal analysis

Digestion

The samples were weighed accurately around 0.2 g in a pre-cleaned, dry 50 mL volume microwave digestion vessel. The volume of 6 mL HNO_3 (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 min. 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.

APPENDIX – VIII

PROCEDURES ADOPTED FOR NUTRIENT ANALYSIS

(a) Test for Crude Fiber:

i) Fibra Plus Method:

According to Center for Food and Safety, Fibra plus method of analysis with AOAC (2023) 985.29 procedure, the test for crude fiber was carried out. The dry samples of eucalyptus barks (*E. grandis)* and annatto seeds (*Bixa Orellana)* were defatted with ether if greater than 10 percent fat content id present in the sample. Then the samples' were weighed for duplicating the test portions with difference in weight of not more than 20 mg. Then the samples were added with phosphate buffer with the pH of 6.0±0.2 with added enzyme incubated at 95-100^{0}C in 30 minutes of water bath. Likewise, protein and starch substances were removed from the samples with protease enzyme added in pH of 7.5±0.2 at 60^{0}C for 30 minutes and for starch the pH of 4.0 to 4.6, added with amyloglucosidase enzyme at 60^{0}C for 30 minutes.

The prepared sample substances are precipitated at room temperature for 60 minutes with 95 percentage of Ethyl alcohol in 280ml added to the substances and the residues are collected in preweighed crucibles. The test portion is divided and using N x 6.25 as conversion factor, protein is calculated. The other portion is incinerated at 525^{0}C for five hours and the fiber is calculated using the formula:

$$\text{Total Fiber} = \frac{\text{Weight of the residue} - \text{Protein content} - \text{Ash} - \text{Blank}}{\text{Weight of the Test Portion}}$$

(b) Test for Fat:

i) Soxhlet or solvent Extraction Method:

The "**Soxhlet**" method is recognized by the *Association of Official Analytical Chemists (AOAC, 2023)* as the standard method for crude fat analysis. In Soxhlet method crude fat content of the food sample is determined by solvent extracting method, then determining the weight of the fat recovered.

The procedure followed is that accurately weigh five grams of samplesof eucalyptus barks (*E. grandis)* and annatto seeds (*Bixa Orellana)* into a flask. Dry the sample in an oven at 102ºC for five hours. Then insert the flask in a Soxhlet liquid/solid extractor. Accurately weigh a clean, dry 150 ml round bottom flash and put about 90 ml of petroleum ether in the flask. Assemble the extraction unit over in an electric heating mantle or a water bath. Heat the solvent in the flask until it boils (Adjust the heat source so that solvent drips from the condenser into the sample chamber at the rate of about six drops per second). Continue the extraction process for six hours.

Remove the extraction unit from the heat source and detach the extractor and condenser. Replace the flask on the heat source and evaporate off the solvent. Place the flask in an oven at 60 to 80ºC and dry the contents until constant weight is obtained for one to two hours. Cool the flask in a desiccator and weigh the flask with contents. The fat percentage of the samples are calculated as: Weight of empty flask (g) = W1; Weight of flask and extracted fat (g) = W2; and Weight of sample = S

$$\text{Total percentage of Crude Fat} = (W2 - W1) \times 100 \div S$$

(c) Test for Protein:

i) Kjeldahl Method:

According to *Carmen (2022),* Kjeldahl method is 125-year-old method for the determination of organic nitrogen has long been the international reference method for the determination of the protein content of dairy products. Although it involves a long analysis time and the use of hazardous reagents at high temperatures, the method is extremely reliable and precise, which is probably why it has stood the test of time so well. Currently, there is micro and macro Kjeldahl determination equipment that will differ in the quantity of sample and chemicals required.

In the procedure (*AOAC, 2023*), a weighed sample of the eucalyptus barks (*E. grandis)* and annatto seeds (*Bixa Orellana)* is heated together with concentrated H_2SO_4 in the presence of K_2SO_4 and Se, Hg, or Cu catalyst in a heat-resistant tube at a temperature of approximately 400 °C. This step is essentially a wet oxidation of the sample, and the organic constituents are initially carbonized as evidenced by the appearance of a dirty black color. Continued heating results in complete oxidation to CO_2 and the digest becomes clear. This digestion step converts sample N into a soluble nonvolatile form, $(NH_4)_2SO_4$. The clear digest is cooled, suitably diluted, and made alkaline by the addition of an excess of strong base (NaOH), which releases the N in a quantifiable form as free NH_3.

The neutralized digest is then subjected to steam distillation, which separates the volatile NH_3 from the other constituents, and the condensed NH_3 is trapped in dilute boric acid. The final step involves quantification of the trapped NH_3 by titration with a standard acid. The amount of acid used in titration can easily be related to the N content of the sample:

$$\text{N (\% w/w)} = \frac{\text{Volume of acid (ml) x molarity of acid (mol – 1) x 14 (g mol – 1)}}{\text{Weight of sample (g) x 100}} \text{ X } 100$$

To obtain the protein content of a food, the N content must be multiplied by a factor that reflects the percentage of N in the sample protein. Dairy proteins contain an average of 15.67 percent of N, giving a correction factor of 6.38 (100/15.65); thus,

Protein (%, w/w) = N (%, w/w) x 6.38

For samples where the exact protein profile is not known, a general conversion factor of 6.25 may be applied. As the Kjeldahl method determines N rather than protein *per se*, the protein content so determined is termed the crude protein value. The main source of inaccuracy in the method arises from the contribution of non-protein nitrogenous species.

(d) Test for Calorific Value:

i) DGHS Method:

The calorific value defines the energetic content of the materials; it is the amount of heat a substance produces after complete combustion. The calorific value can also be expressed as gross calorific value (GCV) or high heating value. Also, the calorific value of a substance (generally, solid biofuel or food) is the specific energy of combustion for a unit mass.

Effectiveness of the Calorific Value: The effectiveness of a food fuel depends on the calorific value; the higher the value, the higher the efficiency and vice versa. For example, Hydrogen fuel has the highest calorific value, that is150 KJ/Kg. Therefore, the efficiency of the substance is proportional to the calorific value. Also, water vapor that is generated in the combustion process contains heat and if it is recovered through different techniques, the substance will have a higher calorific value (GCV) and vice versa when it results in a lower or Net Calorific Value (NCV). NCV is the result when the products of combustion are allowed to escape.

With AOAC, *(2023)* guidelines, the calorific value of the fuel is the amount of heat or energy a fuel generates during complete combustion. It is the variable of the heat or energy released, which is either measured in Gross Calorific Value (GCV) or Net Calorific Value (NCV). The calorific value of a fuel can be determined using a bomb calorimeter. With the above understanding of calorific value, we can write the calorific value formula as follows:

Net calorific value (NCV) = Gross calorific value (GCV) - Latent heat of water vapors

(or)

Gross calorific value = Net calorific value + Latent heat of water vapors

(e) Test for Moisture Content:

i) Hot Air Oven Method:

According to *Kalne (2022),* the moisture content is the ratio of weight of water to the solid in the food substance, that is eucalyptus barks (*E. grandis)* and annatto seeds (*Bixa Orellana).* This ratio is expressed in percentage of wet basis and dry basis.

Check the weight of a clean container as W1 in grams. Around five to six gram of the sampe is added to the container and weighed as W2 in grams. Place the container in oven and heat it with the sample at 130^{0}C for about 16 to 24 hours. Again check the weight of the sample as W3 in grams. The moisture content can be calculated in percentage of wet basis and dry basis.

Percentage on Wet basis = [(W2 – W3) ÷ (W2 – W1)] x 100

Percentage on Dry basis = [W3 ÷ (100 – W2)] x 100

The other nutrients like carbohydrates is tested using the AOAC recognized method of IS 1656:2006 and other phyto nutrients like phenols, tannins, beta carotene, lycopene and phytic acid were calculated using the general guidelines used to analyze preliminary phytochemical (*Shaikh et al., 2020)*

APPENDIX – IX FORMULATION OF RECIPES INCORPORATED WITH FOOD COLOURANTS

TRADITIONAL SWEET RECIPES INCORPORATED WITH THE SELECTED NATURAL FOOD COLOURANTS

KESARI

Ingredients

- Semolina – 20 grams
- Sugar – 20 grams
- Ghee – 5 grams
- Cashew nuts – few
- Water – 1 cup

Method of Preparation

- Add few drops of ghee in a kadai, let it heat, roast cashews in it and keep aside.
- Then add the semolina to it and dry roast it. On the other hand, boil water.
- When the semolina turns light gold colour, add sugar, water, ghee and natural food colours (AnV_1 – 1 mg, AnV_2 – 2 mg and AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg).
- Mix well till it reaches a thick semi-solid consistency.

MOTICHOOR LADOO

Ingredients

- Besan – 50grams
- Ghee (melted) – ½ teaspoon
- Water - ¼ cup + 3 tablespoons (divided, 20 ml + 45 ml)
- Oil - for frying

Sugar Syrup

- Sugar (granulated) – 100grams
- Water – 60ml
- Cardamom powder – 2 grams
- Lemon juice – 5 grams

Method of Preparation to Fry the Boondi

1. To a large bowl add besan and food color (AnV_1 – 1 mg, AnV_2 – 2 mg and AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg). Then add little ghee and mix.

2. Start adding water. Add around 1/2 cup (60 ml) to first form a thick batter without lumps. Then add 1 ½ tablespoons (22.5 ml) water and mix well. Let the batter sit for 15 minutes.
3. Then add remaining 1 ½ tablespoons (22.5 ml) water and mix. The total water used for batter will be60 ml + 22.5 ml + 22.5 ml = 105 ml. Batter should be very thin and flowing consistency without any lumps.
4. Heat ghee in a kadai on medium flame.
5. Hold the porous laddle 3-4 inches above the oil. Shake the laddle after pouring the batter. The handle of the laddle should be placed on the canister (a small metal container).
6. Fill a ladle with the batter.
7. Once ghee is hot, start pouring the batter through the laddle.
8. Pour the batter on laddle and you should move it up and down quickly (with the handle of the laddle placed on canister) to drop all batter in hot ghee in the kadai.
9. Let the tiny boondis cook in hot ghee for 30 to 40 seconds only, color should not change.
10. Remove fried boondi in a large sieve. Repeat until all tiny boondis are fried and batter is over. Remember to completely wipe clean the laddle with a wet cloth between frying each batch, else the boondis can clump up. Set aside.

Make the Sugar Syrup

1. To make the syrup, to a large kadai, add sugar and water. Add cardamom powder. Also add lemon juice and food color.
2. Let the sugar dissolve and mixture come to a boil.
3. As soon as the mixture comes to a boil, turn off the heat and add the fried boondi to the pan.
4. Stir and then turn on the heat again (low flame).
5. Cook on lowest heat for around 3 minutes, stirring continuously. You don't want to dry the boondi but the excess sugar syrup should become less. It takes around 2 to 3 minutes.

Shape the Ladoo

1. Remove on a plate and let cool completely.
2. Once the mixture has cooled down, take a small portion of the boondi mixture and roll between the palms to make motichoor ladoo. Repeat with the remaining boondi.

JALEBI

Ingredients

- All-purpose flour – 125 g
- Corn flour – 16 g

- Natural food colourant (AnV_1 – 1 mg, AnV_2 – 2 mg and AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg)
- Curd – 120 ml
- Water – 120 ml
- Baking soda – ½ tsp
- Lemon juice – 1 tsp
- Oil – as required

For Sugar Syrup

- Sugar – 200 ml
- Water – 120 ml + 180 ml
- Cardamom Powder – ¼ tsp
- Lemon Juice – 1 tsp

Making Sugar Syrup

1. Add sugar and water to a pan/pot, boil on a medium heat until it reaches a string consistency.
2. Take a small portion of the syrup in a spoon and cool it slightly. Take it between the thumb and fore finger, gently remove the finger away from each other, to see a single string.
3. Pour lemon juice, cardamom powder, remove from heat, stir an set aside.

