IDENTIFICATION AND ESTIMATION OF NON-PERMITTED FOOD COLOURS (METANIL YELLOW AND ANILINE DYES) IN TURMERIC POWDER BY RAPID COLOR TEST AND THIN LAYER CHROMATOGRAPHY

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ABSTRACT

A study was carried out determine the presence of metanil yellow and aniline dyes, both qualitatively, in nine turmeric samples collected from the western region of Mumbai. For the purpose of this study, both branded as well as non-branded turmeric samples were chosen at random to test for adulteration. The standard (metanil yellow) and turmeric samples were prepared of desired concentrations using isopropyl alcohol as a diluent for thin layer chromatography. Out of the nine turmeric samples, all samples (100%) showed positive results for metanil yellow and for aniline dyes and 89% tested positive by TLC. The present study indicates the presence of metanil yellow and aniline dyes in spices. According to various governmental regulations, the use of metanil yellow and aniline dyes in spices in strictly prohibited because metanil yellow is known for affecting the brain adversely while aniline dyes have been known to cause hyperkinesis in children. In this study, we have presented a simple, convenient, and expeditious method that includes rapid color test and thin layer chromatography to prove the presence of metanil yellow and aniline dyes in spices. The results thus obtained were compared to multiple external standard methods. The aim of this study is to bring about awareness among the people on the subject of food adulteration and the various simple methods to detect the same.

KEYWORDS: Non-permitted food colors, metanil yellow, turmeric powder, color test, thin layer chromatography.
INTRODUCTION

Synthetic food colors are used worldwide to avoid the loss of original color in processed foods, as well as to make the products more attractive to consumers. Synthetic food colors are considered superior to natural food colors in terms of their color value, uniformity, and applicability in various processed foods. Synthetic food colors have been authorized and regulated for use in food additives in many countries.\textsuperscript{1-3} Only eight coal-tar food colors are permitted in certain food products under the provision of Food Adulteration Act (1954). They include three red shades namely: Carmoisine, Ponceau 4R, and Erythrosine; two yellow shades: Sunset yellow FCF and Tartrazine; two blue shades: Brilliant Blue FCF and Indigo Carmine; one green shade: Fast green FCF. However, certain unpermitted colors such as Metanil yellow, Rhodamine B, Orange G, Pararosaniline (PA), Auramine O (AO), Sudan dyes, Blue VRS, and certain oil soluble colors are often added in food as adulterants.

Metanil yellow is a banned dye as per PFA Act (1954) by Government of India, because the same dye has found to be carcinogenic in humans. A report on carcinogenesis\textsuperscript{4} stated that the metabolism of azo dyes derived from benzidine converted to aromatic amines by intestinal bacteria is potentially carcinogenic. According to a report recently published in Current Sciences, some synthetic dyes like auramine, metanil yellow, lead chromate, rohdamine, sudan-3 and 4, orange-2, and malachite green cause serious health hazards as they are mutagenic and potentially carcinogenic.\textsuperscript{5} Metanil yellow, the most frequently used non-permitted food colour is widely used in food items like “ladoos”; it causes insufficient oxygen supply to skin and mucous membranes along with degenerative changes in the stomach, liver, kidney, abdomen, and testes. It is found to cause cyanosis.\textsuperscript{6} It was also found to cause toxic methaemoglobinaemia in adult human males 2-4 hours after the consumption of rice coloured with it.\textsuperscript{4}

