Detection of Non-Permitted Food Colors in Edibles

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Abstract

Background: Food colors are used to give an attractive appearance to food articles. Food colors are categorized as permitted and non-permitted colors. Use of non-permitted food colors has led to loss of real quality of food. Adulteration of food products is a cause of concern these days.

Objective: The objective was to detect the presence of non-permitted food colors in edibles using preliminary color test and thin layer chromatography. Materials and methods: Four types of samples i.e. turmeric, jelly, green peas and namkeen were collected from different locations for analysis. 20 samples of each were collected from different locations to be analysed for adulteration with most commonly used food colors i.e metanil yellow and malachite green. Non permitted food colors in collected edibles were detected using preliminary color test and thin layer chromatography.

Results: The colour change and Rf values of the test samples were analysed and it was observed that 46% of food products tested were adulterated with metanil yellow whereas adulteration with malachite green was about 44% of the tested samples.

Conclusion: The used methods of detection can be used in the area of food quality control to detect such harmful and banned colors in edibles without massive instrumentation.

Keywords: Forensic sciences; Food colours; Adulteration; Colour tests; TLC

Introduction

Food colors are added to edibles to make them look more attractive and tempting [1]. Food colours are pigments, dyes or any other compound which when added to any food article imparts color. When a non-permitted or non-food substance is added to increase the quantity and quality of the substance then it is termed under adulteration [2]. It leads to loss of real quality of the product. Food colours are divided into two categories, permitted food colors and non-permitted food colors [1,3].

There are several reasons due to which colours or dyes are added to foods such as identification of various products, preserve the attractive and colourful appearance and make it look more tempting, these colors are differentiated into 3 categories i.e. straights colors, lakes and mixtures [4,5]. Straight colors are used without being mixed with any other substance whereas as when straight colors are mixed with other chemicals then they are termed as lakes. Mixtures are formed by mixing one or more colorants with each other [4-6].

There are many regulations given by FDA and other regulatory organizations regarding use of food colors. According to FDA, food colours are classified under 2 basic categories i.e. those colors which require certification and those which do not require certification [4,7]. Colors which are produced synthetically are categorized under those colors which need certification. These are used due to their specific properties such as intense color, uniform color, cheap and easy blending.

Naturally occurring colors such as colors extracted from vegetables and animals are classified under colors which do not need certification [4,7,8]. A survey conducted in all the four zones of India, according to which East zone showed maximum adulteration (80.3%). 72.3% of products contained permitted colours and about 58% were used above their permitted limit. Use of non-permitted colours was analysed in about 17% samples and Rhodamine B was maximum used. Orange II and Metanil yellow were also among the non-permitted colours used; intake of Sunset Yellow FCF saturated the acceptable daily intake limit to a maximum of 47.8% in children, which is a cause of concern. Tartrazine, a lemon yellow color, was the most common permitted color and 36% of food products in India had exceeding amount of this color [10].

A study in 2013 determined red synthetic dyes in traditional sweets commercially sold at traditional open Easters market using high performance liquid chromatography. Four red synthetic dyes: azorubine, amaranth, cochineal red, and Allura red were determined, it was observed that the most commonly used dye in traditional sweets was cochineal red – it occurred in 17 samples. Allura red was used only in one sample, whereas no sample contained azorubine. Six samples of sweets contained two kinds of red dyes. The maximum usage level of synthetic dye exceeded only in one product [11].
Materials and Methods

Sample collection

To determine presence of various non-permitted food colors in edibles using preliminary color test and thin layer chromatography, standard colour samples of metanil yellow and malachite green used were purchased from HIMEDIA. Metanil yellow is orange to yellow colour powder soluble in water, alcohol, benzene (slightly), acetone (very slightly), and ether and malachite green are green coloured metallic lustrous crystals soluble in alcohol and water.