For Jalebi Making:

1. Add purpose flour, corn flour and food colourant to a mixing bowl. Mix everything well. Add curd, pour water and make a thick lump free batter.
2. The batter has to be thick but of flowing consistency. If needed add water, beat the batter well with a whisk in one direction in a circular motion for 3-4 minutes. The batter will turn smooth.
3. Heat oil on a medium heat to fry jalebis with 1-2 tbsp of ghee.
4. Pour 1 tsp lemon juice to the batter and mix. Add soda and mix gently until it combines
5. Prepared batter must be smooth free flowing and thick. Spoon just 2-3 tbsp of batter to a sauce bottle or a zip lock cover to check if the consistency is right.
6. Check if the oil is hot enough by dropping a small portion of the batter. It has to come up immediately without browning.
7. Now squeeze the batter gently and move in the circular motion to get spiral.
8. While jalebi is getting fried, check the syrup, it must be slightly hot or warm when the jalebi is dipped into it. If not heat up a bit.

9. The last 1 minute, turn the flame to low and fry the jalebi. Remove the jalebi from flame and add to the warm sugar syrup.
10. Allow to rest for 2 minutes. Remove to a plate. Continue to make more jalebis.

COMMON SNACK RECIPES INCORPORATED WITH THE SELECTED NATURAL FOOD COLOURANTS

CHILLI GOBI

Ingredients: For Frying Gobi

- Small to medium sized cauliflower – 300 grams (chopped small cauliflower florets)
- 1 cup of water (for blanching cauliflower)
- All-purpose flour – 30 g
- Corn flour – 15 g
- Red chili powder – 5 g
- Black pepper powder – 2 g
- Salt – as required
- Oil (deep frying) – 250 ml

Method of Preparation: Blanching Cauliflower

- Rinse and then break or chop the cauliflower in small florets.
- In a pan or take 2.5 cups water and add ¼ teaspoon salt. Bring the water to boil.
- Switch off the flame and keep the pan on the kitchen countertop. Add the cauliflower florets. Blanch for 5 minutes.
- Drain all the water and keep the blanched cauliflower aside.

Making Batter for Chilli Gobi

1. Take all-purpose flour and corn flour in a mixing bowl.
2. Also add salt as required.
3. Add red chilli powder, black pepper powder.
4. Add 1 cup water in parts and begin to mix.
5. Add remaining water and make a smooth batter without lumps. Depending on the quality and texture of flours, you can add ¾ to 1 cup water. Add the food colour (AnV_1 – 1 mg, AnV_2 – 2 mg and AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg), as well.
6. The batter should have a medium consistency and should not be thick or thin. If the batter looks thin, then add some more all-purpose flour. If batter looks thick then add some more water. Heat oil for deep frying on medium flame.

Frying Gobi

1. When the oil becomes hot then place some cauliflower florets in the batter. Coat them with the batter evenly.

2. Slid off the extra coating at the sides of the bowl and place the batter cauliflower florets in the hot oil. Add less portion of the florets in the oil and do not overcrowd the pan or kadai.
3. When one side becomes golden and crisp, turn over each florets. Flip for a couple of times more, so that the florets are evenly fried, golden and crisp.
4. Do note that the cauliflower fritters will stick to each other while frying. Thus add less portion of the florets in oil. Also as soon as you add them stir with a spoon so that they do not stick. In case they stick, then fry them together as one whole. Once you place them on paper towels then break them when they become less hot.
5. Remove the fried gobi florets with a slotted spoon or spider spoon. Fry gobi florets in 3 to 5 batches depending on the size of the kadai.

CHILLI CHICKEN

Ingredients

- Chicken – 300 g
- Oil – for frying

For the batter

- Corn flour – 30 g
- All-purpose flour – 15 g
- Chilli powder – 2.5 g
- Black pepper powder – 2.5 g
- Garlic and ginger paste – 2 g
- Pinch of salt
- Vinegar – 15 g
- Water – 15 g

Method

1. Heat oil in a wide pan. In a mixing bowl add the corn flour, plain flour, chilli powder, black pepper and ginger garlic paste along with the salt and food colour (AnV_1 – 1 mg, AnV_2 – 2 mg, AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg).
2. Add the vinegar and water; mix to make a thick batter. Add the chicken pieces to it and mix well coating all the pieces with the batter.
3. Fry the chicken in the hot oil in batches for 1-2 minutes. They should have a slight colour all over. Drain the chicken on kitchen paper and set aside.

POTATO BAJJI

Ingredients

- Potato – 1
- Gram flour – ¾ cup
- Rice flour – 2 tbsp
- Natural Food Colourants (AnV_1 – 1 mg, AnV_2 – 2 mg, AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg)
- Red chilli powder – ½ tsp
- Chaat masala – ½ tsp
- Asafoetida – a pinch
- Ginger garlic paste – 1 tsp
- Salt – ½ tsp
- Water – ½ cup
- Oil – to fry

Method of Preparation

- Peel the skin of potato and slice thinly. Rine in cold water to remove off starch from potatoes.
- Pat dry to remove off excess water, keep aside.
- Prepare besan batter by taking ¾ cup besan and 2 tbsp rice flour in a mixing bowl.
- Add ¼ tsp turmeric, ½ tsp chilli powder, ½ tsp chaat masala, a pinch of asafoetida, 1 tsp ginger garlic paste and ½ tsp salt. Combine the ingredients well.
- Add in ½ cup water and whisk smooth, making a smooth batter without forming any lumps.
- Add a pinch of baking soda and mix gently. Do not over mix as baking soda will lose its property. Make sure the batter is in flowing consistency.
- Dip the sliced potato into prepared besan batter and coat it completely. Deep fry in hot oil.
- Stir occasionally and fry on both sides. Fry the potatoes till they turn golden brown.

POTATO BONDA

Ingredients: Aloo Bonda Batter

- 1 cup gram flour
- ¼ cup Rice Flour
- ⅓ to ½ cup water or add as required
- Natural Food Colourant (AnV_1 – 1 mg, AnV_2 – 2 mg, AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg)
- ½ teaspoon red chili powder
- 1 pinch asafoetida

- 1 pinch baking soda (optional)
- salt as required

For Stuffing

- 4 to 5 medium sized potatoes
- 1 medium sized onion – finely chopped
- 1 or 2 green chilies, finely chopped
- 12 to 15 curry leaves
- 2 tablespoon chopped coriander leaves
- 1 teaspoon Ginger Garlic Paste
- ½ teaspoon turmeric powder
- 1 pinch asafoetida (hing)
- 1 teaspoon mustard seeds
- ⅓ to ½ teaspoon lemon juice or as required
- 1 or 1.5 teaspoon urad dal (spilt and husked black gram)
- 1 to 1.5 tablespoon oil
- salt as required
- oil for deep or shallow frying

Making Stuffing

1. First boil the potatoes in a pan or steamer or pressure cooker. Then peel and mash them when still warm.
2. Heat oil in a frying pan. Add the mustard and urad dal.
3. Allow the mustard seed to splutter and the urad dal to get golden.
4. Add the chopped onion and saute till they are translucent and soften.
5. Now add the ginger-garlic paste, curry leaves and green chilies. Saute for a minute.
6. Add the turmeric powder, asafoetida and stir. Now add the coriander leaves and stir.
7. Add the potatoes to this mixture or add this sauted mixture to the potatoes. Stir well.
8. If there is moisture, then you can cook the potato mixture for a few minutes.
9. Add lemon juice & salt. Mix well.
10. Check the seasoning and add more salt or red chili powder or lemon juice if required.
11. Make medium sized balls from this mixture.

Frying Aloo Bonda

1. Heat oil for deep frying in a kadai or pan. Dip each potato ball in the batter and coat it well with the batter.
2. Add the batter coated potato balls in the medium hot oil.
3. Fry till golden on both sides. Drain them on paper towels to remove excess oil.

FRUIT PRESERVES RECIPES INCORPORATED WITH THE SELECTED NATURAL FOOD COLOURANTS

TUTTI FRUTTI

Ingredients

- Raw papaya – 300 g
- Water – 600 ml
- Sugar – 300 g
- Vanilla Essence – a drop/two

METHOD OF PREPARATION

1. Take raw papaya and peel the skin off. Cut the papaya into small cubes and boil the cubes in 4 cup water for 5 minutes.
2. Cook until the papaya cubes turn into semi-transparent and drain off the water, keep aside.
3. Now in a large kadai take 2 cup sugar and 3 cup water.
4. Add semi cooked raw papaya cubes and stir. Boil for 20 minutes stirring in between.
5. Make sure to check for 1 string consistency of sugar syrup and papaya to turn soft, yet retain its shape. Turn off the flame and add vanilla extract. Mix well.
6. Divide the cooked papaya cubes into 3 parts along with sugar syrup.
7. Add food colour (AnV_1 – 1 mg, AnV_2 – 2 mg, AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg) to each part and mix well.
8. Allow to soak for 12 hours or a day making sure papaya absorbs all the colour.
9. Now drain off the sugar syrup and allow them to dry over kitchen towel.
10. Once the tutti-frutti dries off completely it will not be sticky.

APPLE JAM

Ingredients

- Apples – 400 g
- Sugar – ¾ cup
- Apple vinegar – 4 tbsps
- Water – 4 tbsps
- Vanilla extract – a drop
- Cinnamon – 5 nos

Method of Preparation

1. Rinse, peel, core and quarter the apples. Grate them or chop them finely.
2. Add the apples to a heavy bottom pan, add vinegar, water, cinnamon sticks and food colourants (AnV_1 – 1 mg, AnV_2 – 2 mg, AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg).

3. Cover and cook on low heat until the apples are slightly softened.
4. Add sugar and continue to cook stirring often until thick. Add a pinch of grounded cinnamon and vanilla essence.
5. When it thickens test by swiping the bottom of the pan with a spatula. It should leave a trail behind the spatula.

JELLY

Ingredients

- 4 cups of water
- 2 Tbsp gelatin
- 2 Tbsp sugar
- Natural Food Colourants (AnV_1 – 1 mg, AnV_2 – 2 mg, AnV_3 – 3 mg, EuV_1 – 4 mg, EuV_2 – 5 mg and EuV_3 – 6 mg)

Method of Preparation

1. Add 1/2-3/4 cup of the water to a bowl or liquid measuring cup and sprinkle with gelatin powder.
2. Whisk together to combine and allow to sit for 3-5 minutes. The granules will plump and the mixture will look like very thick applesauce or take on a lumpy appearance.
3. Pour the remaining (3 1/4- 3 1/2 cups) water into a medium saucepan. Heat over medium heat until almost boiling.
4. Remove from heat and stir in honey (if using) and the bloomed gelatin mixture. Stir to dissolve.
5. Pour into a dish, or into individual glasses or jars for individual portions and refrigerate.

