



Full Length Research Article

DETECTION OF ADULTERANTS IN SPICES THROUGH CHEMICAL METHOD AND THIN LAYER CHROMATOGRAPHY FOR FORENSIC CONSIDERATION

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ABSTRACT

Spices are any pungent, aromatic plant substances used to flavor food or beverages. This study aimed to detect the presence of non-permitted food colours in spices using preliminary colour test and TLC. Three types of samples that is 15 samples of turmeric powder, 15 samples of chilli powder, and 13 samples of garam masala were collected from local areas of Allahabad to be analysed for adulteration with most commonly used food colours that is metanil yellow, sudan III etc. The colour change and R_f values of the test samples were analysed, the results were compared with standard dye materials and it was found that 10 out of 15 samples of turmeric powder, 9 out of 15 samples of chilli powder and 7 out of 13 samples of garam masala were adulterated with metanil yellow, sudan III and artificial colours.

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INTRODUCTION

Food, one of the fundamental necessities of life, is required for growth and maintenance of our body. A major concern of human societies has been the attainment of sufficient quantities of food to provide to their citizens. When a non-permitted substance is added to increase the quantity and quality of the substance then it is termed under adulteration. Adulteration of food commonly defined as, "The addition or subtraction of any substance to or from food, so that the natural composition and quality of food substance is affected". The substances that is used to lower the quality is known as adulterants. Adulterants are categorized into 3 types i.e.

- **Intentional** – sand, marbles, chips, stone, chalk powder, harmful colours etc.
- **Incidental** – pesticide, residues, larvae in food etc.
- **Metallic** – arsenic from pesticides, lead from water, tin from cans etc.

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Spices

Spice is a seed, fruit, root, bark, bud or vegetable substance primarily used for flavouring, coloring or preserving food. Common and rare spices have their respective adulterants that either are mixed while preparation of the spices or completely replace the original spices. Most of the locally branded spices were found containing harmful substances like white chilli powder is dyed with 'Sudan red', an artificial dye to turn it into an expensive red chilli powder; whole red chillies are adulterated with coal tar to enhance their appearance. Worse still, in many cases the used and exhausted spices are mixed with fresh ones in small quantities to confuse the consumer. The most obvious and simplest reason is to increase profit. A manufacturer may use a cheap filler that is easily disguised in the spice to increase the volume sold thereby cutting the cost of pure spice, and thereby increasing the ultimate profit margin. Sudan dyes are indirect carcinogens therefore they are banned for foodstuffs. Cornet *et al.*, (2006) found that, these Sudan dyes used in animal testing reveals that Sudan III isomers cause allergic reactions. To detect the presence of non-permitted food colors in edibles using preliminary color test and thin layer chromatography Purba *et al.*, (2015). In addition, TLC gives rise to different retardation factor for different non-permitted colors present in spice samples.

MATERIALS AND METHODS

For determination of various non-permitted food colours in spices using colour test and thin layer chromatography, standard colour samples of metanil yellow and Sudan III used were purchased from CDH. 15 samples of Turmeric powder, 15 samples of Chili powder and 13 samples of Garam Masala were collected from local market of Allahabad i.e. Kydganj, Allapur, Mutthiganj, Katra, Naini, Mahewa, etc. as well as standard sample of turmeric, chilli powder etc. were also collected from market. Metanil yellow dye commonly used in turmeric powder to intense the colour, Sudan dyes are red dyes that are used for colouring solvents, have been used in chilli powder, chutneys and seasonings containing chili powder and Garam masala is now adulterated with powdered barn, saw dust and artificial colours to look more colourful. The use of colors are common in countries like India.

Testing method for Metanil Yellow

About 0.1 gm of turmeric sample was taken in test tube and then about 1 ml of propanol was added to dissolve the sample. 5-10 drops of HCl was added to the sample and then the colour of the sample was observed. Presence of pink colouration indicates the presence of metanil yellow.

Testing method for Sudan Dyes

Taken 1 gm of suspected Chili powder in a test tube, added 2 ml of Hexane to it, and shaken well. Allowed it to settle. Decant the clear solution into another test tube. Added 2 ml of Aceto-nitrile reagent in water (7:3) and shake well. The appearance of a red colour in the lower Aceto-nitrile layer indicates the presence of Sudan dye.

Testing method for Artificial Colours

Extract the spice sample with petroleum ether. Added 13N Sulphuric acid solution (88 ml of conc. Sulphuric acid diluted with 250 ml of dist. Water). Appearance of Red colour that persists even upon adding Dist. Water indicates the presence of artificial colour. If the Red colour completely disappears on adding Dist. Water, the sample is free of Colour adulteration.