Various food samples were collected from different regions of Delhi-NCR i.e. Sagar pur, Vikas puri, Possangi pur, Chandni chowk, Uttam nagar, Janak puri, Dwarka and Mayur vihar. Four different types of samples were collected i.e. turmeric, green peas, jelly and namkeen for analysis. Twenty five samples of each were collected from different locations. Turmeric stem was also used and tests were performed for comparison. For thin layer chromatography, EMD/Merck KGaA TLC silica plates were used for analysis.

Sample preparation

All the samples procured were preserved in clean paper and plastic polybags and were kept in clean environment. Standard of metanil yellow (0.1 gms) and malachite green (0.1 gms) were dissolved in propanol (1 ml) at the time of analysis. Turmeric (0.14 gms), turmeric stem flakes (0.14 gms) and jelly samples (0.8 gms) were mixed and shaken with 1-2 ml of propanol for 5-10 mins to dissolve food color if present in the sample and then were used for analysis. Namkeen (0.8 gms) and green peas (2-3 seeds) samples were mixed with 1-2 ml propanol and were kept overnight to dissolve food color if present in them. This extracted sample was further used for preliminary color test and thin layer chromatography.

Color test

About 0.1 gm of standard sample of metanil yellow was taken in a test tube and then about 1 ml of propanol was added to dissolve the sample. 2 ml of freshly prepared neutral sulphite solution. (5 mg of Sodium metabisulphite dissolved in 10 ml of water) was added to the test tube. Disappearance of green coloration indicates presence of malachite green as shown in Figures 2-8. The same procedure was followed for collected food samples of jelly and green peas samples for presence of adulteration. Results according to the color test are tabulated in Table 1 [12].

| S. No. | Sample          | Location | Test                  | Observation               |
|-------|-----------------|----------|-----------------------|----------------------------|
| A     | Turmeric powder | Sagar pur| Sample+Propanol+HCl   | Pink colouration appears   |
|       | Standard sample |          |                       |                            |
|       | (metanil yellow)| i)       | Sample+Propanol+HCl   | Pink colouration appears   |
|       |                 | ii)      | Sample+Propanol+HCl   | Reddish-Pink colouration appears |
|       |                 | iii)     | Sample+Propanol+HCl   | Reddish-Pink colouration appears |
|       |                 | iv)      | Sample+Propanol+HCl   | Pink colouration appears   |
|       |                 | v)       | Sample+Propanol+HCl   | Pink colouration appears   |

Figure 1: Test result of standard of metanil yellow.

