

## International journal of adulteration

### To Cite:

Yadav U. Quantitative and Qualitative Assessment of Synthetic Dye Adulteration in Street-Sold Snacks from Mumbai Suburban Region, India. *International journal of adulteration*, 2026; 10: e2ijad3069  
doi:

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### Peer-Review History

Received: 27 August 2025  
Reviewed & Revised: 07/September/2025 to 09/January/2026  
Accepted: 18 January 2026  
Published: 25 January 2026

### Peer-Review Model

External peer-review was done through double-blind method.

International journal of adulteration  
eISSN (Online) 2456 – 0294



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# Quantitative and Qualitative Assessment of Synthetic Dye Adulteration in Street-Sold Snacks from Mumbai Suburban Region, India

Udaybhan Yadav<sup>1\*</sup>

## ABSTRACT

Street foods are an important part of urban diets in India, especially in the metro cities like Mumbai, Chennai, Kolkata and Delhi. The colour appearance of these foods often comes from synthetic food dyes, which sometimes go over safe limits or include banned substances which ultimately leads to illful impact of humans. The current study focuses more on the presence of synthetic dye adulterants in commonly sold street snacks from the suburbs of Mumbai. I collected eighty food samples, such as jalebi, samosa, bhaji, chaat chutneys, pav bhaji masala, and ice gola syrups, from major street food vending areas or Khau-galli. Here for better and accurate result, I, used thin-layer chromatography and UV-visible spectrophotometry for qualitative screening, while high-performance liquid chromatography (HPLC) was used for quantitative estimation. The dyes we found included allowed colors like tartrazine, sunset yellow, carmoisine, and ponceau 4R, along with banned dyes like metanil yellow. About 32.5% of the samples exceeded the maximum tolerable limits which was approved and given by the Food Safety and Standards Authority of India. Our risk assessment, based on estimated daily intake, suggests potential health issues for regular consumers especially the young kids who are more enthusiastic for eating outside from street vendors. These findings show the need for better monitoring and increased public awareness about food dye adulteration in informal food markets.

**Keywords:** Food adulteration, synthetic dyes, street foods, Mumbai suburban, HPLC, public health, Khaugalli

## 1. INTRODUCTION

Food adulteration, whether accidental or deliberate, continues to be a major and long-lasting public health issue around the world. This is especially true in developing countries where a large part of the population relies on informal food systems, like street food or Khaugalli's, to meet their daily nutritional needs (Dixit

et al., 2007). Adding bad, unwanted, harmful, or illegal things to food which degrade its quality is called adulteration. This makes the food less healthy and could be bad for your health. Street food is a big part of Indian culture, and it's cheap, easy to get, and a big part of the culture (Dixit et al., 2007; Sharma & Verma, 2015).

There are four types of food adulteration: intentional, incidental, accidental, and metallic. Using too many synthetic food dyes is a big problem because it is a form of intentional adulteration. Most of the time, food colors are added to make food look better, to bring back color that was lost during processing, or to make food look good so people will buy it. Street food vendors use synthetic dyes a lot because they are cheap, easy to find, and work well even in small amounts (Sharma & Verma, 2015; Rahnamah et al., 2022). But these benefits often lead to careless use, like using dyes in ways that aren't allowed or using industrial dyes that aren't allowed.

There are only a few synthetic food colors that are allowed to be used in food products by law. The FSSAI (Food Safety and Standards Authority of India), lets you use certain synthetic colors, like Tartrazine, Sunset Yellow FCF, Carmoisine, Ponceau 4R, and Allura Red AC, up to 100 mg/kg, either alone or in combination, depending on the type of food (World Health Organization, 2016a). The World Health Organization's Joint FAO/WHO Expert Committee on Food Additives (JECFA) has also set Acceptable Daily Intake (ADI) values for these dyes to make sure they are safe to eat for life as long as you stay within those limits (Bhattacharyya & Chakraborty, 2023; World Health Organization, 2011).

Even though there are rules, a lot of studies done all over India have found that a lot of allowed levels have been broken, especially in street foods that aren't regulated (Dixit et al., 2007; Rahnamah et al., 2022). Metanil Yellow and other colors that are not allowed in food are still present, even though they are illegal because they can be poisonous, neurotoxic, and cause cancer (Dixit et al., 2007; Rao & Bhat, 2003).

Prolonged contact to chemical based food dyes has been associated with various disease like, allergic reactions, gastrointestinal issues, oxidative stress, alterations in children's behaviour, early adolescence, and DNA damage (Swaroop et al., 2011; Food Safety and Standards Authority of India, 2011). Children are considered particularly more vulnerable because of lack of awareness owing to their disproportionate consumption of vividly colored foods relative to their body weight (Rahnamah et al., 2022; Food Safety and Standards Authority of India, 2011).

People usually think that natural food colors made from plants are safer like saffron, turmeric, annatto, and beetroot. Natural dyes can do more than just color things; they can also be good for your health and work as antioxidants (Rao & Bhat, 2003). They aren't used as much in the street food business, though, because they cost more, don't last as long, and aren't as stable.

Many people in the suburbs of Mumbai rely on street food. People are eating more street food these days because cities are growing quickly, people are spending more time commuting, families have two incomes, kids are under a lot of academic pressure, and people don't have much time to cook at home. People who work in offices, go to school, or only work for a day eat street food because it's easy to find, tastes good, and doesn't cost much. This high demand, though, often makes store owners put looks and profits ahead of following food safety rules.

Regulatory agencies do check things out from time to time, but there isn't any scientific data on how much and how good synthetic dye adulterants are in street food sold in the suburbs of Mumbai. Most of the studies that have been done so far focus on packaged foods. Street foods that aren't packaged don't get regular inspections (Dixit et al., 2007; Sharma & Verma, 2015). This necessitates region-specific analytical studies that examine both adherence to regulatory standards and potential public health risks.

This research addresses the gap by examining the utilization of synthetic/ artificial food colors in street snacks, which are frequently consumed by individuals in the suburbs of Mumbai, through both qualitative and quantitative methods. The study seeks to produce evidence-based data through the integration of various chromatographic techniques with risk assessment and exposure analysis, which can be employed to improve food safety monitoring, law enforcement, and public awareness.