Chromatographic analysis

Samples were taken as 1 gm and dissolve in 1 ml of propanol for 15- 30 mins. The extracted samples were used for thin layer chromatography. Taken a beaker, washed thoroughly and then the solvent ethyl acetate: methanol: ammonia: water (35:11:5:5) were poured in beaker for metanil yellow in turmeric sample. Samples were taken as 0.5 gm, dissolved in 2.5 ml of acetonitrile. 1 gm silica gel and 0.2 gm of anhydrous sodium sulphate were added. The mixture were properly shaken for 30 mins. The colouring components from the silica gel were recovered by 2.0 ml of diethyl ether. The ethereal extract was evaporated in a porcelain dish on hot water bath. The dried extract were re-dissolved in 1 ml of diethyl ether. The extracted samples were used for thin layer chromatography. Taken a beaker, washed thoroughly and then the solvent benzene: hexane: acetic acid (40:60:2) were poured in beaker for Sudan III in chilli sample.

RESULTS

According to the observations based on preliminary colour test, turmeric samples St₂, St₃, St₅, St₆, St₇, St₈, St₁₀, St₁₁, St₁₄, and St₁₅ were collected from local area of Allahabad, after analysis it was found that pink colour were developed after addition of HCl to the sample as shown in Figure: (4.1), were adulterated with metanil yellow.

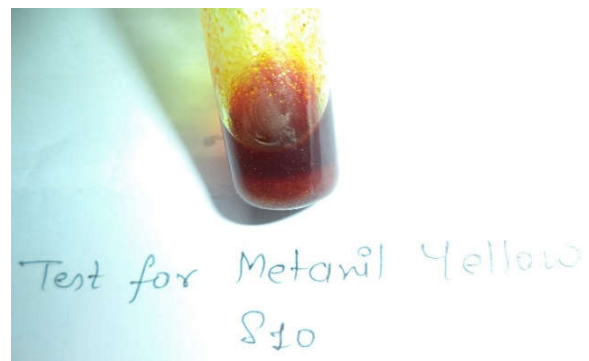


Figure 4.1. Pink colouration appear



Figure 4.2. Reddish-pink colouration appear

According to the observations based on preliminary colour test, chilli samples Sc₂, Sc₃, Sc₄, Sc₇, Sc₈, Sc₁₀, Sc₁₃, Sc₁₄, and Sc₁₅ were collected from local area of Allahabad gave red colouration in lower Aceto-nitrile layer as shown in Figure:(4.3), were adulterated with sudan III.

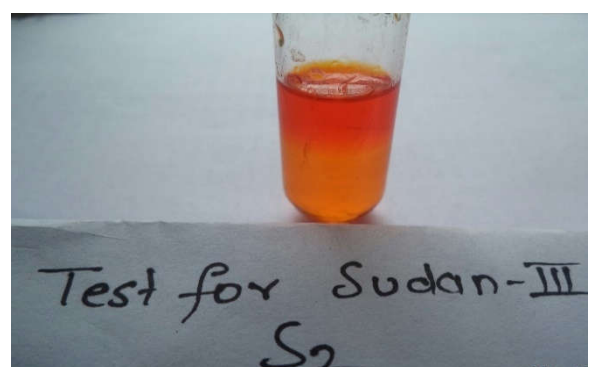


Figure 4.3. Red colouration appear

According to the observations based on preliminary colour test, garam masala samples Sg₄, Sg₇, Sg₉, Sg₁₀, Sg₁₁, Sg₁₂, and Sg₁₃ were collected from local area of Allahabad gave red colouration and persist even after adding water as shown in Figure:(4.5) were adulterated with artificial colours.