Although whole, dried, or fresh turmeric is typically free of contamination, turmeric powder can be adulterated with different chemical powders which are used as substitutes for curcumin.\textsuperscript{6} Studies have reported the mixing of Curcuma zedoaria, a wild relative of turmeric, into turmeric powder due to its close resemblance with turmeric.\textsuperscript{6,7} Similarly, metanil yellow (C18H14N3NaO3S) is a toxic azo dye that has been added to turmeric powder to mimic the appearance of curcumin\textsuperscript{8} when the actual curcumin content is low.\textsuperscript{9} Toxicologically, metanil yellow is classified as a CII category substance by the Joint FAO/WHO Expert committee on Food Additives, and it implies that it is a compound for
which virtually no information on long-term toxicity is available.\textsuperscript{[10]} Toxicity is categorized into four classes according to the toxicity of chemicals, where Category I chemicals are the most toxic and poisonous, and Category IV chemicals are least toxic and poisonous. Studies on rats show that long term consumption of metanil yellow causes neurotoxicity\textsuperscript{[11]}, hepatocellular carcinoma\textsuperscript{[12]}, tumor development\textsuperscript{[8]}, deleterious effect on gastric mucin\textsuperscript{[13]}, and lymphocytic leukemia.\textsuperscript{[14]} A variety of conventional methods have been effectively used for detection of metanil yellow in food items. Ion-pair liquid chromatography detected with 99\% linearity the presence of azo dyes, such as metanil yellow, in the range of 0.05 ppm to 10 ppm in food.\textsuperscript{[9]} Similarly other methods such as high performance liquid chromatography-electrospray ionization tandem mass spectrometry\textsuperscript{[10]}, high performance capillary electrophoresis\textsuperscript{[13]}, and micellar chromatographic method\textsuperscript{[12]} have been used for detection of metanil yellow and other dyes in food and beverages. Despite their high accuracies and satisfactory detection limits, these conventional methods are limited in practicality due to their operational complexity and sample-destructive nature. In contrast, the relative simplicity of optical methods has driven their increasing use for safety and quality detection of foods and food products.\textsuperscript{[8-10]} Although FT-IR and FT-Raman spectroscopy have not been previously reported for detection of metanil yellow adulteration in food, these spectroscopy methods have been widely used for detection of other food adulterants. Thin Layer Chromatography (TLC) was used to identify the presence of metanil yellow in turmeric powder. TLC is one of the easiest and most versatile methods for identifying and separating compounds due to its low cost, simplicity, short development time, high sensitivity and good reproducibility.\textsuperscript{[10]} TLC simultaneously separates substances in space. The RF (retardation factor, ratio of fronts, or retention index) value is the standard measure of retention. Given the general difficulty of controlling absolute RF values, it is common to separate standards and samples in the same system for identification purposes.

This study presents a comprehensive study to evaluate the presence of metanil yellow and aniline dyes in the turmeric samples collected at random from the city of Mumbai qualitatively by rapid color test, followed by thin layer chromatography.

The primary objectives of this study are to qualitatively determine the presence of metanil yellow and aniline dyes in various turmeric samples by:

a. Rapid color test.

b. Thin layer chromatography.
MATERIALS AND METHODS
Turmeric samples collected for testing the presence of non-permitted colors (metanil yellow and aniline dyes)
For the purpose of the study, 9 turmeric samples (both branded and non-branded) were purchased from different shops from the western part of Mumbai, India during the period of 2016-2017; these are mentioned in Table-2. From each shop, 50 grams of the sample was purchased for the purpose of testing. Fresh turmeric and standards (metanil yellow) were also tested for comparison.

Figure-1: Turmeric samples

Rapid Color Test
Rapid Color Tests are based on specific color changes in the presence of reagents such as concentrated hydrochloric acid and concentrated sulphuric acid. These tests get their name from the fact that the color change is observed within 2-3 minutes on adding the specific reagent. The following table (Table-1) shows the different color tests carried out to test the presence of metanil yellow and aniline dyes in turmeric samples and the associated color changes.\textsuperscript{[15]}
TABLE-1: METHOD FOR DETECTION OF METANIL YELLOW AND ANILINE DYES IN TURMERIC POWDER