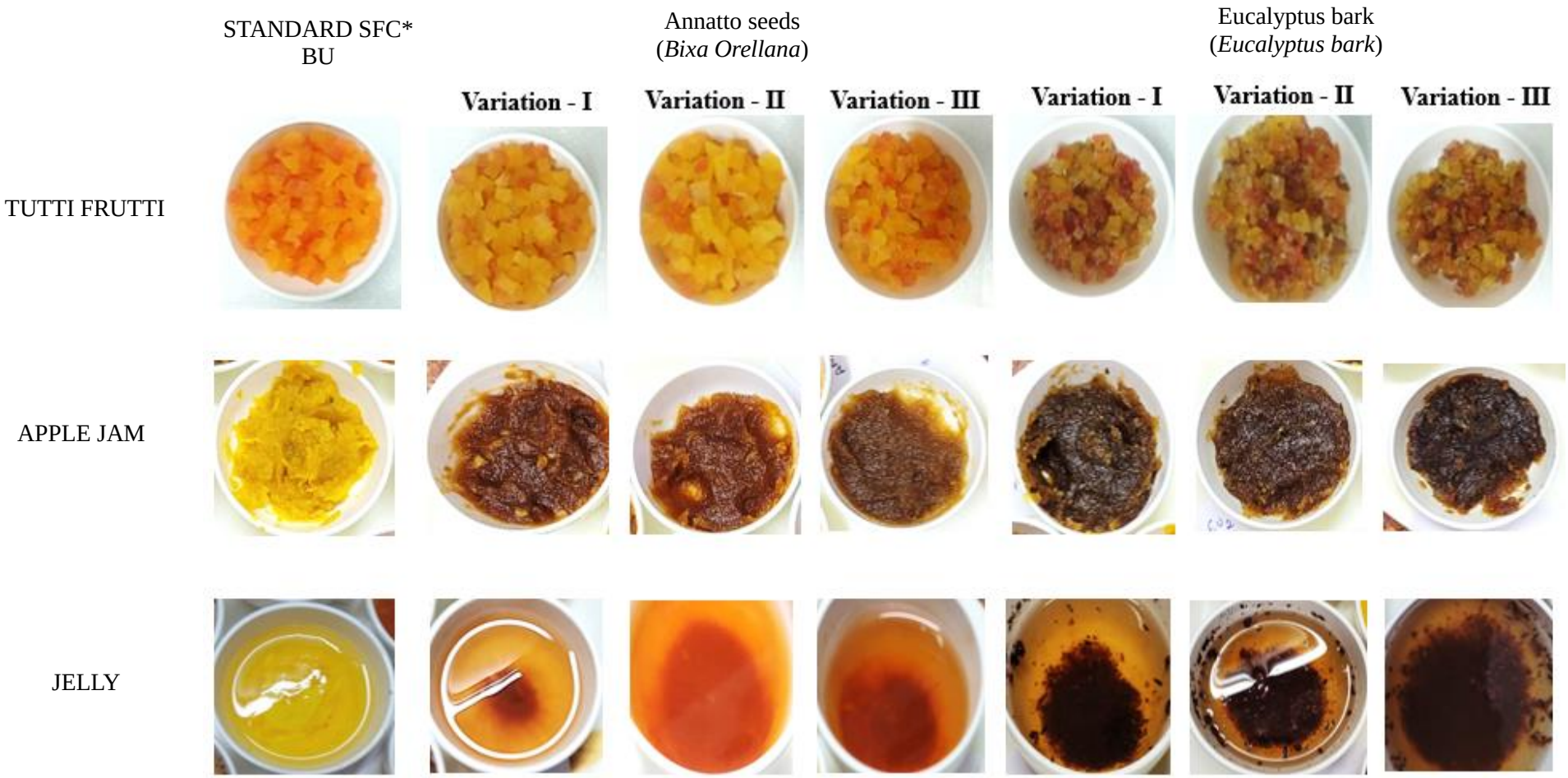
TRADITIONAL SWEET RECIPES INCORPORATED WITH THE SELECTED NATURAL FOOD COLOURANTS

		Annatto seeds (*Bixa Orellana*)			Eucalyptus bark (*Eucalyptus bark*)		
	STANDARD SFC* BU	Variation - I	Variation - II	Variation - III	Variation - I	Variation - II	Variation - III
KESARI							
MOTICHOOR LADOO							
JALEBI							

SNACKS RECIPES INCORPORATED WITH THE SELECTED NATURAL FOOD COLOURANTS

	STANDARD SFC* BU	Variation - I	Annatto seeds (*Bixa Orellana*)	ariation - III	Variation -	Eucalyptus bark (*Eucalyptus bark*)	Variation - III
CHILLI GOPI							
CHILLI CHICKEN							
ALOO BAJJI							
ALOO BONDA							

FRUIT PRESERVATIVE RECIPES INCORPORATED WITH THE SELECTED NATURAL FOOD COLOURANTS



APPENDIX – X

COMPOSITE SCORE CARD USED TO EVALAUATE THE PREPARED RECIPES

PRODUCT – 1

An: Anatto seeds **Eu: Eucalyptus bark**

Name				**Date**	
Name of the Product					
	Appearance	**Consistency**	**Texture**	**Flavour**	**Taste**
Standard					
An: V – I					
An: V – II					
An: V – III					
Eu: V – I					
Eu: V – II					
Eu: V – III					

(5 – Excellent; 4 – Very Good; 3 – Good; 2 – Fair; 1 – Poor)

APPENDIX – XI

SOURCECODE FOR THE DEVELOPMENT AND FABRICATION OF FOOD COLOUR SENSOR

HTMLCODE

```
importos

importurllib.request import http
importpandasaspd import re
fromtimeimportsleep

fromdatetimeimportdatetime
importpickle filename='model.sav'
loaded_model=pickle.load(open(filename,'rb')) base = "http://192.168.137.53/"
deftransfer(my_url):#usetosendandreceivedata try:
    n=urllib.request.urlopen(base+my_url).read() n = n.decode("utf-8")
    returnn
    exceptthttp.client.HTTPExceptionase: return e
#SpecifytheabsolutepathfortheExcelfile
#Createanemptylisttostoredata data_list = []
ct=0
whileTrue:res=transfer(str(ct)) response = str(res) print(response)
# Split the received data values=response.split('-')
iflen(values)==3: r, g, b = values
s=int(input("ENTER1FORKESARI2 FORCHILLIGOBI3FOR LADDU"))
reports= [[s,r,g,b]]
predicted=loaded_model.predict(reports) print(predicted)
ft=predicted[0] if ft == 0:
conn
urllib.request.urlopen("https://api.thingspeak.com/update?api_key=1Z4GBPZ7ZZEV8NCW&fie
ld1=KESARI(LIGHT_ORANGE)"+"&field2="+str(r)+"&field3="+str(g)+"&field4="+str(b))
ifft==1: conn=
urllib.request.urlopen("https://api.thingspeak.com/update?api_key=1Z4GBPZ7ZZEV8NC
W&fiel
d1=CHILLY_GOBI(RED)"+"&field2="+str(r)+"&field3="+str(g)+"&field4="+str(b))
#response=conn.read()
```

```
ifft==2: conn=
    urllib.request.urlopen("https://api.thingspeak.com/update?api_key=1Z4GBPZ7ZZEV8N
    CW&field1=LADDU(ORANGE)"+"&field2="+str(r)+"&field3="+str(g)+"&field4="+st
    r(b))
    #response=conn.read()
ifft==3: conn=
    urllib.request.urlopen("https://api.thingspeak.com/update?api_key=1Z4GBPZ7ZZEV8N
    CW&field1=CHILLY_GOBI(LIGHT_RED)"+"&field2="+str(r)+"&field3="+str(g)+"&
    field4="+s tr(b))#response = conn.read()
ifft==4: conn=
    urllib.request.urlopen("https://api.thingspeak.com/update?api_key=1Z4GBPZ7ZZEV8N
    CW&field1=KESARI(DARK_ORANGE)"+"&field2="+str(r)+"&field3="+str(g)+"&fie
    ld4="+str(b)) #response = conn.read()
    sleep(1)
```

KNNALGORITHM

```
importnumpyasnp importpandasaspd
fromsklearnimport metrics
fromsklearn.model_selectionimporttrain_test_split import matplotlib.pyplot as plt
importseabornassns import pickle
data=pd.read_csv('dataset.csv')
X=data.iloc[:,:-1]
y=data.iloc[:,-1]
X_train,X_test,y_train,y_test=train_test_split(X,y,test_size=0.3,random_state=5)
sns.countplot(x='Target',data=data) plt.show()
fromsklearn.neighborsimportKNeighborsClassifier
model=KNeighborsClassifier(n_neighbors=1) model.fit(X_train, y_train)
filename = 'model.sav' pickle.dump(model,open(filename,'wb'))
y_pred=model.predict(X_test)
acc=metrics.accuracy_score(y_pred,y_test) print("Accuracy is:", acc)
cm=metrics.confusion_matrix(y_pred,y_test) print('Confusion Matrix : \n', cm)
#Classificationreport
classification_report=metrics.classification_report(y_pred,y_test)
print('Classification Report:\n', classification_report)
```

SENSOR INTEGRATION

```
#include
<ESP8266WiFi.h>
#include<ESP8266m
DNS.h> #include
<WiFiClient.h>
//OURSERVER'SPORT,80FORDEFAULT
WiFiServerserver(80); WiFiClient client; String rule;
void start(String ssid, String pass){ WiFi.mode(WIFI_STA);
WiFi.begin(ssid.c_str(),pass.c_str());
Serial.println("");
//Waitforconnection
while(WiFi.status()!=WL_CONNECTED){ delay(500);
Serial.print(".");
}
Serial.println(""); Serial.print("Connectedto"); Serial.println(ssid); Serial.print("IP
address:"); Serial.println(WiFi.localIP());
if(!MDNS.begin("esp8266")){
Serial.println("ErrorsettingupMDNSresponder!"); while (1) {
delay(1000)
}
}
Serial.println("mDNS responder started"); server.begin();
Serial.println("TCP server started"); MDNS.addService("http","tcp",80);
}
boolisReqCame=false;
bool CheckNewReq(){ client=server.available(); if (!client) {
return0;
}
/*
while(client.connected()&&!client.available()){ delay(1);
}*/   //to make data transfer fast Stringreq=client.readStringUntil('\r'); int addr_start =
req.indexOf(' ');
intaddr_end=req.indexOf(',addr_start+1); if (addr_start == -1 || addr_end == -1) {
Serial.print("Invalid request: "); Serial.println(req);
return0;
```

```
}
req=req.substring(addr_start+1,addr_end);
rule=req;
isReqCame=true;
client.flush(); return 1;
}
void waitUntilNewReq(){
do{CheckNewReq();}while(!isReqCame); isReqCame = false;
}

voidreturnThisStr(Stringfinal_data){ String s;

client.print(final_data);

}

voidreturnThisInt(intfinal_data){ returnThisStr(String(final_data));

}

StringgetPath(){ return rule;

}
```

APPENDIX – XII

HEADLINES FROM NEWSPAPERS ON SYNTHETIC FOOD COLOURANTS

Chemicals are present all along the food chain, the increasing use of harmful additives, like artificial colours and preservatives, pose a number of health risks for the consumer

COLOURS OF DANGER

RISHITA KHANNA @ Bengaluru

BIG PICTURE

THE iconic pink hue of cotton candy and enticing red of gobi manchurian will now pale, following the state government's recent ban on artificial colour-enhancing chemicals. While the public speculates about the impact on their favourite dishes, and children mourn the fading pink in their treats, health experts call for a balanced policy framework to educate the public, enforce regulations, and advocate further restrictions on harmful food additives.

An FSSAI official maintained that the regulatory agency carefully selects food samples for inspection, prioritising those lacking proper labelling and those raising surveillance concerns, such as items widely consumed but sold at a lower price, or possessing overly vibrant colours.

Dr US Vishal Rao, member of Vision Group for Biotechnology with the Government of Karnataka, advocated a uniform policy framework. "It is necessary to implement a consistent policy framework that prioritises and maintains food safety standards. Food is universally consumed and if standards are not met, it could lead to a situation where, over the next 10 years, there will be widespread prevalence of non-communicable diseases, including heart disease and kidney disease, affecting a large portion of the population."

"While items are not banned every month, testing undergoes thorough scrutiny to uphold standards set by the FSSAI, and ensure adherence to chemical limits prescribed by the regulating agency," an official from the regulating agency said.

Health Minister Dinesh Gundu Rao said, "Food manufacturers should follow Food Safety and Standards Act 2006 and Food Safety and Standards (Food Products Standards and Food Additives) Regulations 2011, for their products. Food additives should be within the prescribed limit of Food Safety and Standards and Regulations."

Further, as per the FSSAI order, food manufacturers should test their products once in six months in FSSAI-notified laboratories, and upload the same to the FoSCoS website of the FSSAI, Rao said, adding that the Food Safety and Standards department, from time to time, picks random samples to check the quality of food sold in the market.

If the food sample is not in compliance with the Food Safety Act and regulations, the department initiates legal action, the minister added.

Said Dr Vishal Rao, "From farm to plate, chemicals are present throughout the food supply chain. Initially, raw ingredients are treated with pesticides and then processed through various stages, including adulteration, preservation and eventual decomposition."

Dr Rao added that from pastries to bread, processed meats and carbonated drinks, every food item now contains harmful artificial colours. While candies and pastries boast vibrant hues, bread stays mould-free for extended periods because of added chemicals, colours and the most deadly of all, preservatives. This underscores the necessity for ethically disclosing ingredients in both regulated and unregulated food products, ensuring that consumers understand what they consume, and the associated health risks.