**Table 1.** Permissible Synthetic Food Colours and Regulatory Limits (FSSAI / Codex)

Sr. No.	Synthetic Food Colour	INS No.	Regulatory Status	Maximum Permissible Limit*	Acceptable Daily Intake (ADI)**
1	Tartrazine	INS 102	Permitted	Up to 100 mg/kg	0-10 mg/kg body weight/day
2	Sunset Yellow FCF	INS 110	Permitted	Up to 100 mg/kg	0- 4 mg/kg body weight/day
3	Carmoisine	INS 122	Permitted	Up to 100 mg/kg	0-4 mg/kg body weight/day
4	Ponceau 4R	INS 124	Permitted	Up to 100 mg/kg	0-4 mg/kg body weight/day
5	Allura Red AC	INS 129	Permitted	Up to 100 mg/kg	0-7 mg/kg body weight/day
6	Metanil Yellow	--	<b>Not permitted</b>	<b>Not allowed in any food</b>	<b>ADI not assigned (toxic)</b>

Table 1 shows the list of synthetic food colors that are allowed, along with their maximum limits and Acceptable Daily Intake (ADI) values, as set by FSSAI and Codex Alimentarius. Any violation of these restrictions constitutes food adulteration and poses potential health risks.

The FSSAI and Codex guidelines (World Health Organization, 2016b) say that the maximum allowable limit is the most synthetic food color that can be in food, either by itself or with other colors. WHO/JECFA sets ADI values, which tell you how much of a substance is safe to be around for the rest of your life (Bhattacharyya & Chakraborty, 2023; World Health Organization, 2011). Metanil Indian and international laws say that yellow is an industrial color that can't be used in food (Dixit et al., 2007; Rao & Bhat, 2003).

#### Objectives:

- i. To identify, characterize, and classify synthetic food dyes present in street-vended snacks from suburban regions of Mumbai, including Borivali, Malad, and Kandivali.
- ii. To check the levels of dyes and see if they follow the rules set by the FSSAI and WHO.
- iii. To find out what health risks these dyes pose to the general public when used too much.

#### Area of the survey:

The survey was done in the suburbs of Mumbai, including Borivali, Malad, Kandivali, and nearby train stations and shopping areas. A lot of people buy and eat things in these places.

## 2. MATERIALS AND METHODS

**Study Area and Sample Collection:** Selection of location is depends on the density of an individual along with their standard of living so that individuals can come across by walking means which further leads to customer footfall and increase the consumption of the street food items. By taking in to the consideration for study purpose I have selected Mumbai suburban region like Kandivali, Borivali, Malad, covering locations of Railway Station, College vicinity, Kahugallis etc.

The food Samples were collected in sterile plastic containers from different vendors to avoid duplication and to ensure representative coverage of the study area. Total 80 street food samples were randomly collected during June to July month of 2025, a period corresponding to high street food consumption because of Indian Festivals. The food samples contains commonly consumed and visually coloured food items:

- i. Funnel Cake or Jalebi (n=15 samples)
- ii. Stuffed pastry or Samosa (outer coating and filling analyzed separately) (n=15 samples)
- iii. Bhajiya or Pakoda (n=10 samples)
- iv. Green and Red Chutneys (n = 15 samples)
- v. Pav bhaji masala (prepared food) (n = 10 samples)
- vi. Ice gola syrups (n = 15 samples)

Approximately 100-150gm of each sample was collected under sterile condition in a food-grade plastic containers. Samples were labelled properly with time & date and transported under ambient conditions to the Microbiology Laboratory, Thakur Shyamnarayan Degree College, Mumbai, and stored at 4 °C until analysis. All samples were studied and evaluated within 24 hours of collection to minimize degradation of dye compounds.

**Chemicals and Reagents:** Analytical-HPLC grade solvents/ chemicals including methanol, acetonitrile, and distilled water (d/w prepared in our microbiology lab) were used throughout the study. Certified reference standards charts from FSSAI (purity ≥ 98%) of synthetic food dyes, namely Tartrazine, Sunset Yellow FCF, Carmoisine, Ponceau 4R, Allura Red AC, and Metanil Yellow, were procured from authorized suppliers for calibration and validation purposes. All glassware was thoroughly washed, cleaned and rinsed with distilled water, sterilised in autoclave prior to use. All the testing of samples performed under sterile condition in Laminar Air Flow in between the burners.

**Extraction of Synthetic Food Dyes from Food Samples:** Collected food sample was finely homogenized using a sterile mortar and pestle under sterile condition individually. Aqueous methanol (70%) was selected due to its proven efficiency in extracting both permitted and non-permitted synthetic dyes from complex food matrices. Approximately 05gm of homogenized sample was extracted with 70% aqueous methanol by continuous shaking for 30 minutes in the shaker. The mixture sample was centrifuged at 5,000rpm for

10 minutes, and the supernatant was filtered through Whatman filter paper No.-1. The filtrate was concentrated under controlled condition (reduced pressure) when necessary and reconstituted in a known volume of methanol prior to analysis. All extractions were performed in duplicate, and mean values were used for analysis.

### Qualitative Analysis

**i. Thin Layer Chromatography (TLC):** Qualitative screening of synthetic dyes was carried out using silica gel TLC plates. The mobile phase was prepared in the ratio of 4:1:5, which consists of n-butanol: acetic acid: water. Extracts and standard dye (Certified reference standards of synthetic food dyes) solutions were spotted on the plates and developed in a saturated chamber with mobile phase. After development, plates were air-dried and observed under visible and UV light. Rf values (Retention factor or Retardation factor) of obtained sample spots were compared with the of standard dyes values for preliminary identification.

Formula to calculate Rf Values:

$$\text{Rf values} = \frac{\text{Distance travelled by the compound (spot)}}{\text{Distance travelled by the solvent front}}$$

Rf (Retention factor or Retardation factor) values are characteristic features under controlled experimental conditions and are useful for preliminary identification of compounds by comparison with standard references chart.

**ii. UV- Visible Spectrophotometry:** Next step was performed for qualitative confirmation using UV-Visible spectrophotometry. Sample extracts were scanned in the wavelength range of 200nm -700nm, and characteristic absorption maxima ( $\lambda_{\text{max}}$ ) i.e. recorded OD were compared with reference standards chart results.

### Quantitative Analysis

**i. High Performance Liquid Chromatography (HPLC):** Quantitative estimation of synthetic dyes was performed using High Performance Liquid Chromatography (HPLC).

- i. Column: C18 reverse-phase column
- ii. Mobile phase: Acetonitrile- water gradient system
- iii. Flow rate: 1.0 mL/min
- iv. Detector: UV-Visible detector set at dye-specific wavelengths
- v. Injection volume: 20  $\mu$ L

Calibration curves were prepared by evaluating a series of known concentrations of certified dye standards, and quantitative estimation of synthetic dyes in various food samples was evaluated using the external standard calibration tool method.

**ii. Interpretation of Evaluation Results:** Quantitative outcome were obtained by introducing sample peak areas against calibration curves prepared using approved and certified dye as per FSSAI standards. The concentrations of chemical dye were expressed as mg/kg of food sample (fresh weight). The observed values were evaluated and verified against the regulatory limits recommended by FSSAI and Codex Alimentarius, and samples exceeding these limits were considered non-compliant for the consumption of the human beings.

