Identification of synthetic food dyes in beverages by thin layer chromatography

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ABSTRACT

The present study aims to inquire the type of food colors present in different varieties of drinking food stuff. The research was conducted on different types of beverages, soft drinks and juices for sake of making comparison between branded and non branded items which contain different types of synthetic dyes. Synthetic food colors were determined by using TLC (thin layer chromatography) technique. Permitted colors were determined in majority of branded beverage samples while local illegal food colors were present in local samples which may greatly affect the human health. The results show that there is no quality check system for such types of drinks which may result in health related perils. Due to lack of awareness regarding harmfulness of synthetic food colors more than 60% people are consuming these harmful dyes particularly in rustic areas. A stronger quality check system should be maintained to fulfill the needs of a regulated system and also to ensure the use of food dyes in controlled range.

Key words: Food dyes, Health issues, determination, separation, TLC

INTRODUCTION

Beverage industries totally based on synthetic food colors. Food colors can be obtained from synthetic, natural or many other sources, but concerning to food, natural dyes preferred over many other types. But on industrial scale, where industry deals with a bulk of products, natural food colors can’t meet the desired quality, as it soon fades away so synthetic dyes used in replacement to enhance the product color and make it more attractive and palatable. Selection of an appropriate color or dye for a product depends upon nature of dyes and their properties (Anderson and Keiding, 1964). Food industries are relying more on synthetic dyes irrespective of their harmfulness for health.

Synthetic dyes are prepared chemically. Every dye is primarily composed of aromatic rings and azo functional groups, some dyes may be consists of complex, intensive and large structures of tar coal or petroleum (parent chain). Dyes are also famous with the name of ‘food additives’ or ‘food colorants’ as improves the color of food lost during manufacturing or processing, to make food visually appealing, persuade customer (eye catching) and induce temptations. Bright food colors of candies, beverages, bubble gum and cakes added to make these food products more alluring and attractive for children specially. Dyes are generally aqua based powdered substances could easily dissolve in water so easy to apply and could be stored in liquid form also. On other hand “lakes” are such type of colorants which are not soluble in non-polar chemicals with having the advantage of heat and moisture resistance hence could be used in food packing stuff and for oil & waxy based substances efficiently.

Natural dyes are extracted from plant or natural origin and have no health hazardous effects while the synthetic dyes are composed of various chemicals and may have severe effects on health. Synthetic food dyes preferred over natural food dyes due to some reasons like; being inexpensive, more attractive in appearance and due to their long lasting effects (Sarah and Michael, 2010). Synthetic dyes are manufactured in granular, powdered, liquids or other many forms. They can be used in beverages, candies, ice creams, jellies, baked items, dairy products and other various products and household items (Sharma D., 2014).

Elevating risks by using food dyes

It is true that with the passage of time, the usage of food additives has been increasing but it is important to discuss that the higher intake of these artificial additives leads towards the behavioral changes especially in children and teenagers. Research conducted on these issues revealed an elevating list of complications are attention deficit hyperactivity
disorder (ADHD), neuro-developmental behavioral disorders (NDBDs), genetic problems, impulsivity, respiratory & skin problems, headaches, nausea, weakness, obesity, asthma, diabetes etc. Use of most synthetic dyes prohibited due to these health issues, some of these are; FD & C Red No. 2, FD & C Red No. 4 and FD & C Red No. 32 (Irena et al., 2011; Bateman et al., 2004 and Swanson, 1980). The elimination of most food dyes just due their toxicological, mutagenic & carcinogenic properties. Some of the harmful dyes are given below:

**Sudan:** cause mutagenic changes and damages stomach (Fonovich, 2013).

**Metanil yellow:** cause degenerative changes in vital organs (stomach, liver, kidney, abdomen and testis).

**Lead chromate:** causes epigastric pain, anemia, nausea, and constipation.

According to Food and Drug Administration (FDA) there are some term and conditions under which a specific dyes could be used after verification process. It has organized a set of series in which dyes arranged on the basis of their amounts, usage, effects and all those factors which concerns with human health. All these specifications prepared to meet the safe and risk free usage of synthetic food dyes in edible commodities. Food acts and food laws further helps to confirm the quality. Food laws introduced and labeling of standards started in 1938. Standards were made to sustain and assure the food dye quality, used in edibles. Hence, by manufacturing standardize colors, a boundary designed for all food colors that which color could be used as permitted (certified) and beyond this range other colors fall in range of non-permitted colors. Certified colors are good reference for determination, analysis and testing of those unwanted or non-permitted colors due to their unique properties e.g. physical (appearance, color, flavor and taste) or chemical (structure, nature and reaction in solvents). FDA authorities are responsible for all such activities, to study and monitor food dye usage and corresponding health effects. Annual regulation and assessment programs also made a great contribution to ensure dyes quality (CFR, 2004b and Griffiths James 2005). The present study involves the identification of some synthetic food dyes in beverages by simple thin layer chromatographic method.

**MATERIAL AND METHODS**

Sample collection

Samples of different types of beverages including soft drinks, energy drinks, and juices were collected from local markets of Lahore in which different brands of beverages included e.g. Pepsi, Coca cola, Shezan, Strings, Gourmet, Country & Maza. TLC technique was applied to check the quality of samples and random comparison was done with standards to seek out the quality difference in different samples.

**Extraction of food dyes**

Extraction of food dyes was done by using standard method of AOAC 2005. 50ml of sample was taken and 30 ml of 5% acetic acid solution was added to the sample and 20 -30 wool thread of 20 cm length were immersed in the sample. The contents were boiled for 15 minutes until wool absorbed color from solution. Then wool threads were removed from the solution, washed with distilled water. The wool threads re soaked in 20 ml of 1M NH₃ solution. Solution was again boiled for 5 – 10 minutes until whole color removed from the wool.

**Mechanism of Dye Extraction**

Usually food dyes are salts of sulfonic acids. Sulfonic acids are relatively strong acid. Thus it is expected that FD & C colorant will be ionized and carry negative charges at pHs found in most foods. The extraction will be accomplished by binding the colorants to wool and later releasing them into an aqueous solution. Wool protein is a suitable binding agent for this purpose because it is insoluble and its charge can be manipulated by changing the pH. At low pH carbonyl and amino group on the wool protein are protein is protonated giving the wool protein a net positive charge. Food dyes on the other hand remain negatively charged at low pH, because they are salts of strong acids. Acetic acid, a weak acid, used to acidify the food so that when wool is added, it is protonated. Electrostatic bonding between positively charged protein molecules and negatively charged dyes probably accounts for most of the bonding of the colorants to the wool strand although some hydrogen bonding and hydrophobic interaction may also be involved. After extraction of food dyes from samples of beverages were examined by color analysis and chromatographic analysis.
Chromatographic analysis
Thin layer chromatography is a technique to separate mixture of dyes and to identify the individual dye components in chromatographic analysis. This method covers the separation and identification of dyes in beverages. TLC based on adsorption, more polar compounds adsorb more strongly and remain nearer the origin, less polar adsorb weakly, so spend more time in moving solvent and begin to separate.