"Consumers should possess the 'Right to Health', necessitating education about the ingredients they consume to make informed decisions about what to include and what to avoid in their diets. Through a comprehensive disclosure of food content, from manufacturers to retailers, all involved parties will exercise greater vigilance, consequently reducing the prevalence of food adulteration practices," Dr Rao said.

Additives and health risks

Edwina Raj, head of services, Clinical Nutrition & Dietetics at Aster CMI Hospital, highlighted the presence of chemicals in a variety of commonly consumed products. From preserved meats to soup mixes, bread and spreads, these chemicals are often added and consumed without complete awareness of their potential health effects by consumers.

"It is advisable to familiarise ourselves with the additives present in processed foods, and their possible impacts. Rather than depending solely on packaged foods, let's prioritize fresh unprocessed alternatives. These natural sources offer essential nutrients vital for our well-being, while avoiding artificial additives and preservatives."

Dr Suresh Babu, Professor of Medical Oncology at Kidwai Memorial Institute of Oncology, said, "Clora Red and Brilliant Blue, along with the already prohibited Sunset Yellow and Tartrazine, are frequently used to impart an enticing blue colour to beverages and refreshments, like 'blue lime soda'. Despite potentially allowable usage within defined parameters, regular and prolonged consumption of these chemicals poses health risks over time."

Even staple foods like rice and wheat are sometimes extensively adulterated. For instance, high-quality basmati rice may be mixed with lower-quality long-grain rice to give the impression of superior quality. However, in reality, this exposes them to the risks associated with food adulteration, including kidney diseases and various vitamin deficiencies in the body, Dr Suresh explained.

"Dairy products such as cheese, butter and milk, along with oats, brown rice, dry nuts and black beans, remain untainted by artificial colouring and adulteration. Consumers should prioritize millet and other natural foods over processed options that may contain added preservatives to extend shelf life. While these preservatives prolong the product's lifespan, they can potentially reduce the individual's overall health and longevity."

CHEMICALS & EFFECTS

BUTYLATED HYDROXYANISOLE (BHA) AND BUTYLATED HYDROXYTOLUENE (BHT)
Synthetic antioxidants commonly used as preservatives in a wide range of everyday consumables such as food products, cosmetics and pharmaceuticals. However, their usage poses significant risks to human health. BHA has been linked to the development of both benign and malignant tumours, as well as disruptions in hormone function and induced dysfunction within the body. BHT can potentially trigger allergic reactions such as skin rashes, itching or respiratory difficulties.

SODIUM NITRITE / NITRATE
A commonly used salt which is infused in various food products such as bacon, beef jerky, ham, hot dogs and smoked fish. While nitrate is naturally present in our diet, excessive consumption can elevate the risk of colorectal cancer, as well as other diseases, including leukaemia, heart disease, thyroid issues and ovarian cancer.

PROPYL GALLATE
A widely used chemical found in food items such as meat products, mayonnaise, soup mixes, popcorn and chewing gum. Additionally, it serves as an antioxidant in hair products, cosmetics, and lubricants to prevent the oxidation of oils and fats. While generally considered safe for consumption, some individuals may encounter skin rashes, itching, swelling, or respiratory issues upon exposure.

FOODS TO AVOID
- With elevated salt content such as packaged pickles, marinated fish
- Designed for prolonged shelf life
- Ready-to-cook soups
- Colourful foods
- Preserved meats

WHAT TO CONSUME
- Dairy products such as cheese, butter, milk
- Oats, brown rice, dry nuts, black beans

"Consumers should possess the 'Right to Health', necessitating education about the ingredients they consume to make informed decisions about what to include and what to avoid in their diets"
Dinesh Gundu Rao, Health Minister

HOME / NEWS / CITIES / COIMBATORE

FSSAI destroys half tonne sweets for excessive use of colourants during Deepavali special drive in Coimbatore

Updated - November 08, 2023 07:42 am IST

Published - November 07, 2023 07:03 pm IST - COIMBATORE

Nine teams of FSSAI conducted a special drive at 104 places, including manufacturers, wholesale dealers, those who manufacture only during festival seasons and retailers, from Nov. 1 to 6

THE HINDU BUREAU

Diwali: Food safety officials go into overdrive

TIMES NEWS NETWORK

Chennai: Food safety teams are lifting daily samples of sweets and savouries from manufacturing units, storehouses and stores to assess quality of ingredients, manufacturing practices, storage and labelling of finished products, officials said. The food safety department has also launched a drive to sensitise food business operators about hygienic practices.

Health secretary Beela Rajesh, who is also the commissioner of food safety, has issued orders directing designated food safety officials of all districts to send samples for testing. Samples of oil, ghee, sweets and savouries have to be lifted as per prescribed format and sent to lab for checks, the letters samples and meeting with manufacturers.

"We have asked them (manufacturers) to avoid synthetic colours in sweets and maintain proper hygie- been asked to check the labels of ghee and oil used and buy only from trusted sources with proper bills/ invoices," said food safety deputy director K Vanaja. "Manu- standards will lead to convictions. We will track the quality of ingredients and will book their suppliers if the raw material is adulterated," she said.

... ed up samples of ghee and butter from manufacturing units in T Nagar and Saidapet along with a red dye. The dye was found to be cancer-causing, oil-soluble colour that is not permitted to be used in food. Samples of butter and ghee picked from these units also failed food safety standards. Government food analysts reported that as much as 50% of fat in the samples tested was not exclusively dairy fat.

Staff in the manufacturing units told officials that the dye, a combination of red powder and mustard oil, was used as it gave the butter texture and odour. These products cost ₹200 a kg compared to ₹435 per kg of ghee charged by brands such as Aavin of the state-run milk cooperative. Similarly, some oils meant for lighting lamps

BITTER SWEET

Food safety teams are lifting daily samples of sweets and savouries from manufacturing units, storehouses and stores to assess quality of ingredients, manufacturing practices

CUSTOMERS SHOULD
- **Avoid buying sweets with artificial colouring agents.** The dyes can contain cancer-causing substances
- Don't buy packs of assorted sweets that have milk sweets mixed with other kinds. **Milk sweets tend to have a shorter lifespan**
- If you are buying milk sweets, **check the label for expiry date**
- Look around the shop to check its hygiene. **If it's a sweetspot for flies, it can upset your tummy**
- See if the workers are healthy and following sanitation rules: use gloves and cover their hair. The **store should also display details of oil and ghee it uses**
- **Plastic containers used** for packing snacks **should be of food grade**
- Packages must have details of all ingredients, **display FSSAI certificate, complete address of manufacturer, expiry date** of products and customer care numbers

அனுமதியில்லாத செயற்கை நிறங்களை பயன்படுத்தினால் கடும் நடவடிக்கை

உணவு பாதுகாப்பு அலுவலர் எச்சரிக்கை

பழநி, அக். 24: உணவுப் பொருட்களில் அனுமதிக்கப்படாத செயற்கை நிறங்களை பயன்படுத்தினால் கடும் நடவடிக்கை எடுக்கப்படுமென உணவு பாதுகாப்பு அலுவலர் மோகனரங்கம் எச்சரிக்கை விடுத்துள்ளார்.

ஐயப்ப பக்தர்கள் சீசன் துவங்க உள்ள நிலையில் பழநிக்கு ஏராளமான பக்தர்கள் வந்து செல்கின்றனர். இவ்வாறு வரும் பக்தர்களிடம் விற்பனை செய்வதற்காக அடிவார பகுதிகளில் ஏராளமான இடங்களில் தற்காலிக சாலையோர கடைகள் அமைக்கப்பட்டு சிப்ஸ், கற்கண்டு, பேரீட்சை, அல்வா போன்ற பொருட்கள் விற்பனை செய்யப்படுகின்றன. சில கடைகளில் கலப்பட மற்றும் தரமற்ற தின்பண்டங்கள் விற்

மான, பாதுகாப்பான, கலப்படமில்லாத, காலாவதியாகாத உணவு பொருட்களையே விற்பனை செய்ய வேண்டும். உணவுப்பொருட்களை கையாள்வோருக்கு தொற்று நோய் எதுமில்லை என உடல்நலத் தகுதி சான்று கட்டாயம் பெற்றிருத்தல் வேண்டும். உணவுப் பொருட்களை தயாரிக்க பாதுகாக்கப்பட்ட குடிநீரையே பயன்படுத்த வேண்டும். அனுமதிக்கப்பட்ட இயற்றை அல்லது செயற்கை நிறங்களை சரியான அளவில் மட்டுமே பயன்படுத்த வேண்டும். காலவதியான எண்ணெய்கள் மற்றும் அனுமதிக்கப்படாத செயற்கை

நிறங்களை பயன்படுத்தக் கூடாது. உணவுப் பொருட்களை தயாரிக்க பயன்படும் நெய், வனஸ்பதி மற்றும் எண்ணெய் வகைகளை வாடிக்கையாளர்கள் அறியும் வகையில் அறிவிப்பு பலகையில் தெரியப்படுத்த வேண்டும்.

விற்பனைக்கான உணவுப்பொருட்களை மூடிய நிலையில், ஈக்கள், பூச்சிகள் மற்றும் தூசிகள் விழாதவாறு முறையாக

விபரங்களை குறிப்பிட வேண்டும். உணவுப் பொருட்களை சாக்கடையின் மேல் வைத்து பொதுமக்களுக்கு விற்பனை செய்யக்கூடாது. மினரல் ஆயில் போன்ற எண்ணெய்களை பேரிட்சையில் தடவி விற்பனை செய்யக்கூடாது. நகர்ப்பகுதியில் உணவுத் தொழில் செய்வதற்கு உணவுப் பாதுகாப்பு மற்றும் மருந்து நிர்வாகத்துறையினரிடம்

வைத்திருக்க வேண்டும். எச்சரிக்கை ஏதாவது இருந்தால் அதுகுறித்த

BEWARE OF COLOUR IN YOUR FAVOURITE SNACKS

Artificial colouring agents are added to some of the most popular food items in the city such as Gobi fry, Chicken 65 etc., often exceeding the allowed levels, making them unfit for human consumption

Healthy Living

+3 more SHWETA TRIPATHI

Pani Puri, Gobi 65, chats, cotton candy and other snacks are some of the fastest selling food items in the city. However, artificial colours in the form of dyes used in such items can cause several ailments including cancer because of the presence of carcinogenic agents.

The recent incident of Pani Puri samples containing cancer-causing elements has shocked a lot of people.

NATIONAL 12th March 2024

KARNATAKA BANS ARTIFICIAL FOOD COLOUR IN GOBI MANCHURIAN, COTTON CANDY

KARNATAKA BANNED HARMFUL ARTIFICIAL COLORS IN COTTON CANDY AND GOBI MANCHURIAN AFTER TESTS FOUND UNSAFE LEVELS. THE BAN PROHIBITS ALL ARTIFICIAL COLORS IN GOBI MANCHURIAN AND LIMITS SPECIFIC DYES IN COTTON CANDY.

VIOLATORS TO FACE SERIOUS PUNISHMENT INCLUDING LIFETIME IMPRISONMENT.

Avinashilingam Institute for Home Science and Higher Education for Women
(Deemed to be University Estd. u/s 3 of UGC Act 1956, Category 'A' by MHRDRe-accredited with A++ Grade by NAAC. CGPA 3.65/4, Category I by UGC Coimbatore - 641 043, Tamil Nadu, India

Appendix L2

(Item No 5 of Check List) Details of Research Publications

S.No	Article	Journal	Other Details Vol/No/Page No/ Year	Published in UGC-CARE / Scopus Indexed/ Web of Science
1	A Study on Extraction Methods and Primary Toxicity Level of Bio Colourants	Indian Journal of Nutrition and Dietetics	Vol. 60 No. 3 Pg. No: 431-446 Year: 2023 ISSN: 0022-3174	UGC – CARE I
2	Toxicity Study on Eucalyptus grandis Bark as Natural Food Colourant in Wistar Albino Rats	Gujarat Agricultural University Research Journal	Vol.49 No.1 ISSN: 0250-5193	UGC – CARE I (Received Acceptance with Reference number of MS – RA 2924)

*Proof of list of Journals from Internet to be attached along with copies of reprints.