### Quality Control and Method authentication

Quality control approach were implemented throughout the analytical process to ensure accuracy, precision, and reproducibility of the results. Reagent blanks were analysed alongside samples to check for any background contamination arising from solvents or glassware. Sample analysis were performed in duplication to assess analytical repeatability.

To analyse sample extraction efficiency and valuation method accuracy, spike recovery experiments were performed by fortifying selected food samples with known concentrations of certified dye standards prior to extraction. Percentage recovery of solution sample was obtained by comparing the measured concentration with the added concentration, and acceptable recovery ranges were considered in accordance with standard analytical guidelines.

Linearity of the method was assessed by analysing a series of standard solutions of each dye across a defined concentration range. Calibration curves were drawn by plotting peak area against concentration, and linear regression analysis was performed to determine correlation coefficients ( $R^2$ ). Only calibration curves showing good linearity were used for quantification.

The LOD (limit of detection) and LOQ (limit of quantification) for each dye were determined based on the signal-to-noise approach, where LOD i.e. limit of detection was defined as the minimal amount concentration producing a signal-to-noise ratio of approximately 3:1, and LOQ as the concentration corresponding to a signal-to-noise ratio of approximately 10:1. These parameters ensured the sensitivity of the method for detecting low levels of dye adulteration.

Method precision was evaluated by analysing replicate samples within the same day (intra-day precision) and on different days (inter-day precision), and results were expressed as relative standard deviation (RSD). The overall validation parameters confirmed the reliability and suitability of the analytical method for qualitative and quantitative determination of chemically synthesized food dyes in street-sold food samples.

### Statistical Analysis of samples

Quantitative data procured from the evaluation of synthetic food dyes were subjected to descriptive statistical analysis and represented as mean  $\pm$  standard deviation (SD) to finalise the central tendency value and variability of synthetic dye concentrations in different food samples from different vendors. Comparative statistical quantitative analysis was carried out to evaluate differences in dye concentrations among various categories of street foods. If suitable to samples, one-way analysis of variance (ANOVA) was used to determine statistically significant differences between food groups, followed by suitable post-hoc comparisons when required. In cases involving comparison between two groups, Student's t-test was applied. A p-value less than 0.05 was considered statistically significant. All statistical evaluation were performed using statistical computer based software, ensuring accuracy and reproducibility of results.

### Ethical Considerations

The study did not involve any human or animal or living subjects. Food vendor's identities and exact locations were kept confidential, and the analysis was conducted solely for academic and public health assessment purposes.

## 3. RESULTS

### Occurrence of Synthetic Dyes in Street-Sold Food Samples

Out of the 80 street food samples from Khaugallis were analysed, synthetic food dyes were detected in a substantial proportion of samples collected from the Mumbai suburban region like Borivali, Malad & Kandivali. Qualitative screening by TLC and UV-Visible spectrophotometry revealed the presence of both permitted and non-permitted synthetic dyes across different food categories.

Among the detected dyes, Tartrazine, Sunset Yellow FCF, Carmoisine, Ponceau 4R, and Allura Red AC were frequently identified. Notably, the non-permitted dye Metanil Yellow was detected in a few samples, particularly in intensely coloured snacks and syrups.

**Table 2.** Occurrence and Permissibility of Synthetic Food Dyes Detected in Street-Sold Food Samples from Mumbai Suburban Region

Food Category	Tartrazine	Sunset Yellow FCF	Carmoisine	Ponceau 4R	Allura Red AC	Metanil Yellow	Remarks
Jalebi	✓	✓	–	–	–	–	Permitted dyes detected
Samosa (outer coating)	✓	✓	–	–	–	–	Permitted dyes detected
Bhaji / Pakoda	✓	–	–	–	–	–	Permitted dye detected
Chaat chutneys (green & red)	–	✓	✓	–	–	✓	Non-permitted dye detected
Pav bhaji masala (prepared food)	–	–	–	✓	–	–	Permitted dye detected
Ice gola syrups	–	–	–	–	✓	✓	Non-permitted dye detected

Note: ✓ = Detected & – = Not detected



Table 2 shows the qualitative occurrence of permitted and non-permitted synthetic food dyes in commonly consumed street-sold foods collected from the Khaugallis of Kandivali, Malad & Borivali region of the Mumbai suburban, which is identified and plotted by TLC and UV-Visible spectrophotometry.

### Qualitative Identification of Synthetic Dyes

TLC (Thin Layer Chromatography) result revealed distinct and well-highlighted spots in the various food sample extracts that matches closely with those of certified reference standards values developed and provided by under identical chromatographic conditions by FSSAI. The R<sub>f</sub> (Retention or Retardation factor) values obtained for the sample extracts showed minimal or least variation from standard referred values, confirming the presence of specific synthetic dyes after comparing.

Further qualitative analysis confirmation was achieved by using instrument UV- Visible spectrophotometry, where sample extracts exhibited characteristic of absorption maxima ( $\lambda_{\text{max}}$ ) consistent with the respective dye standards reference number. The combined and comparative result of use of TLC and UV-Visible spectrophotometry enhanced the reliability of qualitative identification and minimized the possibility of false positives. The observed R<sub>f</sub> spotted values and absorption maxima for the identified dyes are summarized in Table 3.

**Table 3.** Qualitative Identification of Synthetic Food Dyes in Street-Sold Food Samples from Mumbai Suburban Region

Dye Identified	Permissibility	R <sub>f</sub> Value (Mean $\pm$ SD)	$\lambda_{\text{max}}$ (nm)
Tartrazine	Permitted	0.42 $\pm$ 0.02	427
Sunset Yellow FCF	Permitted	0.48 $\pm$ 0.03	482
Carmoisine	Permitted	0.36 $\pm$ 0.02	515
Ponceau 4R	Permitted	0.40 $\pm$ 0.02	508
Allura Red AC	Permitted	0.44 $\pm$ 0.02	504
Metanil Yellow	Non-permitted	0.55 $\pm$ 0.03	430

Minor variations or differences in R<sub>f</sub> data values were attributed to matrix effects of various complex food samples and standard experimental conditions.

### Quantitative Estimation of Synthetic Food Dyes from samples

Quantitative estimation using HPLC demonstrated considerable variation in synthetic dye concentrations across different street food categories. The highest dye levels were predominantly observed in ice gola syrups, chaat chutneys, and jalebi, indicating deliberate addition of colouring agents to enhances visual attractiveness and consumer appeal.