Table 1. Rf Values of Identified Synthetic Dyes in Beverage Samples

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample Name</th>
<th>Obtained Rf Values</th>
<th>Identified Color</th>
<th>Standard Rf Values</th>
<th>Color given on label</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Coca Cola</td>
<td>No color</td>
<td>Not present</td>
<td></td>
<td>Not present</td>
</tr>
<tr>
<td>2-</td>
<td>Marinda</td>
<td>0.42</td>
<td>Sunset Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
</tr>
<tr>
<td>3-</td>
<td>Shezan</td>
<td>0.43</td>
<td>Sunset Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
</tr>
<tr>
<td>4-</td>
<td>Dew</td>
<td>0.66</td>
<td>Tatrazine</td>
<td>0.68</td>
<td>Tatrazine</td>
</tr>
<tr>
<td>5-</td>
<td>Salice mango</td>
<td>0.42</td>
<td>Sunset Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
</tr>
<tr>
<td>6-</td>
<td>Pepsi</td>
<td>No color</td>
<td>Not present</td>
<td></td>
<td>Not present</td>
</tr>
<tr>
<td>7-</td>
<td>String Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
</tr>
<tr>
<td>8-</td>
<td>String Red</td>
<td>0.55</td>
<td>Ponceau 4R</td>
<td>0.57</td>
<td>Ponceau 4R</td>
</tr>
<tr>
<td>9-</td>
<td>String green</td>
<td>0.67</td>
<td>Tatrazine</td>
<td>0.68</td>
<td>Tatrazine</td>
</tr>
<tr>
<td>10-</td>
<td>Malt</td>
<td>0.27</td>
<td>Orange dye</td>
<td>0.26</td>
<td>Orange dye</td>
</tr>
<tr>
<td>11-</td>
<td>Cola</td>
<td>No color</td>
<td>Not present</td>
<td></td>
<td>Not present</td>
</tr>
<tr>
<td>12-</td>
<td>Twister</td>
<td>0.69</td>
<td>Tatrazine</td>
<td>0.68</td>
<td>Tatrazine</td>
</tr>
<tr>
<td>13-</td>
<td>Salsaleb mango</td>
<td>0.44</td>
<td>Sunset Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
</tr>
<tr>
<td>14-</td>
<td>Tropico</td>
<td>0.29</td>
<td>Orange dye</td>
<td>0.26</td>
<td>Orange dye</td>
</tr>
<tr>
<td>15-</td>
<td>Maza</td>
<td>0.45</td>
<td>Sunset Yellow</td>
<td>0.43</td>
<td>Sunset Yellow</td>
</tr>
<tr>
<td>16-</td>
<td>Tops</td>
<td>No color</td>
<td>Not present</td>
<td></td>
<td>Not present</td>
</tr>
<tr>
<td>17-</td>
<td>Nurpur</td>
<td>0.29</td>
<td>Orange dye</td>
<td>0.26</td>
<td>Orange dye</td>
</tr>
</tbody>
</table>

The mobile phase used for analysis is Propanol: ammonia in ratio of 60:15 ml respectively.

Developing chromatograms
Spots of samples and standards were applied at the bottom of thin layer chromatographic plate and placed vertically in TLC chamber having 50ml mobile phase (propanol: NH₃). Solvent moves upward on the plate by capillary action and spots of sample also travels in same direction. When solvent reaches at the top of the plate, plate removed and examined. By comparing the colors and migration distance of standards and samples, it is possible to identify components in samples (AOAC, 2005).

RESULTS AND DISCUSSION
Beverages are very popular among customers throughout the world. People prefer to drink soft drinks rather than water. Special trend observed in western countries that carbonated drinks consider a necessary part of meal. As concerning to spicy and heavy meal, beverages also help to promote the digestion process. On other hand some local beverages also famous for their bad effects. Beverages produced by carbocation process (CO₂) in water containing sugar, food color, flavors and
preservatives. Food laws bound food industries to use legal certified dyes.

To identify these food dyes in various kinds of beverages TLC (thin layer chromatography) is one of the best techniques (Dixon et al., 1982). This technique based on separation of components between mobile and stationary phase so also helpful in separation of mixture of dyes. Conclusive result on basis of above data shows satisfactory results.

A total 17 beverage samples were analyzed by using TLC technique to find the dyes. 5% acetic acid was used to remove the dyes on wool threads. Rf (retention factor) values obtained by calculating the distance covered by each solute (spot) and solvent. The results given in table 1, describes the Rf values including both for samples and standards, type of dye and dye name mentioned on labels. Identification of dyes by TLC also a way to check the quality of a product by comparing with the specifications (given on labels) and local food dyes detection.

By examining the results of previous researches (in 2008 to 2014), confirms the usage of all types of dyes in beverages. It was found that sunset yellow was commonly used in most of juices and soft drinks (Al-Degs, 2009). No color found in Pepsi, Coca cola and gourmet cola, the brown color produce during processing. Dyes found in most of juices were sunset yellow and orange dye. Tartrazine found in twister and strings (De Andrade et al., 2014).

CONCLUSION

It is direly needed to confirm and to determine the type of illegal food dyes present in food (beverages) and to identify the levels and labels complies with the recommendations of food dyes legislation. The extensive use of food dyes on industrial level not only reveals their significance but also shows their requirement in beverage industry but despite of these facts their vast usage have raised some heath issue that’s why food colors need to be regulated to assure the quality. Thin layer chromatography is an easiest and cheap method for the identification of synthetic food dyes present in beverages.

REFERENCES