Scholar : Yoshia Leela 12/07/24

Supervisor : P.K. Adisivalam 12/7/2024

Checked By: 12/7/24

HoD/Dean of Respective School

The scholar miss. Yoshia Leela(21PHFDF001) has published her articles in the following journals:

1. Indian Journal of Nutrition and Dietetics - indexed and active in UGC care List Gp I from January 2021 to present
2. Gujarat Agricultural University Research Journal - indexed and active in UGC Care List Group 1 from June 2019 to present.

She got acceptance in this journal.

This may be considered.

12.07.24

ISSN 0250-5193

GUJARAT AGRICULTURAL UNIVERSITIES RESEARCH JOURNAL

S.D. Agricultural University
Sardarkrushinagar

Anand Agricultural University
Anand

Junagadh Agricultural University
Junagadh

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Navsari

VOLUME : 49 NUMBER : 1 JANUARY, 2024

GAU RES. J. 49 (1)

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GAU Research Journal **49** (1) : 64-71 (January 2024)

Toxicity Study on *Eucalyptus grandis* Bark as Natural Food Colourant in Wistar Albino Rats

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Received : 26/04/2024 Accepted : 25/06/2024

ABSTRACT

The toxicity of the powdered substance of Eucalyptus bark (Eucalyptus grandis) was investigated. The level of feed to be given was optimized in the rats and later in the experimental period of 56 days, same procedure was carried out, where according to the OECD guidelines, oral feed ofaqueous solution with 2ml/100g body weight was fed. The histopathology of the rats showed no alternative changes in liver, kidney, intestine, stomach, brain and spleen proving that E. grandis can be used as natural food colourant. The hematology and biochemical parameters were checked for both the control group and the experimental group. The average values were highly similar to each other proving that there were no biochemical changes that had taken place in the experimental rats. Thus, this study proves that eucalyptus barks can be used as natural food colourants, as an alternative for harmful synthetic food colourants.

Keywords : Eucalyptus grandis, Wistar albino rats, optimization of dosage, fixed dosage, Subchronic toxicity

INTRODUCTION

Traditional medicinal practices are defined to have their origin from plants and plant extracts, precisely known as herbalism. Since 6000-4000 BC, Indians have practiced traditional medicinal therapy that has vital retention in curing various diseases. Currently, natural products especially traditional medicines have gained their significance through their unique therapeutic attributes with phytochemicals, their efficacy and safety.In such case, eucalyptus is considered the best traditional remedy for ailments like arthritis, ulcer, diabetes and bladder diseases. Considering the therapeutic values of *E. grandis,* few researchers and food analysts have recommended *E. grandis* as a substitute for synthetic food colourants (*Abuajah et al., 2015*).

Color additives also known as synthetic coloursare used in a wide variety of foods such as beverages, dairy products, cereals, bakery goods, snack foods and ice creams. Although there are strict guidelines for chemicals to be approved as food additives, the safety of food colorants has not been rigorously proven and acceptable daily intake (ADI) has been used to minimize any possible unfavorable effect of the dyes (*Sarah Kobylewski et al, 2012*).

With proper evidences the artificial food colors (AFCs) have proved to affect

children's behavior, through various studies carried out for around 35 years. Food and Drug Administration Food Advisory Committee convened to evaluate the current status of evidence regarding attention-deficit/hyperactivity disorder (ADHD) caused due to artificial food colours. AFCs appear to be more of a public health problem than an ADHD problem, where it seems to affect children and have an aggregated additive or synergistic effect on most children who suffer from behavioral decrement (*Eugene et al., 2012*).

Naseer (*et al., 2019*) has expressed that "in the investigations carried out to establish the safety and nutritional value of natural dye produced from the bark of *Eucalyptus globules*. The natural brown dye obtained from the *Eucalyptus* bark was applied in the production to access stability of dye in the candies, as *E. grandis bark* poses health benefits. Thus this study is carried out to prove that *E. grandis* can be used in place of harmful synthetic food colours.

MATERIALS AND METHODS

Plant material collection

The bark of *E. grandisbark*were collected from Forest College and Research Institute College in Coimbatore, Tamil Nadu, India. The freshly collected barks were cleared from dust and other particles. The barks were dried in shade, powdered using sterilized mixture jar and stored in zip lock bags. The mixture jar and the zip lock bags were made sure that no moisture content was found in it.

Animals used for Toxicity Testing

Wister Albino rats of 230-250g of male were used for the study. Five rats per group was allocated. Animals were purchased from the Animal House located in Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore*, Tamil Nadu, India*. The University animal ethical committee has approved of animal ethical clearance, providing the clearance number as AIW: IAEC.2023:03 for the study.

The animals were housed under standard environment condition of 25±2°C with sufficient light and dark cycles. The rats were fed with standard commercial feed and normal 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 the rats settled down and the experimentation was progressed.

Toxicity Testing

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 in parts per million/million per liter of solution in kilogram. In 100 ml of water, the dosage is prepared using the following formula:

$$V_1 N_1 = V_2 N_2$$

$$V_1 = \frac{V_2 N_2}{N_1}$$

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 ppm = 100 × 2 ÷ 1000 = 0.2 ml

Likewise, ppm level is increased for every specific dosage fed for 5 days continuously, if there are no changes found in the animals then the dosage is increased gradually with two days wash-out period.

Table 1 : Level of Water Given with Natural Food Colourants

Powdered Forms	Name of the Rat	Calculation of Level of Water to be given	Determined Level of Water (in ml)
Eucalyptus barks (*Eucalyptus grandis)*	Test rat – 1 (TR1)	376 × 2 ÷ 100	7.5

According to the *table 1,* the determined level of water in which the natural food colourant are to be optimized are 7.5 ml for the test groups of rats with powdered forms of *E. grandis.*

Pilot Study

A pilot study was carried out with two Wistar Albino rats, where the consumption level of the colourant was finalized by monitoring the animals for eight 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 for the eucalyptus bark powder was higher than expected. After determining the maximum threshold level, the experimentation was carried out with the other rats.

Table 2 : Profile of the Rat used for Pilot Study

Profile of the Rat	Eucalyptus Bark (*Eucalyptus grandis*)	
Type of Group	Test Group	
Name	Eu 1	
Age (months)	8-12 months	
Sex	Male	
	During Purchase	Eighth Week
Weight (g)	344 g	452 g
Water Consumption (in ml)	20-25 ml from 120 ml of water	10 ml + 8 ml of Sample
Food Consumption (g)	25 g	25 g
Body Parts		
	During Purchase	Eighth Week
Fur/Skin	Normal White	Normal White
Eyes	Normal Red	Normal Red
Ears	Normal Pink	Normal Pink
Nose and Mouth	Normal Pink	Normal Pink

Profile of the Rat		Eucalyptus Bark (*Eucalyptus grandis*)
Limbs and Nails	Normal	Normal
Tail	Normal Pink	Normal Pink
	During Purchase	Eighth Week
Active	9	9
Sluggish	X	X
Aggressive	X	X
Irritated	X	X
Sleepy	X	X

The test groups for the powdered form of *E. grandis* barkswere named as test rat – 1. The Wistar Albino rats' level of water to be given was calculated with the body weight of the rats during the purchase. The calculation for the feeding of rats with natural food colourant is as follows for *E. grandis*barks, where the rat weighed 376 g. The level of natural food colourant that was added to the oral feed was optimized in the pilot study for eight weeks. The optimized level was orally fed continuously for 56 days during the experimentation period.

Table 3 : Optimization of *Eucalyptus grandis*

No of Weeks	Water (ml)	ppm	Calculation of ppm	ppm of Natural Food Colourants added in water (mg)
1	7.5	2	7.5 × 2 ÷ 1000	0.01
	7.5	12	7.5 × 12 ÷ 1000	0.08
2	7.5	14	7.5 × 14 ÷ 1000	0.10
3	7.5	28	7.5 × 28 ÷ 1000	0.20
4	7.5	41	7.5 × 41 ÷ 1000	0.30
5	7.5	54	7.5 × 54 ÷ 1000	0.40
6	7.5	67	7.5 × 67 ÷ 1000	0.50
7	7.5	80	7.5 × 80 ÷ 1000	0.60
8	7.5	94	7.5 × 94 ÷ 1000	0.70

2.3.1 Experimentation

The animals were divided into two groups with five animals in experimental group and two animals in control group. Group - 1 is the control group and Group - 2 is experimental group were the animals are fed with *E. grandis*for 56 days.

Table 4 : Experimentation Plan for Toxicity Testing

Groups	No. of animals	Food and dosage given during experimentation
Control Group	2	Commercial Feed (25 g) + Normal Drinking water
Experimental Group – 1	5	Commercial Feed (25 g) + Normal Drinking water + Specific Dosage of Food Colourants Eucalyptus barks (*Eucalyptus grandis)* (calculated according to the weight of the rat)

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.

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 solution.

The blood was used for hematology screening such as - hemoglobin, red blood cells and white blood cells. Other part of blood was centrifuged at 3000 rpm for five minutes and serum was separated to estimate the biochemical parameters like - SGOT, SGPT, Glucose, Total Protein, Albumin, Creatinine, Sodium, Potassium and Chloride.

In the estimation of biochemical parameters - SGOT and SGPT also known as Serum glutamic-pyruvic transaminase and Serum glutamic-oxaloacetic transaminase are the markers of liver functions. The analysis for albumin, creatinine, sodium, potassium and chloride are carried out to analyze if the functioning of kidney is normal. If there is an elevation in the values of biochemical parameters, it indicates that there has been malfunctioning of kidney in the tested organism. A decrease in total protein and albumin is a sign of the reduced synthetic functioning of the liver or due to impaired hepatocellular function.

From the *table 2,* it is interpreted that *E. grandis*of 6 mg as natural food colourantwas mixed in 7.5 ml of water was given to the rats orally from week one to week eight.

RESULTS AND DISCUSSION

Clinical Signs

The rats fed with *E. grandis*did not show any symptoms of presence of toxicity andno clinical changes were found in the rats throughout the experimental period. The physical observation in their skin, fur, eyes, mucus, eating pattern, behavioural change and sleep were all normal.

Table 5 : Mean weight gain in the rats

Groups	Mean weight on Day zero (g)	Mean weight on Day 56 (g)	Difference in weight (g)
Control	201	292	92
Experimental Group 1	272	354	82

Table 5 represents the mean weight gain that has taken place in the animals. All the animals in both the control group and the experimentation group have gained weight.

The difference in the weight gain of the control group after the eighth week is 92 g, whereas the difference of weight gain in the group that consumed *E. grandis*is 82 g.

The increase in weight is due to increase in intake of water and food. Likewise, loss of appetite will be due to some metabolic change in intake of either carbohydrates, protein or fat. The change in metabolism may lead to loss of appetite and loss of appetite will lead to loss of weight gradually. But, in this experimentation there is no loss of weight observed in the rats.

Hematology and Biochemical Analysis

Table 6 represents the hematology and biochemical parameter of the rats to find if there has been any alteration in the renal functioning that was influenced by the powder mixed in water. Liver and kidney analysis is very important as the toxicity evaluation of the plant substance will be noted through the tissues, which is responsible for the survival of the organism.

The hematology value of hemoglobin is 15.9 g/dl, RBCs and WBCs are 8.35 ten million per cubic meter and 4.2 thousand per cubic meter. Whereas the SGOT and SGPT obtained are 27U/L and 25U/L respectively. The other parameters like glucose and cholesterol are 45 g/dL and 40 g/dL, which showed the same level of absorption of glucose and cholesterol in blood.

Table 6 : Mean hematology and biochemical parameter values

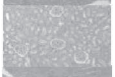
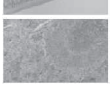
Biochemical Parameters	Estimated Values	
	Control Group	Experimental Group
Hemoglobin (g/dl)	14.53	15.9
Red blood cells (10^6/mm^3)	7.56	8.35
Total White blood cells (10^3/mm^3)	4.93	4.2
SGOT (U/L)	27.17	27
SGPT (U/L)	20.18	25
ALP (U/L)	72.45	72.45
Glucose (g/dL)	46	45
Cholesterol (g/dL)	42	40
Total Protein (g/dL)	6.5	6.5
Albumin (g/dL)	4.2	3.5
Creatinine (mg/dL)	0.9	0.65
Sodium (mg/dL)	137.3	107.9
Potassium (mg/dL)	4.1	6.5
Chloride (mg/dL)	88	89

Serum concentration of total protein 6.5 g/dL and albumin 3.5 g/dL in the treated and control group confirms that the substance has not damaged the hepatocellular or secretory functions of the liver. Renal dysfunction is assessed through the concurrent values of creatinine valuing 0.65 mg/dL and their normal value reflects the normal renal functioning in the rats. The proper functioning of kidney is again proved through the standard values of sodium 107.9 mg/dL, potassium 6.5 mg/dL and chloride 89 mg/dL.The values have proved that the *E. grandis* substance did not affect the hepatocytic functions of the rats.