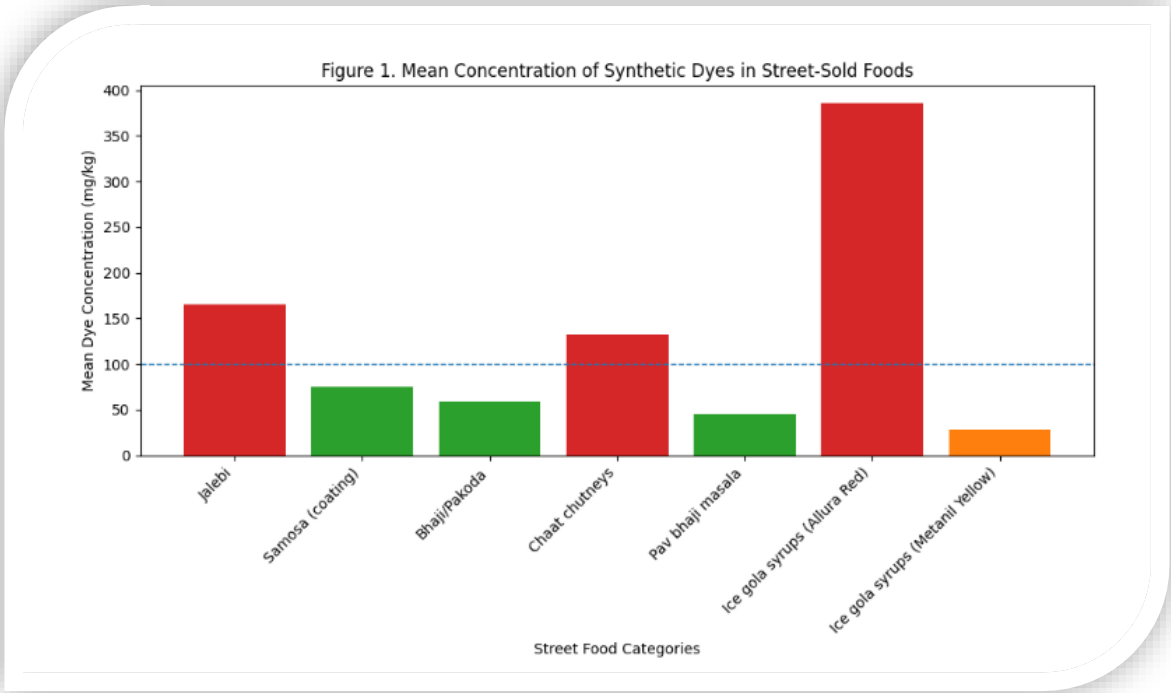
Overall, dye concentrations ranged from minimum concentration 12.4mg/kg to maximum 385.6 mg/kg. Several samples exceeded the maximum permissible limits prescribed by the FSSAI and Codex Alimentarius, particularly for commonly used dyes such as Tartrazine and Sunset Yellow FCF. Alarmingly, the non-permitted banned chemical dye i.e. Metanil Yellow was detected in ice gola syrups, confirming the informal food adulteration from food vending systems. In several samples, multiple dyes were detected simultaneously, which may increase cumulative dietary exposure beyond individual dye limits which further leads to various diseases to human kind. The detailed quantitative mean concentration values of detected dyes, along with FSSAI regulatory compliance status, is presented in Table 4.

**Table 4.** Quantitative Levels of Synthetic Food Dyes Detected in Street-Sold Foods from Mumbai Suburban Region

Food Item	Major Dye Detected	Mean Concentration (mg/kg)	FSSAI Permissible Limit (mg/kg)	Compliance Status
Jalebi	Tartrazine	165.3 $\pm$ 42.6	100	Non-compliant
Samosa (outer coating)	Sunset Yellow FCF	74.5 $\pm$ 18.2	100	Compliant
Bhaji / Pakoda	Tartrazine	58.9 $\pm$ 14.7	100	Compliant
Chaat chutneys	Carmoisine	132.7 $\pm$ 36.4	100	Non-compliant
Pav bhaji masala	Ponceau 4R	45.2 $\pm$ 11.3	100	Compliant

Ice gola syrups	Allura Red AC	385.6 ± 72.8	100	Non-compliant
Ice gola syrups	Metanil Yellow	28.4 ± 6.9	Not permitted	Non-compliant

Table 4 suggest that Quantitative concentration (mean ± SD) of major synthetic food dyes detected in commonly consumed street-sold food items collected from the Mumbai suburban region, as determined by HPLC analysis. The observed concentrations were compared with the maximum permissible limits prescribed by the Food Safety and Standards Authority of India (FSSAI), and samples exceeding these limits or containing non-permitted dyes were classified as non-compliant for consumption.



**Figure 1.** The Mean Concentration of Synthetic Dyes in Street-Sold Foods.

The Figure 1 represents a Bar chart showing the mean (average) concentration of synthetic food dyes (mg/kg) detected in different street-sold food categories collected from the Mumbai suburban region of Kandivali, Malad & Borivali (X-axis: food categories; Y-axis: mean dye concentration in mg/kg). Graph Bars are in different colour-coded to indicate regulatory status, where red bars represent non-compliant samples exceeding FSSAI limits, green bars indicate compliant samples, and orange bars denote the presence of non-permitted or banned dyes. The dashed horizontal line at 100 mg/kg represents the maximum permissible limit approved by the FSSAI. The figure 1 highlights markedly elevated dye concentrations in ice gola syrups and regulatory exceedance in jalebi and chaat chutneys, along with detectable levels of the banned dye Metanil Yellow.

**Prevalence of Non-Permitted Dye (Metanil Yellow)**

Metanil Yellow was detected in 7.5% of the total street food samples analysed, with its presence mainly confined to ice gola syrups and chaat chutneys, which are visually appealing and liquid or semi-liquid in nature texture wise. Although the concentrations of Metanil Yellow were lower than those observed for permitted synthetic dyes, its detection is of serious concern because Metanil Yellow is completely prohibited for use in food products under Indian food safety regulations (FSSAI).

The presence of this non-permitted dye, even at low levels, indicates deliberate or uninformed adulteration practices within informal street food systems and reflects gaps in regulatory awareness and enforcement. Given its reported toxicological effects, the continued use of Metanil Yellow poses a potential health risk, particularly to children and frequent consumers of street foods.

Comparative Statistical Analysis

The Comparative statistical analysis of synthetic dye concentrations used across different street food categories disclose statistically significant variation ( $p < 0.05$ ) among the various analysed samples. The analysis demonstrated that sweet, liquid, and semi-liquid food items, particularly ice gola syrups and jalebi, contained significantly higher concentrations of synthetic dyes when compared to savoury solid foods such as samosa and bhaji/pakoda.

A biostatistics method ANOVA was used to assess differences in mean dye concentrations among the various food categories. The obtained results indicated a significant effect of food type on dye concentration, suggesting that the extent of colour adulteration is strongly influenced by the location of the food preparation, physical nature and preparation method of the respective food. Post-hoc multiple comparison evaluation result further confirmed that liquid and syrup-based foods revealed significantly higher dye levels than solid food items.