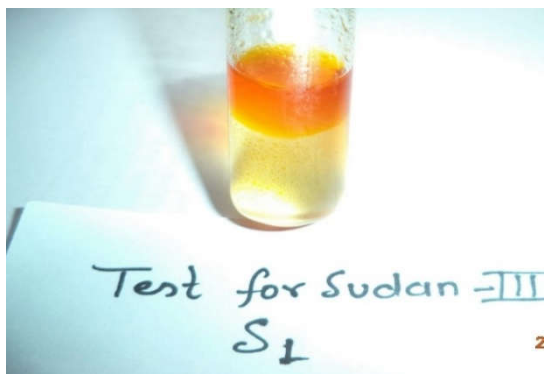


Figure 4.4. Orange colouration appear

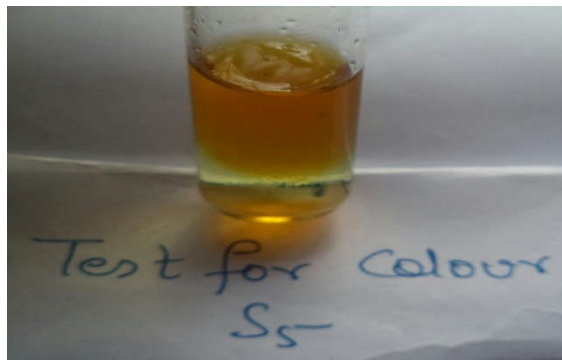


Figure 4.5. Red colouration appear

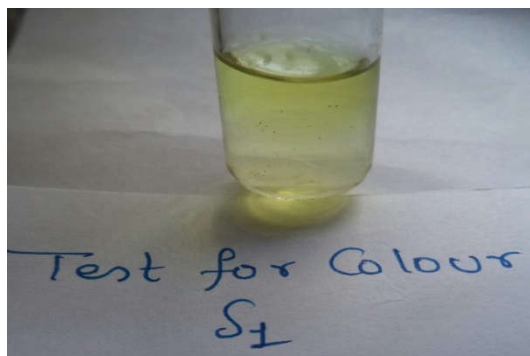


Figure 4.6. No colouration appear

Table 4.4. Result of chromatographic analysis of turmeric sample

| Sr.no. | Sample no. | Distance travelled by solvent (cm) | Distance travelled by sample (cm) | Rf value |
|--------|---------------------------|------------------------------------|-----------------------------------|----------|
| 1. | Metanil yellow (standard) | 7.0 | 5.3 | 0.75 |
| 2. | St ₂ | 7.0 | 5.2 | 0.74 |
| 3. | St ₃ | 7.0 | 5.2 | 0.74 |
| 4. | St ₅ | 7.0 | 5.4 | 0.77 |
| 5. | St ₆ | 7.0 | 5.1 | 0.72 |
| 6. | St ₇ | 7.0 | 5.2 | 0.74 |
| 7. | St ₈ | 7.0 | 5.4 | 0.77 |
| 8. | St ₁₀ | 7.0 | 5.3 | 0.75 |
| 9. | St ₁₁ | 7.0 | 5.2 | 0.74 |
| 10. | St ₁₄ | 7.0 | 5.1 | 0.72 |
| 11. | St ₁₅ | 7.0 | 5.5 | 0.78 |

The data collected from the chromatogram were recorded, and the Rf values were calculated using formula distance travelled by solute/ distance travelled by solvent. The chromatogram of

samples as shown in Figure: (4.7) shows the similarities between standard and controlled samples of metanil yellow.



Figure 4.7. Developed TLC plate for turmeric sample

Table 4.5 Result of chromatographic analysis of chilli samples

| Sr. no. | Sample no. | Distance travelled by solvent (cm) | Distance travelled by sample (cm) | Rf value |
|---------|----------------------|------------------------------------|-----------------------------------|----------|
| 1. | Sudan III (standard) | 7.5 | 6.0 | 0.80 |
| 2. | Sc ₂ | 7.5 | 5.5 | 0.73 |
| 3. | Sc ₃ | 7.5 | 5.9 | 0.78 |
| 4. | Sc ₄ | 7.5 | 5.8 | 0.77 |
| 5. | Sc ₇ | 7.5 | 5.9 | 0.78 |
| 6. | Sc ₈ | 7.5 | 5.2 | 0.69 |
| 7. | Sc ₁₀ | 7.5 | 5.7 | 0.76 |
| 8. | Sc ₁₃ | 7.5 | 5.8 | 0.77 |
| 9. | Sc ₁₄ | 7.5 | 6.0 | 0.80 |
| 10. | Sc ₁₅ | 7.5 | 5.4 | 0.72 |



Figure 4.8. Developed TLC plate for chilli sample

The data collected from the chromatogram were recorded, and the Rf values were calculated using formula distance travelled by solute/ distance travelled by solvent. The chromatogram of samples as shown in Figure: (4.8) shows the similarities between standard and controlled samples of Sudan III.

DISCUSSION

The study was conducted to determine the presence or absence of non-permitted colours such as metanil yellow, Sudan III, artificial colours etc. in spices. Metanil yellow is azo dye synthesized from the coupling of metanilic acid and diphenylamine and is carcinogen. Sudan dyes are illegal dyes which are carcinogenic. Sudan dyes which are added in spices only to enhance the colour of spices, their redness, they are continuously harming the human health. The samples collected randomly from various areas of Allahabad for determination of these harmful dyes in spices. According to the analysis 10 out of 15 samples of turmeric powder, 9 out of 15 samples of chilli

powder and 7 out of 13 samples of garam masala were adulterated with metanil yellow, sudan III and artificial colour. The study includes use of colour test as well as thin layer chromatography. The chromatogram were obtained and the results were expressed in Rf values. The samples were checked by internal standard procedure. Dar *et al.* (2013) detected Sudan dyes III and IV in four samples. It was found that in all four samples Sudan III and IV both were not found. Summary: In the present study the spice samples were purchased from local areas of Allahabad. After collection of samples, the preliminary tests were performed then after TLC was performed. For different samples, different solvents used like for turmeric sample ethyl acetate: methanol: ammonia: water (35: 11: 5: 5) was used and for chilli sample benzene: hexane: acetic acid (40: 60: 2) was used. After that the Rf values of standard and controlled were compared for further confirmation of addition of non-permitted dyes in spices.

Conclusion

From this study, it was concluded that Sudan dye, metanil yellow etc. are harmful colours that are added in various spices to enhance the colour of spices but studies show that these

colouring dyes which are frequently added in spices of daily use and are harmful for health as they can cause cancer and are hazardous also. Such colours and dyes should be checked and banned also so that they may not be used for enhancing the colour of spices and human health may be protected from the threats of these dyes and their cancerous effect.

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