Histopathological Study of the Organs

The microscopic examination of the organs of the rats treated with *E. grandis*powder did not show any change in colour compared with the control group rats' organs. Hypertrophy of organ indicates the toxicity of chemical and biological substance. In addition, the microscopic examination revealed that none of the organs from the treated rats have shown any alteration in cell structure or any unfavourable effects under the light microscope using multiple magnification powers

Table 7 : Microscopic view of the harvested organs from the rats

Organs	Control Group	Experimental Group
Brain		
Liver		
Kidney		
Heart		
Stomach		
Intestine		
Spleen		

No pathologies were recorded in the histological sections of the vital organs (brain, liver, kidney, heart, stomach, intestine and spleen) of the control group. Equally, there was no change in the creatinine after administration of *E. grandis*powder when compared to the control group. Any alteration found in the level of creatinine show that there has been damage in the functioning of nephrons. The observations in the histopathology study of the kidney tissue have been recorded no changes. Thus, this study proves that *E. grandis*is non toxic in nature and can be used as natural food colourant.

CONCLUSION

The results demonstrate that natural form of powders substance of *E. grandis* barks is practically proven to be non toxic in nature when administered orally and is safe for consumption. So, the study has proved that in place of synthetic food colourants, eucalyptus barks can be powdered and used as it is in foods making the food therapeutically valuable.

REFERENCE

Abuajah CI, Ogbonna AC, Osuji CM. (2015) Functional components and medicinal properties of food: a review. *J Food Sci Technol.*;52:2522–2529

Eugene Arnold. L, Nicholas Lofthouse and Elizabeth Hurt (2012), Artificial Food Colors and Attention-Deficit/Hyperactivity Symptoms: Conclusions to Dye Neurotherapeutics, Jul; 9(3): 599–609, *PMCID*:

PMC3441937, PMID: 22864801, doi: 10.1007/s13311-012-0133-x

Mohamad Faizal Mohamad, Daniel Joe Dailin (2019), Natural Colourant for Food: A Health Alternative, *Int J Sci Technol Res*; *IJSTR;* Volume 8 (11): 3161-3166

Naseer. S, Afzal. M.S, Nisa A, Hussain. S, Ahmad M. A, Parveen. S, Anjum. F, Riaz. M, (2019), Research Article, Extraction of brown dye from *Eucalyptus* bark and its applications in food storage; *QASCF*; 11 (8)- Pages: 769 – 780, https://doi.org/10.3920/QAS2019.1569, ISSN: 1757-8361, Online ISSN: 1757-837X

Sarah Kobylewski, Michael F Jacobson (2012), Toxicology of food dyes, Review; *Int J Occup Environ Health*; Jul-Sep 2012;18(3):220-46, PMID: **23026007;**DOI: 10.1179/107735 2512Z.00000000034

Print ISSN : 0022-3174
Online ISSN : 2348-621X

THE INDIAN JOURNAL OF NUTRITION AND DIETETICS

UGC CARE List Group 1 - Sciences
NAAS Rating - 4.87

IJND

Vol. 60 | No.3 | 321 - 475 | JULY - SEPTEMBER 2023
http://www.informaticsjournals.com/index.php/ijnd

THE INDIAN JOURNAL OF NUTRITION AND DIETETICS

Journal of Avinashilingam Institute for Home Science and Higher Education for Women

(Established under section 3 of the UGC Act 1956 vide Notification No. F.9-20/84-U.3 dated 8th June 1988 of the Government of India)

Re-accredited with A++ Grade by NAAC, CGPA 3.65/4, Category I by UGC

Vol. 60 No. 3 **July - September 2023**

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Original Articles

The Indian Journal of Nutrition and Dietetics
ISSN: 0022-3174; eISSN: 2348-621X
www.ijndonline.org

DOI:10.21048/IJND

A Study on Extraction Methods and Primary Toxicity Level of Bio Colourants

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(Received 9th January, 2023)

Abstract

Bio-colourants for food are extracted from plant sources that it is becoming a huge demand in the global market of processed foods and confectionaries. Natural colours play a vital role in food safety and also in meeting the needs of food consumption practices for the growing crisis in the availability of healthy foods. Aim of the bio colours is to replace synthetic colours like sunset yellow FCF, Allura Red, Ponceau 4R, Amaranth and Brown HT as they are becoming a wide range of threat to the human race, especially in bringing psychological changes in children and adolescents. Usually bio-colourants are extracted from almost all the parts of a plant. But majority of the colours are extracted from leaves and barks, whereas few colours in specific are extracted from fruits, seeds and some from petals of flowers. In this study, bio-colouring agents such as madder roots (Rubia cordifolia), eucalyptus bark (Eucalyptus grandis), annatto seeds (Bixaorellana), roselle petals (Hibiscus sabdariffa) and tamarind seeds (Tamarindus indica) were identified and used in extraction of colours. The colours obtained were of different shades of red like: sangria red, rusty red and yellow red. These natural food colours were extracted from their natural sources through two processes:10 per cent aqueous extraction (reflux, boiling) and powdering of substances. The pH of the extracts was taken to know their level of hydrogen ion concentration. To verify,if these extracts are edible and stable, preliminary toxicology study was carried out in brine shrimp assay. On the other hand, every fourth day the discolouring of extracts was constantly measured using Food Colour Reader. The aqueous extracts showed discolouring and formation of microbial layer even when preserved in refrigerator in sterilized glass bottles, whereas the powdered substances remained the same with no discolouring or microbial activity in it. The toxicity study has practically proved that the powdered substances in various concentrations of 100, 250, 500, 1000 and 1500 µg/ml has the overall lowest mortality rate in consideration to mean and standard deviation (M ± SD)

of 11.7 ± 10.7, than to the aqueous extraction samples of 73.7 ± 22.8 respectively. This study is carried out to promote and incorporate bio-colours in foods that can be consumed on daily basis, replacing the commercially available hazardous food colourants.

Keywords : Colourants, extracts, maddar roots, eucalyptus bark, annatto seeds, powdered substance, pH, food colour reader, brine shrimp assay, toxicity

Introduction

Food colourants from plant sources have grabbed the attention of global markets. As quoted by few connoisseurs of food, the colours in foods affect the psychological behavior of humans; in addition, they also have further effects on positive mannerism[1]. Few countries around the world have started exploring the field of developing bio food colourants for industrial purposes to introduce healthy eating to the society. Scientific investigations on natural food colourants considering economy, legislative, technological feasibility to identify natural colourants and consumer acceptance aspects, have been proved to be less promising. It is likely believed that there are possibilities in introducing completely new bio-colourants into today's food manufacturing industry, as long as current food legislations and consumer attitudes remain unchanged[2].

With proper evidences, the Artificial Food Colors (AFCs) have proved to affect the health status of the children's behavior, through various studies carried out for around 35 years. Food and Drug Administration Food Advisory Committee convened to evaluate the current status of evidence regarding Attention-Deficit Hyperactivity Disorder (ADHD) caused due to artificial food colours[3]. AFCs appear to be more of a public health problem than an ADHD problem, where it seems to affect children and have an aggregated additive or synergistic effect on most children who suffer from behavioral decrement[4]. The edible dyes used in the food industries are not proved to improve the safety and quality of nutrition in foods, indicating the removal or replacement of currently used food colourants and their supplies, for the betterment of booming food industries.

Beet root is one common substance used for colouring and its pigment responsible for the colour is betalains. These red dyes along with beta xanthins (yellow) are considered as irreversible colouring pigments with the presence of nitrogen in it extending the colour shades to red, yellow and bluish-red. Betalains have antimicrobial, antiviral and antioxidant properties. Carrots are the best source of beta-carotene. They are pro vitamin A agents that provide colour. Grapes skin extracts are soluble in water and it gives reddish purple colour. Anthocyanin is the pigment responsible for the colour of the extract.

Investigations were carried out to establish the safety and nutritional value of natural dye produced from the bark of *Eucalyptus globules*. The natural brown dye obtained from the *Eucalyptus* bark was applied in the production to access stability of dye in the candies, as eucalyptus bark (*Eucalyptus grandis*) poses health benefits against arthritis, ulcer, diabetes and bladder diseases[6]. Madder (*Rubia cordifolia*) is one of the traditional dyes used mostly as food dye, as they are commonly available in India and Pakistan, *Rubia peregrina* (Wild Madder). The roots of *R. cordifolia* have been used in Indian and Chinese traditional system of medicines to treat haematemesis, haematuria, inflammations, ulcers and skin diseases. Experimental studies confirmed madder as prophylactic agent, the antioxidant and free radical scavenging activities of phyto- components isolated from this plant give an impression that Indian madder might be the future drug for diversified panel of tumors and cancers[7].

Paprika gives oranges colour which is mainly the extract from the pods. The red coloured pigment is dominated by canthaxanthin and capsorubin, whereas yellow xanthophylls include cryptoxanthin, zeaxanthin, antheraxanthin and carotene. Turmeric is a commonly used colouring agent extracted from the rhizomes of turmeric plant, which is water soluble. It is also considered as an ayurvedic plant used as antitumor, anti-allergic, anti-inflammatory, antiseptic agent, also used in treating anemia, diabetes, indigestion and food poisoning. The seeds of the *Bixaorellana* tree produce an orange-red food colouring condiment known as annatto. Bixin and norbixin, carotenoid pigments produce colours that are found in the reddish waxy coat of the seeds. Annatto (*Bixa orellana*) extracts are widely used in many processed food products as a coloring agent and is also a natural alternative to synthetic food coloring compounds (*Food RGB, 2021).*

Food Safety and Standards Authority of India (FSSAI) has permitted bio-colours in India according to the Rule 26 of the Prevention of Food Adulteration Act, 1954 (PFA) and the Prevention of Food Adulteration Rules, 1955 and 1999. The following permitted bio-colours can be isolated from their natural sources: beet root concentrates annatto, beta-carotene, cochineal extract, grape extract, paprika oleoresin, turmeric oleoresin and saffron. Even though annatto is recommended as a natural alternative to synthetic food colourants, the permissible amount to be used as food colourant (other than milk products) is not precisely recommended by FSSAI. Thus, this study is carried out to analyze the requirement of bio food colourant to be added in foods. The objective of the study was to replace the synthetic and artificial food colourants with natural food colours by analyzing

the characteristics of the locally available natural food colourants.

Materials and Methods

Using questionnaire, a survey was conducted on the basis of knowledge related to the sources and usage of bio-colourants among the selected 50 home makers. From the survey, the frequently used bio-colourings, their sources, uses and healthy benefits were collected and is listed in Table I.

Table I represents the bio-colouring agents that were commonly known to the maximum number of the home makers (78%). The home makers were familiar with all the bio-colours except for annatto seeds (*Bixa orellana*) (18%), eucalyptus bark (*Eucalyptus grandis*) (23%), madder roots (*Rubia cordifolia*) (18%), roselle petals (*Hibiscus sabdariffa*) (19%), tamarind seeds (*Tamarindus indica*) (21%) and tanner's cassia petals (*Senna auriculata*) (16%). Thus, the above mentioned samples were proceeded for further analysis.

Tamarind seeds (*Tamarindus indica*) did not give colour and tanner's cassia (*Senna auriculata)* was a seasonal flower (unavailable in all seasons). Roselle petals (*Hibiscus sabdariffa*) gave beet red colour which showed quick discolouring and the presence of toxins in primary toxicity test using brine shrimp assay. Thus, tamarind seeds (*Tamarindus indica*), tanner's cassia (*Senna auriculata)* and roselle petals (*Hibiscus sabdariffa*) were ruled out for further processing of bio-colours.