The raised dye concentrations observed in liquid foods may be attributed to the ease of dye dissolution, lack of visual dosing control, and consumer preference for bright colours, whereas solid savoury foods showed comparatively lower and more regulated dye usage. These findings helps us to understand the importance of targeted regulatory monitoring of high-risk food categories, particularly street-sold beverages and syrups.

Table 5. Comparison of Mean Synthetic Dye Concentrations among Street Food Categories

Food Category Type	Number of Samples (n)	Mean Dye Concentration (mg/kg)	Statistical Significance
Liquid / Syrup-based foods	30	High ( $\geq 150$ mg/kg)	Significant ( $p < 0.05$ )
Semi-solid sweet foods	15	Moderate ( $\geq 130$ mg/kg)	Significant ( $p < 0.05$ )
Solid savoury foods	35	Low ( $\leq 75$ mg/kg)	Reference group

Significance determined using one-way ANOVA followed by post-hoc comparison.

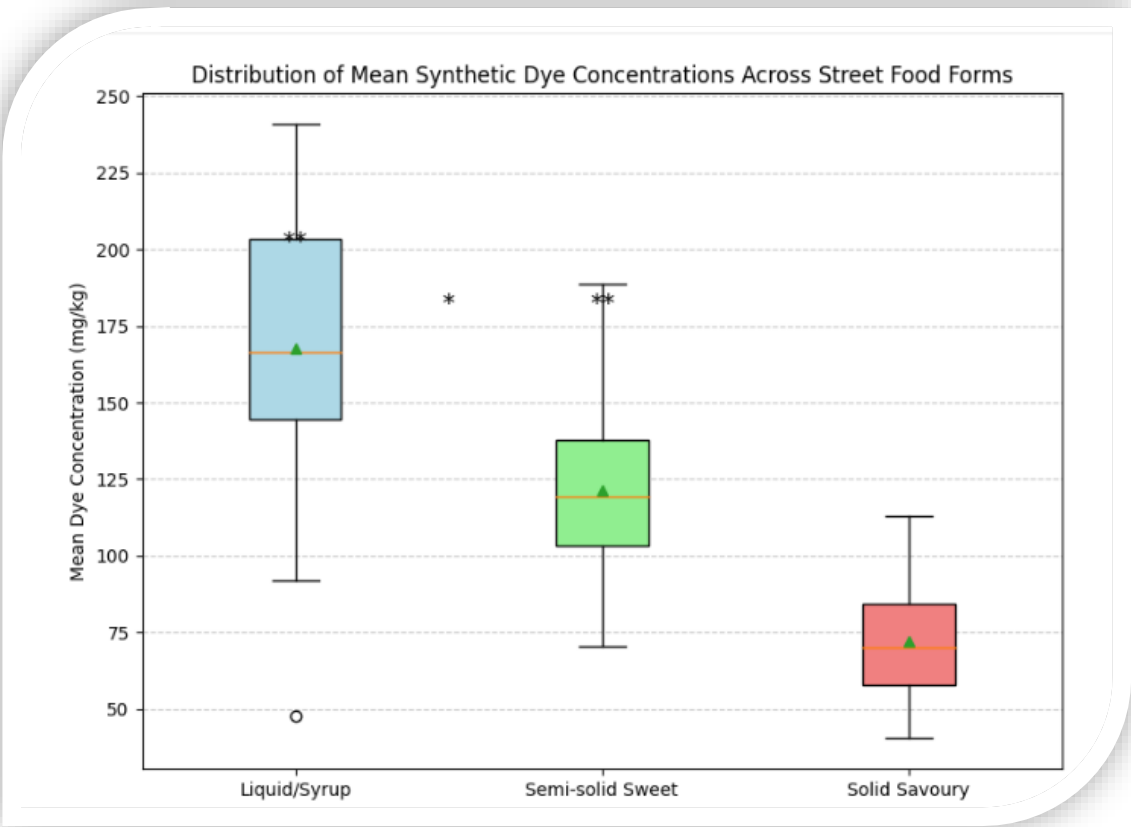


Figure 2. Distribution of Synthetic Dye Concentrations across Street Food Categories



Table 5 & figure 2 Summarizes the mean concentrations of synthetic dyes in liquid/syrup-based, semi-solid sweet, and solid savoury street foods. Statistical significance was confirmed using one-way ANOVA followed by post-hoc (after this in Latin) tests, with liquid and semi-solid foods showing significantly higher dye levels than solid savoury foods ( $p < 0.05$ ).

Box plot illustrating the distribution, median, and mean (marker) of synthetic dye concentrations in liquid/syrup-based, semi-solid sweet, and solid savoury street foods collected from the Mumbai suburban region. Liquid and semi-solid food categories exhibit higher dye concentrations and greater variability compared to solid savoury foods. (\*\*) Double asterisks indicate statistically significant differences between various groups at  $p < 0.05$  as determined by one-way ANOVA followed by post-hoc (after this) analysis.

### Quality Control (QC) and Method Performance Analysis Report

#### *i. Method Accuracy and Recovery Evaluation:*

Method accuracy was analysed and evaluated through spike recovery method experiments by strengthen dye-free snack matrices with known concentrations of standard synthetic dyes at three levels (low, medium, and high) as per FSSAI. The percentage recovery ranged from 86.2% to 108.5%, indicating satisfactory extraction efficiency and minimal matrix interference across different food types. Recoveries were within the acceptable range (80-110%) recommended for trace-level food adulterant analysis, confirming the reliability of the extraction and analytical protocol (Table 6).

**Table 6.** Recovery (%) of Synthetic Dyes in Spiked Street Food Samples

Synthetic Dye	Spiking Level (mg/kg)	Mean Recovery (%) $\pm$ SD
Tartrazine (E102)	50	92.4 $\pm$ 3.1
	100	98.6 $\pm$ 2.4
	200	104.2 $\pm$ 2.9
Sunset Yellow (E110)	50	86.2 $\pm$ 3.8
	100	94.7 $\pm$ 2.6
	200	101.3 $\pm$ 3.2
Allura Red (E129)	50	89.5 $\pm$ 3.5
	100	96.8 $\pm$ 2.1
	200	108.5 $\pm$ 3.7