Figure 2: Test result of standard of malachite green.
| Location         | Sample Preparation | Result                        |
|------------------|--------------------|-------------------------------|
| Vikas puri       | Sample+Propanol+HCl| Reddish-Pink colouration appears |
|                  |                    | Reddish colouration appears  |
|                  |                    | Pink colouration appears     |
|                  |                    | Pink colouration appears     |
|                  |                    | Reddish-Pink colouration appears |
| Possangi pur     | Sample+Propanol+HCl| Pink colouration appears     |
|                  |                    | Reddish-Pink colouration appears |
|                  |                    | Pink colouration appears     |
|                  |                    | Pink colouration appears     |
|                  |                    | Reddish-Pink colouration appears |
| Chandni chowk    | Sample+Propanol+HCl| Reddish-Pink colouration appears |
|                  |                    | Reddish-Pink colouration appears |
|                  |                    | Reddish-Pink colouration appears |
|                  |                    | Pink colouration appears     |
|                  |                    | Reddish-Pink colouration appears |
| Uttam nagar      | Sample+Propanol+HCl| Pink colouration appears     |
|                  |                    | Reddish-Pink colouration appears |
|                  |                    | Reddish-Pink colouration appears |
|                  |                    | Pink colouration appears     |
|                  |                    | Reddish colouration appears  |
| Turmeric Stem    | Sample+Propanol+HCl| Red colouration appears      |
| B                | Jelly              |                               |
| Standard sample (malachite green) | Sample+Propanol+Neutral sulphite solution | Green color disappears |
| Janak Puri       | Sample+Propanol+Neutral sulphite solution | Green color disappears |
|                  | Sample+Propanol+Neutral sulphite solution | No change in colouration |
|                  | Sample+Propanol+Neutral sulphite solution | Green color disappears |
|                  | Sample+Propanol+Neutral sulphite solution | No change in colouration |
|                  | Sample+Propanol+Neutral sulphite solution | Green color disappears |
| Dwarka           | Sample+Propanol+Neutral sulphite solution | No change in colouration |
|                  | Sample+Propanol+Neutral sulphite solution | No change in colouration |
|                  | Sample+Propanol+Neutral sulphite solution | Green color disappears |
| Location | Sample + Propanol + Neutral sulphite solution | Reaction |
|----------|---------------------------------------------|----------|
| Sagar Pur | Sample + Propanol + Neutral sulphite solution | Green color disappears |
| Vikas Puri | Sample + Propanol + Neutral sulphite solution | No change in colouration |
| Possangi Pur | Sample + Propanol + Neutral sulphite solution | No change in colouration |
| C | Green peas | No change in colouration |
| Janak puri | Sample + Propanol + Neutral sulphite solution | No change in colouration |
| Mayur vihar | Sample + Propanol + Neutral sulphite solution | No change in colouration |
| Dwarka | Sample + Propanol + Neutral sulphite solution | No change in colouration |
| Region       | Sample + Propanol + Neutral Sulphite Solution | Reaction                          |
|--------------|-----------------------------------------------|-----------------------------------|
| Sagar Pur    |                                               |                                   |
| i)           | Sample + Propanol + Neutral Sulphite Solution | Green color disappears            |
| ii)          | Sample + Propanol + Neutral Sulphite Solution | Green color disappears            |
| iii)         | Sample + Propanol + Neutral Sulphite Solution | Green color disappears            |
| iv)          | Sample + Propanol + Neutral Sulphite Solution | No change in colouration          |
| v)           | Sample + Propanol + Neutral Sulphite Solution | No change in colouration          |
| Possangi Pur |                                               |                                   |
| i)           | Sample + Propanol + Neutral Sulphite Solution | No change in colouration          |
| ii)          | Sample + Propanol + Neutral Sulphite Solution | Green color disappears            |
| iii)         | Sample + Propanol + Neutral Sulphite Solution | Green color disappears            |
| iv)          | Sample + Propanol + Neutral Sulphite Solution | No change in colouration          |
| v)           | Sample + Propanol + Neutral Sulphite Solution | Green color disappears            |
| Namkeen      |                                               |                                   |
| Standard sample (metanil yellow) | Sample + Propanol + HCl | Pink colouration appears |
| Uttam Nagar  |                                               |                                   |
| i)           | Sample + Propanol + HCl                      | Pink colouration appears          |
| ii)          | Sample + Propanol + HCl                      | No Pink colouration appears       |
| iii)         | Sample + Propanol + HCl                      | No Pink colouration appears       |
| iv)          | Sample + Propanol + HCl                      | Pink colouration appears          |
| v)           | Sample + Propanol + HCl                      | Pink colouration appears          |
| Possangi Pur |                                               |                                   |
| i)           | Sample + Propanol + HCl                      | No Pink colouration appears       |
| ii)          | Sample + Propanol + HCl                      | No Pink colouration appears       |
| iii)         | Sample + Propanol + HCl                      | Pink colouration appears          |
| iv)          | Sample + Propanol + HCl                      | Pink colouration appears          |
| v)           | Sample + Propanol + HCl                      | No Pink colouration appears       |
| Vikas Puri   |                                               |                                   |
| i)           | Sample + Propanol + HCl                      | Pink colouration appears          |
| ii)          | Sample + Propanol + HCl                      | No Pink colouration appears       |
| iii)         | Sample + Propanol + HCl                      | No Pink colouration appears       |
| iv)          | Sample + Propanol + HCl                      | Pink colouration appears          |
| v)           | Sample + Propanol + HCl                      | No Pink colouration appears       |
| Sagar Pur    |                                               |                                   |
| i)           | Sample + Propanol + HCl                      | Pink colouration appears          |
### Table 1: Observations after preliminary color test.