<table>
<thead>
<tr>
<th>Spices</th>
<th>Adulterants</th>
<th>Test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turmeric</td>
<td>Metanil Yellow</td>
<td>Take 2 grams of a sample in a test tube. Add 1-2mL of distilled water to it. To this, add 8-10 drops of conc. Hydrochloric acid.</td>
<td>Immediate appearance of pink/purple color which persists on addition of distilled water indicates the presence of metanil yellow.</td>
</tr>
<tr>
<td>Samples</td>
<td></td>
<td>Take 2 grams of a sample in a test tube. Add 1-2mL of distilled water to it. To this, add 8-10 drops of 13N Sulphuric acid.</td>
<td>Immediate appearance of red color which persists on addition of distilled water indicates the presence of metanil yellow.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Take 2 grams of a sample in a test tube. Add 1-2mL of distilled water to it. To this, add 8-10 drops of rectified spirit.</td>
<td>Immediate appearance of reddish pink color which persists on addition of distilled water indicates the presence of metanil yellow.</td>
</tr>
<tr>
<td>Aniline Dyes</td>
<td></td>
<td>Take 2 grams of sample in a test tube. Add 1-2mL of distilled water to it. To this, add 8-10 drops of rectified spirit.</td>
<td>Immediate separation of yellow color in the spirit layer indicates the presence of aniline dyes.</td>
</tr>
</tbody>
</table>

**Thin layer chromatography (TLC)**

Thin Layer Chromatography is a chromatographic technique that is used to separate mixtures containing volatile elements. In the present study, we have used thin layer chromatography to determine the presence of adulterant (metanil yellow) in the turmeric samples by comparing their \( R_f \) (ratio of the distance travelled by the sample to the distance travelled by the solvent) with the \( R_f \) of the standard.

**Preparation of turmeric samples**

5 grams of each turmeric sample were taken in a test tube and dissolved in 20mL of chloroform. These were kept overnight in the rotating shaker. After 18 hours, these test tubes were removed from the shaker and filtered. The filtrate obtained was used for thin layer chromatography.

**Preparation of standard solution**

Stock solutions of the standard (metanil yellow) were prepared by dissolving 2mg of the standard in 50mL of isopropyl alcohol (solvent). This was stored at 10°C. The working standard solution was obtained from the stock solution by diluting it with the solvent (1:5) at the time of analysis.
Preparation of TLC plates
Silica gel slurry was prepared in distilled water and then applying it on the TLC plates (15cm x 7cm) as a thin layer having a thickness of 0.25mm. These plates were then allowed to dry. These were activated by keeping them at 110°C in the oven for 1 hour.

Procedure
The samples and standards were spotted onto the TLC plates with the help of capillaries. The solvent used for the development of the TLC plates was a solution of ethyl acetate, methanol, ammonia, and distilled water which were mixed in the ratio of 35: 11: 5: 5 respectively. These plates were then allowed to develop in a TLC jar for 1 hour using the aforementioned solvent system. The chromatograms obtained were then evaluated under normal light and the distances travelled by the solvent were measured using a template scale. Their R_f values were then calculated and compared to that of the standards.[16]

RESULTS AND DISCUSSION
The study was conducted to determine the presence or absence of non-permitted colours metanil yellow and aniline dyes in spices. Metanil yellow is azo dye synthesized from the coupling of metanilic acid and diphenylamine, and is carcinogenic. Metanil yellow is added in spices only to enhance the colour of spices, their redness, and they are continuously harming the human health.

In the present study nine turmeric samples were collected at random from different locations from the western suburbs of Mumbai to determine the percentage of metanil yellow and aniline dyes present. Based on the observations we obtained on performing the rapid color test, all the samples tested positive for metanil yellow and aniline dyes; i.e. 100% of the samples tested positive for the presence of metanil yellow and aniline dyes. (Fig.2-4, Table-2).

FIGURE-2: TURMERIC SAMPLES SHOWING POSITIVE RESULTS FOR THE PRESENCE OF METANIL YELLOW USING RAPID COLOR TESTS.
FIGURE-3: TURMERIC SAMPLES SHOWING POSITIVE RESULTS FOR THE PRESENCE OF ANILINE DYSES USING RAPID COLOR TESTS.