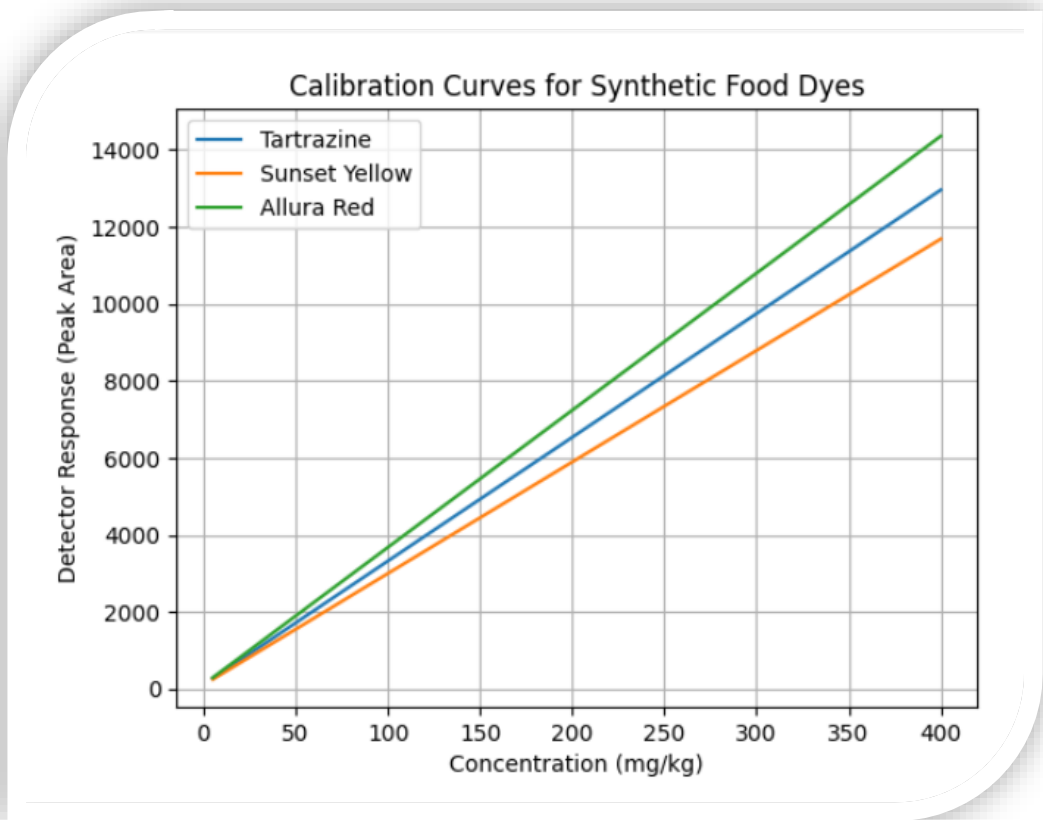
#### *ii. Linearity and Calibration Curve Performance:*

Calibration curves were formed using external standard solutions over a concentration range of 5 mg/kg to 400 mg/kg. All dyes exhibited excellent linearity, with coefficients of determination is ( $R^2 > 0.995$ ), demonstrating proportional detector response across the working range of standard dye (Table 7).

**Table 7.** Calibration Curve Parameters for Synthetic Dyes in food samples

Dye	Linear Range (mg/kg)	Regression Equation	$R^2$
Tartrazine	5–400	$y = 32.14x + 105.6$	0.9987
Sunset Yellow	5–400	$y = 28.97x + 98.4$	0.9979
Allura Red	5–400	$y = 35.62x + 112.3$	0.9968

The figure 3 represents linear detector response for all three synthetic dyes over the concentration range of 5 mg/kg to 400 mg/kg. Excellent linearity was observed with coefficients of determination ( $R^2$ ) exceeding 0.995 for all dyes, confirming the robustness, accuracy, punctual and suitability of the analytical method for quantitative estimation of synthetic dye adulteration in various street food samples obtained from Mumbai Suburban Region.



**Figure 3.** Calibration curves of Tartrazine, Sunset Yellow, and Allura Red procured using external standard solutions.

*iii. Sensitivity Test: LOD (Limit of Detection) and LOQ (Limit of Quantification):*

LOD and LOQ values were evaluated from the SD (standard deviation) of the response and the slope of the calibration curve, following ICH (International Conference on Harmonization) and AOAC (Association of Official Analytical Collaboration) recommendations, using signal-to-noise ratios of the samples are 3:1 and 10:1, respectively (Table 8). The obtained sensitivity allows authentic detection of chemical synthetic dyes at concentrations well below those typically reported in unethically colored street foods, enabling pre-identification of food adulteration even when dyes are added at sub-regulatory or border levels. Furthermore, the low LOD and LOQ values ensure effective consent monitoring at concentrations significantly lower than the maximum permissible limits prescribed by FSSAI, supporting routine food safety surveillance and regulatory enforcement by the organisation.

**Table 8.** Sensitivity Parameters of the Analytical Method

Synthetic Dye	LOD (mg/kg)	LOQ (mg/kg)
Tartrazine	0.72	2.38
Sunset Yellow	0.85	2.81
Allura Red	0.69	2.29

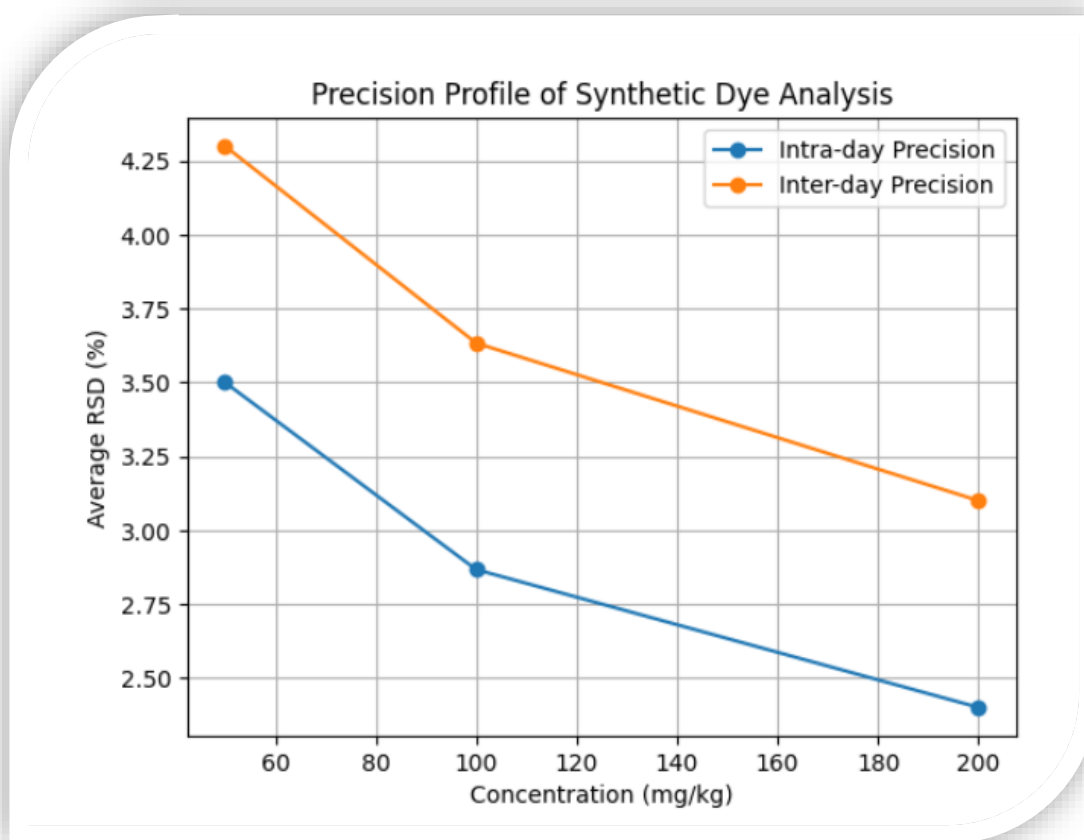
*iv. Precision Studies (Repeatability and Intermediate Precision):*