| Sample Type | Solvent | Colouration Appearance |
|-------------|---------|------------------------|
| ii) Chandni chowk | Sample+Propanol+HCl | No Pink colouration appears |
| iii) Chandni chowk | Sample+Propanol+HCl | No Pink colouration appears |
| iv) Chandni chowk | Sample+Propanol+HCl | Pink colouration appears |
| v) Chandni chowk | Sample+Propanol+HCl | Pink colouration appears |
| i) Sample | Sample+Propanol+HCl | Pink colouration appears |
| ii) Sample | Sample+Propanol+HCl | No Pink colouration appears |
| iii) Sample | Sample+Propanol+HCl | No Pink colouration appears |
| iv) Sample | Sample+Propanol+HCl | No colouration appears |
| v) Sample | Sample+Propanol+HCl | No colouration appears |

#### Thin layer chromatography

For thin layer chromatography, tanks containing solvents were prepared according to ascending chromatography in which solvents were kept in the bottom of the tank. The tank was washed thoroughly and then the solvents Ethyl acetate: methanol: ammonia: water (35: 11: 5: 5) were poured in the tank for metanil yellow in turmeric and namkeen and in other tank carbon tetrachloride: methanol (4:1) were added for detection of malachite green in peas and jelly in their respective samples [12].

After preparation of the tank, it was kept for saturation for about 30 mins. The silica TLC sheet was spotted using capillary tubes; separate capillary tube was used for each sample. The spotted plate was placed inside the tank, the tank was covered and the plate was run up to 8 cm as shown in Figures 9-13. Rf value (distance travelled by sample in relation to the distance travelled by solvent) was measured. It should be considered that environmental conditions such as, air flow, atmosphere pressure and freshness of the eluents temperature influence the distance travelled by the colorants. Results according to the Rf value are tabulated in Table 2 [12,13].

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**Figure 3: TLC turmeric samples.**