The selected samples madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were collected from Forest College and Research Institute College in Mettupalayam, Coimbatore. The above mentioned samples were handpicked washed and dried to remove all the inedible particles. The selected samples of madder roots and eucalyptus barks were stored in sterile glass jars whereas annatto seeds were stored in zip lock bags. During storage at room temperature, the bio food colour samples of madder roots (*Rubia cordifolia*) eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were examined for no moisture content present in them.

Extraction process

The extraction process was carried out through two different methods.

- Powdered substances

The stored natural substances of madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were grinded into fine powder using sterilized desiccated mixing jars and the powdered substances were stored in separate zip lock covers and individual sterile glass jars.

- 10% aqueous extraction method

i) Soaked extraction: In the process of extraction of colourants through the

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TABLE I

An Overview of the Bio-colourants

Common name	Botanical name	Parts used	Colour obtained	Uses	Health benefits
Annatto	*Bixa orellana*	Seeds	Orange Red	Colouring cheese and flavored milk	Antioxidant, antimicrobial and anticancer properties
Beet root	*Beta vulgaris*	Roots	Red, yellow and Bluish-red (depending on betanin content)	Yogurts, ice cream, ice bars, candy, noodles and pasta	Reduces the risk of cancer, cardiovascular, cerebrovascular diseases, liver and kidney damage
Carrot	*Daucus carota*	Roots	Orange yellow	Pro-vitamin A agent and colouring agent in dietary supplements	Promotes vision, aids in weight loss, skin health, improves immunity and brain health
Eucalyptus	*Eucalyptus grandis*	Bark	Yellow Brown	Medicines – cough syrup, oil	Antioxidants, cold relief, treats dry skin, promotes relaxation and pain relief
Grapes	*Vitis vinifera*	Fruit skin	Red to deep purple	Soft drinks, ice creams, yogurts, jams and confectioneries	Rich in antioxidant, antidiabetic, antiobesity and anti-inflammatory activities
Jamun fruit	*Syzygium cumini*	Fruit	Pink to crimson black	Juice, squash, jam, jelly and toffee	Improves hemoglobin count, manages diabetes, improves gut health and acts as an immune booster
Marigold	*Calendula officinalis*	Petals	Vibrant yellow, orange	Cheese and butter	Anti-inflammatory that promotes wound healing for eczema, sunburns, bruises and varicose veins
Madder	*Rubia cordifolia*	Roots	Brown Red	Medicine for kidney stones, menstrual disorders and urinary tract disorders	Treating jaundice, obstruction in spleen, palsy and bruises
Paprika	*Capsicum annuum*	Fruit	Orange red	Meat products, confectionery, cheese, snacks, soups and salad	Rich in antioxidant properties, reduces the risk of cancer, heart diseases and improves immunity
Roselle	*Hibiscus sabdariffa*	Petals	Dark Red	Wine, syrup, ice cream, pies, snacks, tarts and other desserts	Laxative effect, used in treating cracks in feet, sores and wounds

Sandal wood	*Santalum album*	Bark, roots	Yellow	Sandalwood oil used as flavoring agent	Lowers blood pressure, urinary tract infection, reduces common cold, liver and gallbladder problems
Tamarind	*Tamarindus indica*	Pulp, Seeds	Brown, no colour	Pulp is used for preparing jams, jelly and toffee	Rich source of antioxidants, has anticancer properties, improves heart health, liver protective and anti-diabetic effects
Tanner's cassia	*Sennaauriculata*	Flowers	Yellow	Candy, jam and tea powders	Anti-diabetic, relief from rheumatism, conjunctivitis, ulcer and constipation
Turmeric	*Curcuma longa*	Roots	Bright yellow to deep orange	Pickles, seasoning, cheese, pies, candies, frozen desserts, beverages and snacks	Traditionally used against respiratory infections, arthritis, allergies, digestive disorders and liver disease

aqueous extraction, the compounds madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*) and roselle petals (*Hibiscus sabdariffa*) for extraction were taken in the ratio of 1:10, where 25 g of the natural substances were immersed in 250 ml of distilled water for 24 hours. On the 24th hour the aqueous solutions in the form of extracts were filtered and stored in sterilized glass jars.

ii) **Reflux extraction**: In the process of extracting the colourants, boiling of 10% aqueous extraction also known as the reflux extraction method was used. The compounds for extraction from madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*) and roselle petals (*Hibiscus sabdariffa*) were taken in the ratio of 1:10 with 25 g of extraction compounds (roots barks and seeds) were immersed in 250 ml of distilled water and placed in hot water bath of 60°C for an hour. The water bath was stirred constantly every 10 minutes to avoid clogging. Then the extracts were cooled, filtered and stored in individual sterilized glass jars.

Filtration process

i) **Soaked:** After 24 hours of soaking process, the natural food colour substances from madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*) and roselle petals (*Hibiscus sabdariffa*) were filtered into a measuring jar using Whatman filter paper.

ii) **Reflux extraction:** After an hour of boiling at 60°C,the substances in the water bath, the extracts of madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*) and roselle petals (*Hibiscus sabdariffa*) were left aside to cool for 10 minutes. Then the extracts were stirred and filtered using Whatman filter paper into a measuring jar. The extracts varied in their yields when measured and so each extract was made up to 250 ml. The yield of reflux extraction is tabulated in Table II.

pH values

The pH of the extracts (reflux extraction) was measured using pH meter. The pH values were tabulated along with the quantity of the extracts.

From Table II, it is evident to note the pH values of the extracts from madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*), annatto seeds (*Bixa orellana*) and roselle petals (*Hibiscus sabdariffa*) in comparison with the normal pH values. All the extracted compounds were analyzed for pH ranges and were not acidic in nature, where eucalyptus bark (*Eucalyptus grandis*) and roselle petals (*Hibiscus sabdariffa*) extracts were measured to be 4.57 mol/Land 3.43 mol/L of base, whereas madder roots (*Rubia cordifolia*) extract was slightly acidic with 5.69 mol/L and annatto seeds (*Bixa orellana*) extract had very mild acidity of 6.33 mol/L.

Food colour reader

The natural colour of the extracts of madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were measured on fourth day to know the rate of discolouring. To detect discolouring, food colour reader was used to analyse the colours in the extracts from the four samples. The food colour reader was calibrated using black

TABLE II

Yield of the Extracts and pH Range of the Bio Food Colours

Extraction compounds	Raw form (g)	Distilled water (ml)	Extract yield (ml)	pH (mol/L)
Madder (roots) (*Rubia cordifolia*)	25	250	170	5.69
Eucalyptus (bark) (*Eucalyptus grandis*)	25	250	160	4.57
Annatto (seeds) (*Bixa orellana*)	25	250	230	6.33
Roselle (petals) (*Hibiscus sabdariffa*)	25	250	180	3.43

and white cavity covers, placing the food colour reader's lens evenly on the cavity. Then the food samples were placed on a watch glass, with a plain white base and the colours were measured. Around 0.15 ml (that is 150 microliters) of the extracts of madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were pipetted out on the watch glass. The cavity of the lens in the food colour reader was placed over the liquid extracts and the reading was measured using L* as lightness, a* as either red or green and b* as either yellow or blue.

The colour scale used on the food colour reader is Commission International de l'Eclariage (CIE) system. In the colour space, numerical differences between values roughly correspond to the amount of change that can be visualized between colours. CIE LAB works under the principle of hue angle with relative saturation where the lightness of the measuring object remains unchanged.

CIE LAB: CIE LAB is colour space based on the fact that a colour cannot be both red and green, or both blue and yellow because these colours oppose each other. So a single data could be used to describe red/green and yellow/blue. When CIE L*a*b* is used to describe the colour, here L* means lightness, a* means red/green and b* means yellow/blue.

The colour spacing of HCL is Hue Chroma-Luminance or LCH that relates to any cylindrical models that are in accord with human perception of colours with the parameters. The principle of LCH has been initiated by visualizing the object without bias using varying saturation models.

TABLE III

Values used for Analyzing the Intensity of the Extracted Bio Food Colours

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	
ΔL +	White	
ΔL -	Black	
Δa +	Red	
Δa -	Green	
Δb+	Yellow	
Δb -	Blue	

CIELCH: CIELCH adopts same colour spaces as L*a*b*, but its L* represents lightness, c* represents saturation and h* represents hue.

Table III, represents the colours and the formula used in analyzing the colour intensity of the extracts. Delta-E is a single number representing the distance between two colours. It is the comparison of Euclidean distance (also known as distance between two points) difference between the red, green and blue hue of the food colours.

Brine shrimp lethality assay

For identification, the extracts of madder roots (*Rubia cordifolia*), eucalyptus bark (*Eucalyptus grandis*) and annatto seeds (*Bixa orellana*) were coded with the first two alphabets of the source as MA for madder roots, EU for eucalyptus bark and AN annatto seeds. The extracted samples were taken in different concentrations of 100 µl, 250 µl, 500 µl, 1000 µl and 1500 µl in each beaker containing saline solution. 10 shrimps were introduced into the sample solution of the above mentioned concentrations. The movement of the shrimp was monitored at different intervals of 1, 2, 4, 6 and 24 hours. There were three groups, in which the shrimp were introduced into blank solution group, control group [Potassium dichromate –$K_2Cr_2O_7$ 3 mg/ml)] and prepared samples solutions of different concentrations. The mortality percentage of shrimp was calculated after 24 hours. For each of the samples from madder roots, eucalyptus bark and annatto seeds, 50 shrimps were added to each of the solution of extracts in the coded as food colourants. The mortality of the shrimp was monitored as that of blank and control group.

Identification of Phytoconstituents

Qualitative screening for proteins, carbohydrates, flavonoids, alkaloids and cardiac glycosides were carried out to know the phytoconstituents present in the substances.

Test for Protein: Development of violet colour on addition of biuret reagent (2 ml) to the test solution (2 ml) indicates the presence of proteins.

Test for carbohydrates (Molisch's test): The development of purple ring at the interface between the test material and the acid on the addition of Molisch's reagent (α-naphthol dissolved in ethanol) to the extracts followed by addition of a few drops of concentrated sulfuric acid indicates the presence of carbohydrates.

Test for flavonoids

Alkaline reagent test: Development of intense yellow colour on addition of a few drops of sodium hydroxide solution to test solution, which turns to colourless after addition of few drops of dilute acid indicates the presence of flavonoids.

Shinoda test: Development of a few pink scarlet, crimson red or occasionally

green to blue colour appearance after addition of few magnesium turning followed by drop wise addition of conc. hydrochloric acid regarded as the presence of flavonoids.

Test for saponins: Development of emulsion on vigorous shaking after addition of three drops of olive oil to froth extracted by adding 0.1g of extract to 1 ml of distilled water indicate the presence of saponins.

Test for cardiac glycosides: Development of a brown ring at the interface after addition of 2 ml of glacial acetic acid that contained one drop of ferric acid chloride solution followed by further addition of 1 ml of concentrated sulfuric acid to 0.5mg of extract diluted with 5 ml of water indicates the presence of cardiac glycosides.

Statistical analysis

The statistical analysis has been carried out for the lethality of the brine shrimp between the aqueous extraction and the powdered substance solution using one way ANOVA and t-test.

Results and Discussion

Food colour reader

The extracted colours from madder roots, eucalyptus bark and annatto seeds were analyzed for discolouration using food colour reader and the change in colour was recorded and compared between aqueous food colour extract and powdered substances calculating the hue.