Method precision was analysed in two ways i.e. intra-day (repeatability) and inter-day (intermediate precision) performance by evaluating triplet samples at three different concentration levels (50 mg/kg, 100 mg/kg, and 200 mg/kg). As summarized in Table 9, the relative standard deviation (RSD) values for all synthetic dyes obtained from various food samples were consistently below 5%, meeting the acceptance criteria recommended by ICH and AOAC guidelines. Intra-day relative standard deviation (RSD) values ranged from 2.1% to 3.8%, while inter-day relative standard deviation (RSD) values ranged from 2.9% to 4.6%, suitable for excellent accuracy and reproducibility of the method. A decreasing range in RSD values with increasing concentration of the dye was observed,

reflecting improved measurement stability at higher analyte levels. The slightly higher inter-day RSD values compared to intra-day results are accountable to normal day-to-day analytical variations but remained well within acceptable limits, confirming the robustness and reliability of the method for routine analysis.

**Table 9.** Precision Evaluation of Synthetic Dye Determination

Dye	Concentration (mg/kg)	Intra-day RSD (%)	Inter-day RSD (%)
Tartrazine	50	3.2	4.1
	100	2.6	3.4
	200	2.1	2.9
Sunset Yellow	50	3.8	4.6
	100	3.1	3.9
	200	2.7	3.3
Allura Red	50	3.5	4.2
	100	2.9	3.6
	200	2.4	3.1



**Figure 4.** Precision profile of synthetic dye determination.

Average intra-day and inter-day relative standard deviation (RSD) values plotted against concentration levels of food samples demonstrate stable analytical precision across the various studied range, with RSD values remaining below 5% at all concentrations.

#### ***v. Overall Method Suitability:***

The evaluated analytical method exhibited a high extraction efficiency rate, excellent linearity ratio across the studied concentration range, and low levels of detection & quantification, assuring its high sensitivity towards trace-level determination of synthetic dyes from the samples. Accuracy and precision standard achieved established validation criteria, with consistent recovery and relative standard deviation (RSD) values within acceptable limits. Importantly, the method showed reliable performance in complex street food matrices, indicating minimal matrix interference and good analytical robustness. Collectively, these performance characteristics confirm that the method is fit for purpose for routine surveillance, quality control, and regulatory monitoring of synthetic dye adulteration in street-sold foods.

## **4. DISCUSSION**

The findings provides a comprehensive qualitative and quantitative evaluation of synthetic dye adulteration which is commonly available and consumed street-sold foods from the Mumbai suburban region like Kandivali, Malad & Borivali. The study focussed on widespread use of both permitted and non-permitted synthetic food dyes, with marked variation across food categories, reflecting differences in locations, food type, food preparation techniques, and consumer demand for visually appealing or attractive food products.

#### **Occurrence and Pattern Use of Synthetic Dye Usage:**

Qualitative preliminary results (Table 2) expressed the frequent presence of permitted dyes such as Tartrazine, Sunset Yellow FCF, Carmoisine, Ponceau 4R, and Allura Red AC in several street-sold food items from Mumbai suburban region. These synthetic dyes were mainly detected in brightly coloured foods, including jalebi, chutneys, paybhaji, and ice gola syrups etc. The adulteration pattern observed suggests intentional addition of synthetic dyes to enhance colour intensity and consumer appeal, for more profit, a practice commonly reported in informal food vending systems from streets of Mumbai.

Of particular concern was the detection of the non-permitted dye Metanil Yellow in chat chutneys and ice gola syrups (Table 2). Although its occurrence was limited to a quite smaller proportion of samples, the presence of a banned dye underscores continued regulatory non-compliance, cheaply availability and possible lack of awareness among street food vendors who are continuously using for the food preparation. The detection of Metanil Yellow in visually intense, liquid or semi-liquid foods supports the notion that such matrices are more susceptible to unethical colour addition due to ease of mixing and absence of visual dosage control, and more or less similar to natural color like turmeric.

#### **Reliability of Qualitative Identification:**

The combined use of TLC and UV-Visible spectrophotometry gives authentic qualitative identification and classification of synthetic dyes. The close meeting point between  $R_f$  values and absorption maxima of food sample extracts with certified reference standards (Table 3) given by FSSAI confirms the specificity of the qualitative screening or preliminary approach towards dye analysis. Minor differences in  $R_f$  values were likely due to matrix complexity and experimental conditions, and preparation methods, a common limitation when analysing heterogeneous street food samples. Nevertheless, the harmonious nature of TLC and UV-Visible spectrophotometry reduces the false or wrong identification and strengthened confirmedness in the detected dye profiles.

#### **Quantitative Levels and Regulatory Compliance:**

Quantitative HPLC (High-performance liquid chromatography) analysis identifies substantial variation in food dye concentrations among the various different food categories (Table 4). The maximum concentrations were found in ice gola syrups, chaat chutneys, and jalebi, which is regularly with their intense visual appealing appearance and consumer preference for bright colours. In contrast, solid savoury foods such as samosa and bhaji / pakoda generally expressed low dye concentrations and high compliance with regulatory limits provided by FSSAI.

Most of the samples exceeded the maximum permissible limits prescribed by FSSAI, particularly for Tartrazine and Carmoisine, indicating excessive or uncontrolled dye addition in the food samples. (Figure 1) clearly expresses used of regulatory exceeds in jalebi, chaat chutneys, and ice gola syrups, highlights these foods as high-risk categories for food dye adulteration. The identification and detection of Metanil Yellow in ice gola syrups, even at particularly lower concentrations, is frighten given its complete prohibition for food use and reported toxicological concerns among the individuals.

The occurrence of multiple mixed dyes within the same food category further raises higher concerns regarding cumulative dietary exposure, especially among frequent consumers and children, who are major consumers of coloured street foods such as ice gola syrups in the Mumbai suburban region.

#### **Influence of Food Type on Dye Adulteration:**

Comparative statistical analysis (Table 5 and Figure 2) demonstrated a significant influence of food type on synthetic dye concentration. Liquid and syrup-based foods showed significantly higher mean dye levels compared to semi-solid and solid savoury foods ( $p < 0.05$ ). This trend can be attributed to the ease of dye dissolution in liquids, lack of standardized dosing during preparation, and strong consumer preference for vividly coloured products.