**Figure 4: TLC turmeric stem sample.**

**Figure 5: TLC jelly.**
| S. NO. | Sample                      | Location        | Distance Travelled by Solvent | Distance Travelled by Sample | Rf Value |
|--------|----------------------------|-----------------|-------------------------------|-----------------------------|----------|
| A      | Turmeric powder            | Standard sample (metanil yellow) | 8 cm                           | 6.2 cm                       | 0.775    |
| Sagar Pur          | i)                         | 8 cm            | 6.2 cm                       | 0.775                       |
|       | ii)                        | 8 cm            | 6.3 cm                       | 0.787                       |
|       | iii)                       | 8 cm            | 6.1 cm                       | 0.763                       |
|       | iv)                        | 8 cm            | 6.2 cm                       | 0.775                       |
|       | v)                         | 8 cm            | 6.2 cm                       | 0.775                       |
| Vikas Pur         | i)                         | 8 cm            | 6.3 cm                       | 0.787                       |
|       | ii)                        | 8 cm            | 6.8 cm                       | 0.85                        |
|       | iii)                       | 8 cm            | 6.2 cm                       | 0.775                       |
|       | iv)                        | 8 cm            | 6.2 cm                       | 0.775                       |
|       | v)                         | 8 cm            | 6.1 cm                       | 0.763                       |
| Possangi pur     | i)                         | 8 cm            | 6.2 cm                       | 0.775                       |
|       | ii)                        | 8 cm            | 6.0 cm                       | 0.75                        |
|       | iii)                       | 8 cm            | 6.2 cm                       | 0.775                       |
|       | iv)                        | 8 cm            | 6.2 cm                       | 0.775                       |
|       | v)                         | 8 cm            | 5.8 cm                       | 0.725                       |
| Chandni Chowk    | i)                         | 8 cm            | 6.0 cm                       | 0.75                        |
|       | ii)                        | 8 cm            | 6.0 cm                       | 0.75                        |
|       | iii)                       | 8 cm            | 6.1 cm                       | 0.763                       |
|          |     |     |      |
|----------|-----|-----|------|
|          | iv) | 8 cm | 6.2 cm | 0.775 |
|          | v)  | 8 cm | 6.6 cm | 0.825 |
| Uttam Nagar | i)  | 8 cm | 6.2 cm | 0.775 |
|          | ii) | 8 cm | 6.1 cm | 0.763 |
|          | iii)| 8 cm | 6.3 cm | 0.787 |
|          | iv) | 8 cm | 6.2 cm | 0.775 |
|          | v)  | 8 cm | 6.2 cm | 0.775 |
| Turmeric Stem |     | 8 cm | 6.0 cm | 0.75  |
| B Jelly | Standard sample (malachite green) | 8 cm | 5.9 cm | 0.738 |
| Janak Puri | i)  | 8 cm | 5.9 cm | 0.738 |
|          | ii) | 8 cm | 5.7 cm | 0.712 |
|          | iii)| 8 cm | 5.9 cm | 0.738 |
|          | iv) | 8 cm | 6.0 cm | 0.75  |
|          | v)  | 8 cm | 5.9 cm | 0.738 |
| Dwarka | i)  | 8 cm | 5.6 cm | 0.7  |
|          | ii) | 8 cm | 5.7 cm | 0.712 |
|          | iii)| 8 cm | 5.9 cm | 0.738 |
|          | iv) | 8 cm | 5.9 cm | 0.738 |
|          | v)  | 8 cm | 5.5 cm | 0.687 |
| Sagar Pur | i)  | 8 cm | 5.9 cm | 0.738 |
|          | ii) | 8 cm | 5.8 cm | 0.725 |
|          | iii)| 8 cm | 5.7 cm | 0.712 |
|          | iv) | 8 cm | 5.9 cm | 0.738 |
|          | v)  | 8 cm | 5.9 cm | 0.738 |
| Vikas Puri | i)  | 8 cm | 5.8 cm | 0.725 |
|          | ii) | 8 cm | 5.7 cm | 0.712 |
|          | iii)| 8 cm | 6.0 cm | 0.75  |
|          | iv) | 8 cm | 5.5 cm | 0.687 |
|          | v)  | 8 cm | 5.9 cm | 0.738 |
| Possangi Pur | i)  | 8 cm | 5.9 cm | 0.738 |
|     |     |     |     |
|-----|-----|-----|-----|
| ii) | 8 cm | 5.9 cm | 0.738 |
| iii) | 8 cm | 5.9 cm | 0.738 |
| iv) | 8 cm | 6.0 cm | 0.75 |
| v) | 8 cm | 5.8 cm | 0.725 |

C  Green peas

| Standard sample (malachite green) | 8 cm | 5.9 cm | 0.738 |

Janak Puri

| i) | 8 cm | 5.7 cm | 0.712 |
| ii) | 8 cm | 5.6 cm | 0.7 |
| iii) | 8 cm | 5.9 cm | 0.738 |
| iv) | 8 cm | 5.8 cm | 0.725 |
| v) | 8 cm | 5.8 cm | 0.725 |

Mayur Vihar

| i) | 8 cm | 5.9 cm | 0.738 |
| ii) | 8 cm | 5.4 cm | 0.675 |
| iii) | 8 cm | 5.8 cm | 0.725 |
| iv) | 8 cm | 5.9 cm | 0.738 |
| v) | 8 cm | 5.8 cm | 0.725 |

Dwarka

| i) | 8 cm | 5.7 cm | 0.712 |
| ii) | 8 cm | 5.8 cm | 0.725 |
| iii) | 8 cm | 5.9 cm | 0.738 |
| iv) | 8 cm | 6.0 cm | 0.75 |
| v) | 8 cm | 5.7 cm | 0.712 |