In Table IV, 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 is measured using the hue difference. The madder roots extract's colour changed from 48.67 to 47.04 hue. For eucalyptus bark and annatto seed extract colour increased from 38.13 to 47.10 hue and 42.69 to 47.00 hue respectively. As for the roselle petals extract, the colour decreased from 42.22 to 32.79 hue. The result in the food colour reader has shown that the boiled extracts have shown discolouration for every four days with less stability, whereas the powdered substances have not shown any changes in physical characteristics

Brine shrimp lethality assay: The results of the brine shrimp assay for aqueous extractions and powdered substances is tabulated and highlighted in Table V

From Table V, the mortality rate in percentage of 30 shrimp in each solution has been shown. In the blank solution there was no shrimp found dead. But in the study sample extracts from madder roots (*Rubia cordifolia*) and eucalyptus barks (*Eucalyptus grandis*), the mortality rate was noticed in the annatto seeds (*Bixa orellana*) extract minimum mortality rate of 53 percentage

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TABLE IV

Calculated Values of the Bio Food Colour Extracts on Every Fourth Day in a Week

Days	Natural food colour extracts	L*	a*	b*	$\sqrt{(\Delta L^*)2 + (\Delta a^*)^2 + (\Delta b^*)^2}$	ΔE* ab
1		48.37	-5.24	1.49	√2369.32	48.67
2	**Madder (Roots)**	47.25	-8.58	6.59	√2057.43	48.47
3	(*Rubia cordifolia*)	47.31	-6.80	3.90	√2299.68	47.95
4		46.37	-5.00	6.20	√2213.61	47.04
1		37.55	-5.48	3.71	√1453.79	38.13
2	**Eucalyptus (Bark)**	39.62	-3.80	6.16	√1622.12	40.27
3	(*Eucalyptus grandis*)	45.11	-8.30	5.34	√2129.31	45.35
4		46.26	-4.41	7.70	√2218.71	47.10
1		41.84	-8.43	1.08	√1822.80	42.69
2	**Annatto (Seeds)**	43.60	-8.47	12.43	√2127.20	46.12
3	(*Bixa orellana*)	48.91	-6.27	2.79	√2439.27	49.38
4		47.06	-6.92	3.02	√2271.64	47.66
1		41.36	8.21	2.17	√1782.74	42.22
2	**Roselle (Petals)**	38.98	-1.34	0.68	√1521.69	39.00
3	(*Hibiscus sabdariffa*)	27.88	15.90	6.25	√1069. 16	32.69
4		21.58	24.26	4.60	√1075.39	32.79

that is 16 shrimps were found dead at the rate of highest concentration of 1500 µl, whereas in the maximum of around six hours only two shrimps were found dead. In roselle petals (*Hibiscus sabdariffa*) all the shrimp were found dead and so it was not taken for further analysis.

In madder root (*Rubia cordifolia*) extract at the minimum of 100 µl 70 percentage, that is 21 shrimps were found dead and in the eucalyptus bark (*Eucalyptus grandis*) extract also had the minimum mortality percentage of 76 percentage at its lowest concentration level. But other than annatto seeds (*Bixa orellana*) extract, all the other extracts at the highest concentration level, nearly two-third of the shrimps were found dead. Table VI revealed the brine shrimp lethality assay using powdered substances.

In Table VI, the lethality of the brine shrimp for the powdered substances were expressed and revealed that annatto seeds powder showed the lowest rate of mortality of 7 per cent shrimp death even after 24 hours. Whereas in madder roots,

TABLE V

Brine Shrimp Lethality Assay (Aqueous Extraction)

Natural food colourants	Concentration (µg/ml)	Mortality of Brine shrimp (no. of shrimps dead) (hour)					
		1	2	4	6	24	% Mortality (at 24 h)
Madder (Roots) (*Rubia cordifolia*)	100	0	0	0	1	21	70
	250	0	0	4	4	24	80
	500	0	0	1	1	28	93
	1000	0	0	1	1	30	100
	1500	0	0	0	2	30	100
Eucalyptus (Bark) (*Eucalyptus grandis*)	100	0	0	0	1	23	76
	250	0	0	0	2	24	80
	500	0	0	0	1	25	83
	1000	0	0	7	20	29	96
	1500	0	0	22	27	30	100
Annatto (Seeds) (*Bixa orellana*)	100	0	0	0	0	11	36
	250	0	0	0	0	13	43
	500	0	0	0	1	13	43
	1000	0	0	0	2	16	53
	1500	0	0	0	2	16	53
Roselle (Petals) (*Hibiscus sabdariffa*)	100	0	25	29	30	30	100
	250	0	27	30	30	30	100
	500	0	30	30	30	30	100
	1000	0	30	30	30	30	100
	1500	0	30	30	30	30	100
Control $K_2Cr_2O_7$	**1(mg/ml)**	**30**	**-**	**-**	**-**	**-**	**100**
Blank	**Saline water**	0	0	0	0	0	0

the percentage of mortality at 24 hours was 40 per cent in which nearly 12 shrimp were found dead with the highest concentration of solution. In eucalyptus bark powder solution the highest per cent in rate of mortality in the highest concentration was 13 per cent with the death of 4 shrimp in total.

TABLE VI

Brine Shrimp Lethality Assay (Powered Substances)

Bio food colourants	Concentration (µg/ml)	Mortality of Brine shrimp (no. of shrimps dead) (hour)					
		1	2	4	6	24	% Mortality (at 24 h)
Madder (Roots) (*Rubia cordifolia*)	100	0	0	0	0	1	3
	250	0	0	0	1	2	7
	500	0	0	0	1	7	23
	1000	0	0	1	1	10	30
	1500	0	0	1	3	12	40
Eucalyptus (Bark) (*Eucalyptus grandis*)	100	0	0	0	0	2	7
	250	0	0	0	0	2	7
	500	0	0	0	0	2	7
	1000	0	0	1	1	2	7
	1500	0	0	2	3	4	13
Annatto (Seeds) (*Bixa orellana*)	100	0	0	0	0	1	3
	250	0	1	1	1	2	7
	500	0	1	2	2	2	7
	1000	0	1	1	1	2	7
	1500	0	1	1	1	2	7
Control $K_2Cr_2O_7$	1(mg/ml)	30	-	-	-	-	100
Blank	**Saline water**	0	0	0	0	0	0

Profile of phytonutrients

The profile of phytonutrients was noted and proved that the bio food colourants are applicable for human consumption.

In the Table VII, the presence of the metabolites are indicated as '+' and the absence of the metabolites are indicated as '-'. Overall the phytonutrients present in the substances are carbohydrates, flavonoids and saponins and proteins and cardiac glycosides were not present in the selected bio colourants.

Statistics and analysis

The lethality of the brine shrimp between the aqueous extraction and the powdered substance solution were

TABLE VII

Profile of Phytonutrients in the Selected Bio Colourants

Metabolites	Test Performed	Madder roots (*Rubia cordifolia*)		Eucalyptus barks (*Eucalyptus grandis*)		Annatto seeds (*Bixaorellana*)	
		Aqueous extracts	Powdered substances	Aqueous extracts	Powdered substances	Aqueous extracts	Powdered substances
Proteins	+2% Ninhydrin reagent	-	-	-	-	-	-
	+2% $CuSo_4$+ 95% ethanol+ KOH pellet +Conc. HNO_3	-	+	-	+	-	-
Carbohydrates	Molisch's test	+	+	+	+	+	+
	Fehling's test	-	+	+	+	+	+
Flavonoids	Alkaline test	+	-	+	+	+	+
	Shinoda test	-	-	+	-	-	
Saponins	Shaken with water	+	+	+	-	+	
	+Baljet reagent	-	-	+	-	+	+
Cardiac glycosides	Bromine water test	-	-	-	-	-	
	Keller-killai test	-	-	+	-	-	

statistically analyzed using one way ANOVA and t-test to calculate the mean and standard deviation. The null hypothesis (H_0) indicated that there was no difference between the rate of mortality in the aqueous extraction and the powdered substance solution, whereas the alternative hypothesis (H_A) was noted that there was difference in the rate of mortality between the two sample solutions of aqueous extracts and powdered substances.

From Table VIII, the mean and standard deviations were calculated using t-test. The factor value represented as F is

TABLE VIII

Mortality rate between the Aqueous extracts and the Powdered Substances

Mortality (in 24hour)	Mean and Standard Deviation (M±SD)	Factor (F)	Significance (sig.)
Aqueous Extraction	73.7 ± 22.8	1.81	0.20
Powdered Substance	11.7 ± 10.7		

1.81 and the significant value is 0.20. As the factor and significant value is greater than 0.05, alternate hypothesis H_A is accepted and null hypothesis H_0 is rejected which depicts that there is difference in the mortality percentage of the death in shrimp in aqueous solution and powdered substances solution.

Conclusion

Natural food colourants are becoming a global demand. Nowadays synthetic colours are replaced by natural colours. Present days' consumers are progressively becoming conscious and are being cautious about what they consume. Researchers have proved that synthetic food colours affect physical and psychological health of the population especially vulnerable group of young children and adolescents. Thus natural colourants from plant sources are extracted to incorporate in foods and hence primary level toxicity test was carried out in brine shrimp assay. In the colour intensity test, the colours of madder roots (*Rubia cordifolia*), eucalyptus barks (*Eucalyptus grandis)*, annatto seeds (*Bixa orellana*) and roselle petals (*Hibiscus sabdariffa*) were not very much stable, as the colour intensity kept fluctuating and later returned to their stable level of either increasing or discolouring.

As in the primary level of toxicity test of the madder roots (*Rubia cordifolia*), eucalyptus barks (*Eucalyptus grandis)* and annatto seeds (*Bixa orellana*) using brine shrimp assay are proved less toxic in nature when used in the appropriate proposition. Roselle petals (*Hibiscus sabdariffa*) extract was highly toxic and it was ruled out from further processing. When the concentration level of the natural extracts was increased the components were recorded to be toxic with the mortality rate of shrimp in the minimum number of hours in aqueous extraction process of boiling the natural substances. Conclusively, madder roots (*Rubia cordifolia*), eucalyptus barks (*Eucalyptus grandis)* and annatto seeds (*Bixa orellana*) powdered substance showed less mortality rate, as the bio-toxins are not being ignited due to heating. Thus, the natural powdered substances are considered suitable for replacement of synthetic colours as they also provide health benefits against ulcer, diabetes, cancer, also acts as antioxidants that prevent premature ageing, helps boost the immune system and also acts as zoloft (sertraline – drug) in enhancement of the mental health against anxiety and depression in children and adolescents. Consumption of natural food colourants in its natural form is considered safe for human consumption, playing a vital role as biomarkers in various aspects of health.

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REFERENCES

1. Rozylo, R. Recent trends in methods used to obtain natural food colorants by freeze-drying. *Trends. Food Sci. Technol.*, 2020, **102**, 20-612. https://doi.org/10.1016/j.tifs.2020.06.005
2. Wissgot, U. and Bortlik, K. Prospects for new natural food colorants. *Trends. Food Sci. Technol.*, 1996, 7. https://doi.org/10.1016/0924-2244(96)20007-X
3. Mohamad, M.F. and Dailin, D.J. Natural colourant for food: A health alternative, *Int. J. Sci. Technol. Res.*, 2019, **8**, 3161-3166.
4. Arnold, E.L., Lofthouse, N. and Hurt, E. Artificial food colors and attention-deficit/hyperactivity symptoms: Conclusions to Dye. *Neurotherapeutics*, 2012, **9**, 599-609, PMCID: PMC3441917 PMID: 22864801, doi: 10.1007/s13311-012-0133-x.
5. Naseer, S., Afzal, M.S., Nisa, A., Hussain, S., Ahmad, M.A., Parveen, S., Anjum, F. and Riaz, M Extraction of brown dye from *Eucalyptus* bark and its applications in food storage, *Quality Assurance. Safety. Crops. Foods.*, 2019, **11**, 769 – 780, https://doi.org/10.3920/QAS2019.1500
6. Inggrid, H.M., Jaka, and Santoso, H. Natural Red dyes Extraction on Roselle Petals, Series Materials Science and Engineering 162, Second International Conference on Chemical Engineering (ICCE) UNPAR IOP, 2017, 012029 doi:10.1088/1757-899X/162/1/012029
7. Sarah Kobylewski, Michael F. Jacobson. Toxicology of food dyes- Review. *Int. J. Occup. Environ. Health.*, 2012, **18**, 220-246, PMID: **23026007**; DOI: 10.1179/1077352512Z.00000000034.
8. Devi Priya, M. and Siril, E.A. Traditional and modern use of Indian madder (*Rubia Cordifolia* L.) An overview. *Int. J..Pharmac. Sci. Rev. Res.*, 2014, **25**, 154-164. www.globalresearchonline.net