Solid savoury foods, in contrast, exhibited lower dye concentrations, possibly due to greater control during preparation and reduced visual emphasis on colour intensity. These findings highlight the need for targeted regulatory surveillance focusing on high-risk food categories, particularly liquid and semi-liquid street foods.

#### **Method Performance and Analytical Reliability:**

The reliability of the analytical findings is supported by cumulative method validation results. Recovery studies expressed in (Table 6) expressed satisfactory extraction efficiency result across different types of food matrices, indicating minimal matrix interference coherence. Excellent linearity was found for all analysed dyes from the food samples (Table 7 and Figure 3) ensuring accurate quantification across a wide concentration range of the food dyes.

The low level of LOD and LOQ values (Table 8) confirm the method's capability to identify trace-level dye adulteration in the samples, which is critical for early identification of illegal or unethical practices of food preparation. Precision studies (Table 9 and Figure 4) further exemplify consistent repeatability and reproducibility, confirming the robustness of the analytical approach in detection. Collectively, these evaluated results characteristics reinforce the suitability of the method for routine surveillance and regulatory monitoring.

#### **Public Health measures and Regulatory Implications:**

The widespread identification and detection of synthetic chemical dyes, frequent exceedance of permissible limits, and continued use of non-permitted dyes highlight significant gaps in regulatory compliance within informal street food systems among the Mumbai suburban region. Regular consumption of such foods with chemical synthetic dyes may pose potential health risks, particularly for vulnerable section of the populations. The findings underscore the highlights of the importance of strengthening food safety awareness among the food vendors, enhancing routine surveillance, and implementing targeted enforcement in high-risk vending zones.

Chronic ingestion of excessive synthetic dyes has been linked to skin allergic reactions, behavioral disorders in children, aggressiveness, early growth, genotoxicity, and potential carcinogenic effects. The findings underscore the need for enhanced enforcement and vendor education in Mumbai suburban areas.

## **5. CONCLUSION**

The present investigation offers explicit evidence of omnipresence of synthetic dye adulteration in frequently consumed street-vended foods from the Mumbai suburban area of Kandivali, Malad & Borivali. Both test of qualitative and quantitative analyses expressed that permissible synthetic dyes are often used, sometimes at levels higher than what is allowed, and that the non-allowed dye Metanil Yellow is still found in some food groups. Liquid and semi-liquid foods like ice gola syrups, chaat chutneys, and jalebi had the most dye adulteration. This represent that these foods items are high-risk for synthetic dye misuse.

Quantitative HPLC outcome expressed that some food samples went over the maximum limits set by the Food Safety and Standards Authority of India (FSSAI). The fact that Metanil Yellow was found in some food samples, even though it is completely banned for its use and is a serious concern for food safety and public health. Statistical analysis expressed that food dye concentration was markedly affected by food type, its taste and authenticity with liquid and syrup-based foods exhibiting significantly elevated food dye levels as compared to solid savory foods.

The analytical method employed here to study which exhibited satisfactory accuracy, precision, sensitivity, and robustness, ensuring reliable identification, detection and quantification of both permitted and non-permitted synthetic dyes in complex street food

matrices. The low detection limits achieved further support the applicability of the method for routine surveillance and early identification of adulteration practices.

Overall, the findings highlights persistent gaps in regulatory compliance within informal street food supply systems in Mumbai suburban region and emphasize the urgent need for strengthened monitoring, targeted enforcement, and improved vendor & customer awareness to safeguard consumer health.

### Recommendations:

Based on the findings of this study, the following recommendations are proposed to address synthetic dye adulteration in street-sold food items:

- i. Targeted and Regular Regulatory Surveillance: The Regulatory authorities should organise regular routine monitoring of high-risk food categories, particularly liquid and semi-liquid street foods such as syrups, chutneys, and coloured sweets, which consistently showed to use higher synthetic dye concentrations and regulatory non-compliance given by FSSAI.
- ii. Strengthened Enforcement and Guidelines against Non-Permitted Dyes used in Foods: Strict enforcement measures should be implemented by authorities to eliminate the use of banned or restricted dyes such as Metanil Yellow. On regular basis Periodic market surveillance coupled with penal or legal action may act as an effective deterrent against this deliberate food adulteration.
- iii. Street food Vendors Education and Awareness Programs: Training and awareness session initiatives should be conducted on regular basis to educate street food vendors on permitted colorants, maximum allowable limits, and the health risks associated with illegal or non-permitted food dye usage. Improving regulatory awareness at the street food vendor level a critical for long-term compliance.
- iv. Adoption of Validated Analytical Methods for trace level analysis: The validated analytical approach described in this study can be adopted by government and semi government food testing laboratories for routine screening and enforcement activities, given its demonstrated sensitivity, accuracy, trace availability and suitability for complex food matrices.
- v. Consumer Awareness and Public Health Communication: Public awareness campaigns, notices, displays must be highlighting the risks of excessive and illegal food colouring may help reduce consumer demand for overly coloured foods, indirectly discouraging adulteration practices.
- vi. Expanded, Future and Longitudinal Studies: Future studies should extend surveillance to other urban and rural regions and assess seasonal variations in food dye adulteration patterns among the streets. Long-term exposure assessment and cumulative dietary risk evaluation, health issues are also warranted, particularly for vulnerable populations such as children.

### Limitations:

The research was limited to selected suburban regions of Mumbai and may not fully represent dye adulteration patterns across other urban or rural settings. Seasonal variation beyond the study period was also not assessed.

### Acknowledgments

The author was solely responsible for the conceptualization and design of the study, sample collection, laboratory analysis, data interpretation, and preparation of the manuscript.

### Informed consent

Not applicable.

### Funding

This research was financially supported by Thakur Shyamnarayan Degree College. This research did not receive any external funding like specific grant from funding agencies in the public, commercial, or nonprofit sectors.

### Ethical approval

In this article, the product ethical regulations are followed as per the ethical committee guidelines of Department of Botany & Microbiology, Thakur Shyamnarayan Degree College, Kandivali East Mumbai, Affiliated to University of Mumbai, Maharashtra, India; the authors observed the Quantitative and Qualitative Synthetic Dye Adulteration in Street-Sold Snacks from Mumbai Suburban



Region, India. The “brand name” of the products are not mentioned in content and also the “brand image” not displayed as figure in the article. The product ethical guidelines are followed in the study for observation, identification & experimentation.

### Conflict of Interest

The authors declare that they have no conflicts of interest, competing financial interest or personal relationship that could have influenced the work reported in this paper.

### Data and materials availability

All data associated with this study are present in the paper.

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