Sagar pur

| i) | 8 cm | 5.9 cm | 0.738 |
| ii) | 8 cm | 5.8 cm | 0.725 |
| iii) | 8 cm | 5.9 cm | 0.738 |
| iv) | 8 cm | 6.0 cm | 0.75 |
| v) | 8 cm | 5.9 cm | 0.738 |

Possangi pur

| i) | 8 cm | 5.7 cm | 0.712 |
| ii) | 8 cm | 5.9 cm | 0.738 |
| iii) | 8 cm | 5.9 cm | 0.738 |
| iv) | 8 cm | 5.9 cm | 0.738 |
| v) | 8 cm | 5.8 cm | 0.725 |

D  Namkeen
Table 2: Observations after thin layer chromatography.

Results

According to the observations based on preliminary color test mentioned in Table 1, Turmeric samples i), iv) and v) collected from Sagar pur, samples iii) and iv) collected from Vikas puri, samples i), iii) and iv) collected from Possangi pur, sample iv) collected from Chandni chowk and samples i), iv) and v) collected from Uttam nagar gave pink colouration after addition of HCl to the extracted sample as shown in Figure 3. Turmeric stem samples gave red colouration after addition of HCl as shown in Figure 4. In Jelly, samples i), iii) and v) collected from Janak Puri, samples iii) and iv) collected from Dwarka, samples i), ii), iv) and v) collected from Sagar pur, samples i) and v) collected from Vikas puri and samples i), ii), iii) and v) collected from Possangi pur, green colouration of the test sample disappeared after addition of neutral sulphite solution to the extracted sample as shown in Figure 5.
samples i), iii), iv) and v) collected from Mayur Vihar, samples ii) and iii) collected from Dwarka, samples i), ii), iii) and v) collected from Sagar pur and samples ii), iii), iv) and v) collected from Possangi pur, green colouration of the test sample disappeared after addition of neutral sulphite solution to the extracted sample as shown in Figure 6. Namkeen samples i), iv) and v) collected from Uttam nagar, samples iii) and iv) collected from Possangi pur, samples i) and iv) collected from Vikas puri, samples i), iv) and v) collected from Sagar pur and sample i) collected from Chandni chowk gave pink colouration after addition of HCl to the extracted sample as shown in Figures 7 and 8, wherein the results obtained after calculation of Rf have been tabulated in Table 2 which shows similarities with standard samples.

**Discussion**

The study was conducted to determine the presence or absence of non-permitted food colors such as metanil yellow and malachite green in food products having yellow and green color. According to the analysis, 12 out of 25 samples of turmeric and 11 out of 25 samples of namkeen were adulterated with metanil yellow which implies that adulteration with metanil yellow is more in turmeric powder as compared to namkeen. 12 out of 25 samples of jelly and 10 out of 25 samples of green peas were adulterated with malachite green which
implies that adulteration with malachite green is more in jelly samples as compared to green peas. It can be deduced that about 46% of food products tested were adulterated with metanil yellow whereas adulteration with malachite green was about 44% of the tested samples. The study includes use of color test as well as thin layer chromatography for detection of metanil yellow and malachite green in edibles.

Conclusion

Food adulteration is a matter of concern these days. The forensic interest in the use of food colours is related to health rights and safeguard of the consumer. The regulations related to food dyes consider the health of human beings and the adulteration of foodstuffs and economic needs. This study is focused on detection of non-permitted food colors, metanil yellow and malachite green in selected food samples. The used methods of detection can be used in the area of food quality control to detect and quantify such harmful and banned colors in edibles. It could be concluded that the methods used can be used for detection of metanil yellow and malachite green without any massive instrumentation. Efficient analytical methods are required for evaluating toxicity and authenticity or adulteration, in order to determine whether there are synthetic dyes present in foods and whether they are permitted, to determine the levels, to confirm the absence of added dyes in foods and to check on the stability of dyes during processing and storage. Level of adulteration in edibles is still high and further researches can be conducted to detect various non-permitted colors in other food products so that some effective measures can be undertaken for improving the food quality of the products.

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