Determination of the Concentration of Food Dyes in Powdered Drink Mixes

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ABSTRACT

Abstract: Federal Food, Drug, and Cosmetic Act (FD&C) food dyes make industrial goods like foods and beverages more appealing. These dyes are synthetic and are typically used instead of natural dyes due to their color, stability, and low cost. Research has implied that children are sensitive to the amount of food dye in products. The amount of food dye in products is proprietary information, so it can be challenging to determine how much dye children are ingesting. In this study, ultraviolet-visible spectroscopy (UV-Vis) was utilized to find the concentration of food dyes in various powdered drink mixes. The results show that powdered drink mixes containing Red 40 have higher concentrations of food dye than the rest of the drink mixes. Our data supports that there is a difference between the concentrations of food dyes within drink mixes containing Red 40 versus those without it. These concentrations depend on the dye and how many dyes were in the drink mix.

Introduction

Natural dyes from fruits and vegetables were the first recorded dyes used. Natural dyes appeared in early civilization in China, Greece, and Egypt (Bafana et al. 351). A synthetic dye was accidentally produced in the mid-19th century when natural dyes started to become obsolete (Bafana et al. 351). William Henry Perkin, a British scientist, discovered mauveine, a purple synthetic dye that originated from the chemicals of coal-tar (Bafana et al. 351; Drumond Chequer et al. 27). Other scientists then began their pursuits of discovering more synthetic dyes, in colors like red, yellow, green, and blue. Currently, synthetic dyes are found in various areas of industry, including cosmetics, foods, clothing, and beverages (Bafana et al. 351).

Food dyes consist of functional groups that produce color shown by the visible spectrum. These functional groups consist of auxochromes, chromophores, and chromogens (Orna and Goodstein 246). An auxochrome is an amine or alcohol that enhances a chemical's color. A chromophore is an unsaturated functional group like an azo-group or a conjugated benzene ring that contributes to a compound's color. Chromogens are chromophores with no auxochromes (Orna 37). Red 40 and Yellow 5 have chromophores and auxochromes, and Blue 1 has no auxochromes, making it a chromogen. The chemical structures displaying the functional groups are shown (Figure 1).

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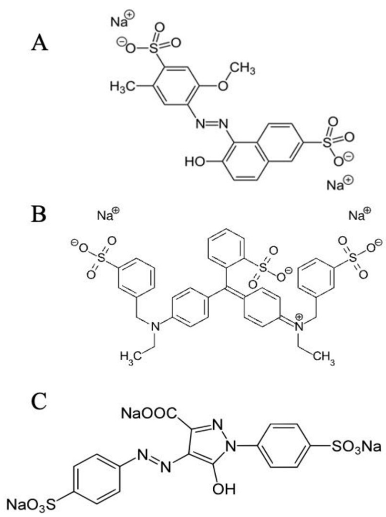

Figure 1: Chemical structures of A) Red 40, B) Yellow 5, and C) Blue 1.

Common food dyes include the approved list of FD&C food dyes: Red 3, Red 40, Blue 1, Blue 2, Yellow 5, Yellow 6, and Green 3. This research focuses on Red 40, Blue 1, and Yellow 5, (Figure 1) due to product availability. These dyes were the most prevalent of the approved list of FD&C food dyes found in drink products that were purchased for testing. The approved list of food dyes are all dyestuffs, which are chemical compounds that can be measured by visible spectroscopy. They are also water soluble and mostly organic (Orna 35).

Previous methods for determining the concentrations of food dyes in products consisted of visible spectroscopy, high performance liquid chromatography (HPLC), paper chromatography, thin-layer chromatography, and capillary electrophoresis (Griffiths 63; Lepri et al. 279; Stevens et al. 137; Watanabe and Terabe 311). This research study of food dye concentrations in powdered drink mixes used visible spectroscopy due to laboratory availability. The concentrations of food dyes in drink mixes were measured in percent mass/mass, % (m/m).

Visible spectroscopy uses light energy from the visible spectrum in order to exhibit the relationship between electromagnetic radiation and matter. The visible spectrum is a measure of wavelengths from 400 nm to 700 nm (Karty 725). The visible spectrum exhibits light by absorbing a photon, which causes electrons in a molecule to shift to a higher energy orbital. This phenomenon is called the molecular orbital theory, and it explains how an electron is transferred from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital, or LUMO (Karty 728).

Since accurate information about food dyes can be proprietary, the current study aims to examine the concentration of food dyes present in powdered drinks. Previous studies have shown that drink mixes containing Red 40 have a higher concentration of dye than drink mixes with other food dyes (Lehmkuhler et al. 4; Sigmann and Wheeler 1475-1478). Lehmkuhler did not report the kinds of drink mixes that they investigated, whereas Sigmann and Wheeler only investigated different Kool-Aid brand flavors. This study evaluates Kool-Aid as well Crystal Light, Great Value, Hawaiian Punch, Starburst, Sunkist, Hi-C, and Gatorade Zero powdered drinks.