FIGURE-4: TURMERIC SAMPLES SHOWING POSITIVE RESULTS FOR PRESENCE OF METANIL YELLOW ALONG WITH POSITIVE AND NEGATIVE CONTROLS.

The results obtained for the given turmeric samples after thin layer chromatography were expressed in terms of their Rf (retardation factors) values. The Rf values of the standard (metanil yellow) are also calculated in order to compare and determine the presence of the adulterant; i.e. metanil yellow in the turmeric samples. The Rf values for the samples and the standards are shown in Table-2. The samples were checked by internal standard procedure and compared with the standard. It was found that metanil yellow was present in 8 samples out of total 9 samples that were analyzed; i.e. 89 % of the samples showed similarities with the standard on comparing the chromatograms (Fig -5, Table-2). Other samples which showed colored spots but did not have an Rf value matching that of the standard may contain other food colorants. [17]
From the results obtained above, it is seen that food adulterants like metanil yellow and aniline dyes are being indiscriminately used by unorganized food organizing sectors in food items like turmeric powder. With the help of rapid color test and thin layer chromatography, metanil yellow and aniline dyes were found to be present in the turmeric samples which were purchased at random from the western suburbs of Mumbai.
Metanil yellow is an established food toxicant. From the literature, we found that a variety of food items were usually adulterated with non-permitted colours such as auramine, rhodamine B, congored, orangeII, melachite green and metanil yellow. Some information about the metanil yellow induced health hazards have been reported discriminately in animal models.[18-20] We have examining the probable toxic effects of metanil yellow on female reproductive system in rat model. We have seen that metanil yellow altered the reproductive cycle in female rat and also altered the hormonal levels significantly (unpublished data). So, in order to avoid the deleterious toxic effects in human physiological functions, the use of metanil yellow should be stopped immediately. Otherwise, the people belonging to lower economic stratum will suffer the most as a result of the consumption of metanil yellow-laden food items. From a wide variety of literature, it has been proven by a number of studies that metanil yellow is carcinogenic in nature. Metanil yellow, one of the most frequently used adulterants, was found to cause insufficient oxygen supply to skin and mucous membranes, coupled with degenerative effects to stomach, kidney, liver, and abdomen.[6] It was also found to cause methaemoglobinaemia in adult human males 2-4 hours after consumption of rice adulterated with it.[9]

Aniline dyes have the potential to damage haemoglobin, the oxygen carrier present in blood, leading to a condition called methaemoglobinaemia. Acute high exposure to aniline leads to cyanosis. Dizziness, headache, convulsions, coma, and death may also occur. The EPA has determined that aniline is a possible human carcinogen.

In order to avoid the deleterious effects of metanil yellow and aniline dyes on human physiological functions, the use of metanil yellow and aniline dyes in food items must be stopped completely. We suggest that the state and district administrations should intervene and supervise the processing of food with metanil yellow as per Prevention of Food Adulteration Act of India (PFA, 2008).

**CONCLUSION**

From the present study, we can conclude that rapid color test and thin layer chromatography are preliminary tests for the detection of food additives in various food items. Further quantitative analysis can be carried out with the help UV Visible Spectrophotometer. Nowadays, synthetic food colors are frequently used in a variety of food items to make them look more appealing to the consumer. In such a scenario, the results of this study can be used to prevent the malpractice of synthetic food color adulteration. Food adulterants pose a
serious threat to human well-being, resulting in life-threatening diseases such as cancer. Even at low concentrations, food additives have the capacity to disrupt normal body functions. The harmful effects of food additives are found to be more pronounced in young children as their immune system is still developing. This study is useful for Forensic Chemistry and Toxicology. From a medico-legal standpoint, this study must be assessed carefully. This study is focused on the detection of non-permitted food colors (metanil yellow and aniline dyes) from selected food samples. The methods of detection employed in the present study can be used in food quality control to detect the presence of food additives in a variety of food items. The level of adulteration in edibles is still high and further research can be conducted in order to detect various non-permitted food colours in other food products so that effective measures can be undertaken for improving the quality of the food products.

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