The determination of food dyes in products is important because of their possible relations with harmful neurological effects in children (Feingold 553). The use of food dyes is now widely popular because it entices children with their vivid colors and is controlled by the FDA using the accepted daily intake (ADI). However, the amounts of food dye in products are uncertain and inaccessible to society, making it difficult to evaluate the dyes' appropriate consumption rates. As a result, concentrations of food dye in powdered drink mixes and other food products require further assessment to ensure that drink mixes are safe for consumption. (Lehmkuhler et al. 1)

Experimental

Standard Curves

Standard curves for Red 40, Blue 1, and Yellow 5 were generated using a UV-Vis Thermo Fisher Scientific Genesys 20 spectrophotometer. To do this, 0.150 grams of each powdered dye was diluted with distilled water. Five different dilutions were then developed, and the dilutions were repeated. Each of the dyes

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had different wavelength values corresponding to the spectrophotometer. Measurements for Red 40 were conducted at 503 nm, Blue 1 at 629 nm, and Yellow 5 at 427 nm. The standard curves are shown in Figure 2.

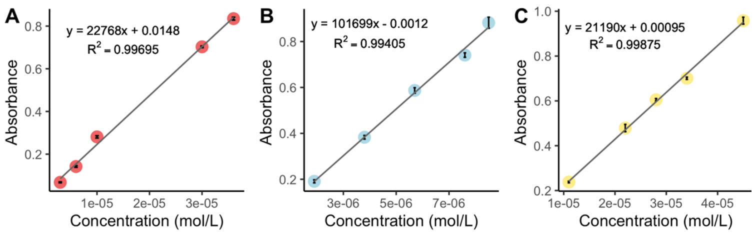

Figure 2: Standard curves for FD&C food dye dilutions for Red 40 (A), Blue 1(B), and Yellow 5 (C).

Analysis of Single Dye Drink Mixes

After the standard curves were produced, solutions of powdered drink mix packets containing solely one FD&C food dye and 1 L of distilled water were produced and their absorbances were recorded. Water was used as the blank for spectrophotometric calibration and, due to availability, 1 cm cuvettes were used. The solutions were kept at room temperature throughout the duration of the experiment. All of the samples were centrifuged for 8 minutes using a Fisher Centrific Model 228 centrifuge at 3400 RPM (Sigmann and Wheeler 1475-1478).

The drink mix packets containing Red 40 included Crystal Light Pink Lemonade, Gatorade Zero Fruit Punch, Hi-C Flashin' Fruit Punch, Kool-Aid Cherry, Kool-Aid Fruit Punch, Starburst Strawberry, and Sunkist Strawberry. The drink mixes containing Blue 1 were Gatorade Zero Glacier Freeze and Starburst Blue Raspberry. The drink mix packets containing Yellow 5 were Crystal Light Citrus, Great Value Lemonade, and Kool-Aid Lemonade. These were purchased from Walmart and Publix.

For the drink mix packets containing only one food dye, the calculation below was completed in order to find the concentration of each food dye in each of the drink mixes. This calculation is a sample computation for the amount of Red 40 in Crystal Light Pink Lemonade. The concentrations of food dyes were found by using the standard curve equations below.

$$Absorbance = 22224(Concentration) + 0.0159$$
$$0.100 = 22224(Concentration) + 0.0159$$
$$Concentration = 3.784 \ x \ 10^{-6} \ mol/L$$

Then the masses of the food dyes were calculated by using the above computed food dye concentrations, the volume of the solutions, and the food dye molecular weights. M is the concentration of the food dye calculated above, V is the volume of the solution, and MW is the molecular weight of the food dye.

$$Mass \ of \ Food \ Dye \ (g) = (M)(V)(MW)$$
$$Mass = \left(3.784 \ x \ 10^{-6} \ \frac{mol}{L}\right)(1.00 \ L)\left(496.00 \ \frac{g}{mol}\right) = 1.877 \ x \ 10^{-3}$$

The concentrations were then calculated using the computed mass of food dye in the powdered drink mix, shown above, as well as the sample mass of each drink packet. These values were multiplied by 100 to obtain the concentrations.

$$\% \ Mass = \frac{Mass \ of \ Food \ Dye \ (g)}{Sample \ Mass \ (g)} \ x \ 100$$
$$\% \ Mass = \frac{1.877 \ x \ 10^{-3} \ g}{4.275 \ g} \ x \ 100 = 0.0439 \ \%$$

Analysis of Multiple Dye Drink Mixes

The same procedure was used to obtain the absorbances for the drink mixes with multiple dyes. The spectrophotometric blank used was water, and the cuvettes used were 1 cm. The solutions were kept at room temperature throughout the duration of the experiment. All of the samples were centrifuged for 8 minutes using a Fisher Centrific Model 228 centrifuge at 3400 RPM (Sigmann and Wheeler 1475-1478).

The drink mix packets containing Red 40 and Blue 1 included Crystal Light Grape, Great Value Blackberry Lemonade, Hawaiian Punch Wild Purple Smash, and Kool-Aid Grape. The drink mix containing Blue 1 and Yellow 5 was Great Value Tropical Limeade. The drink mix packets containing Yellow 5 and Red 40 were Crystal Light Peach Mango and Kool-Aid Orange. All drink mixes were purchased from Walmart and Publix.

For the drink mix packets containing two FD&C food dyes, the final absorbance for each color in the mix was found by equations that correct for interference of another food dye (Sigmann and Wheeler 1478). The equations for each absorbance correction are

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shown in Table 1. The sample calculation below was completed to find the absorbance for Red 40 in Great Value Blackberry Lemonade.

$$A_{Red\ 40} = A_{503\ nm} - 0.028(A_{629\ nm})$$
$$A_{Red\ 40} = 0.570 - 0.028(0.132)$$
$$A_{Red\ 40} = 0.56$$

After the separate absorbances were calculated, the equations used to find the mass of each food dye in the single dye drink mixes and the concentrations were then utilized for each drink mix with two food dyes in them.

Table 1: Corrected Absorbance Calculations for Multiple Dye Drink Mixes

Drink Mix Color	FD&C Food Dye	Corrected Absorbance Calculation
Purple	Red 40	$A_{Red\ 40} = A_{503\ nm} - 0.028(A_{629\ nm})$
	Blue 1	$A_{Blue\ 1} = A_{629\ nm}$
Orange	Red 40	$A_{Red\ 40} = \frac{A_{503\ nm} - 0.028(A_{427\ nm})}{0.992}$
	Yellow 5	$A_{Yellow\ 5} = \frac{A_{427\ nm} - 0.289(A_{503\ nm})}{0.992}$
Green	Blue 1	$A_{Blue\ 1} = A_{629\ nm}$
	Yellow 5	$A_{Yellow\ 5} = A_{427\ nm} - 0.047(A_{629\ nm})$

Table 1: Table showing the corrected absorbance equations for the drink mixes with two food dyes. This table is a summary table from the one reported by Sigmann and Wheeler's experimentation (Sigmann and Wheeler 1478).

Data Analysis

All plots and statistical tests were performed in R 4.1.1 using the packages ggplot2, ggpubr, dplyr, reshape2, multcomp, cowplot, graphics (Wickham 2016; Kassambara; Wickham et al. 2021; Wickham 2007; Hothorn et al.; Wilke; R Core Team). Due to varying sample sizes, differences between food dye concentrations in powdered drink mixes were determined using a Kruskal Wallis test. Pairwise comparisons were determined using the pairwise Wilcoxon rank sum test. To analyze the pairwise comparisons, p-values were adjusted using the Benjamini-Hochberg (BH) correction to control for Type I errors.

Results

Single Dye Drink Mixes

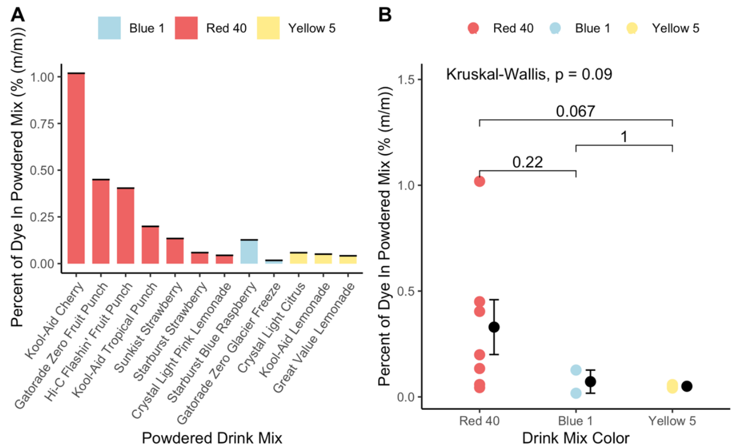

Figure 3: Results for single dye powdered drink mixes. Panel A: The percent mass of single dyes varies widely by drink mix (range: 0.0172 - 1.0186). Panel B: Percent mass of dyes varies between Red 40, Blue 1, and Yellow 5 (H (2) = 0.09). Our data suggests Red 40 percent mass (Median (Mdn) = 0.199) is larger compared to Yellow 5 (Mdn = 0.051, BH adjusted p = 0.067), but not compared to Blue 1 (Mdn = 0.072, BH adjusted p = 0.22).

Percent mass of dye in drink mixes with a single dye varies from 0.0172% to 1.0186%. Based on drink mix availability, mixes containing Red 40 were sampled more, as mixes for Blue 1 and Yellow 5 were harder to obtain. Drinks with Red 40 contain the highest amount of food dye when compared with blue and yellow drink mixes (Figure 3B, H (2) = 0.09). The percent mass of Red 40 and Blue 1 in single dye drink mixes (corrected p = 0.22) showed no detectable difference, though the percent mass of Red 40 was higher compared to Yellow 5 (BH adjusted p = 0.067). There was no detectable difference between percent mass of Blue 1 and Yellow 5 (BH adjusted p = 1). Our results support previous findings by Lehmkuhler et al. and Sigmann and Wheeler (Lehmkuhler et al. 4; Sigmann and Wheeler 1477).

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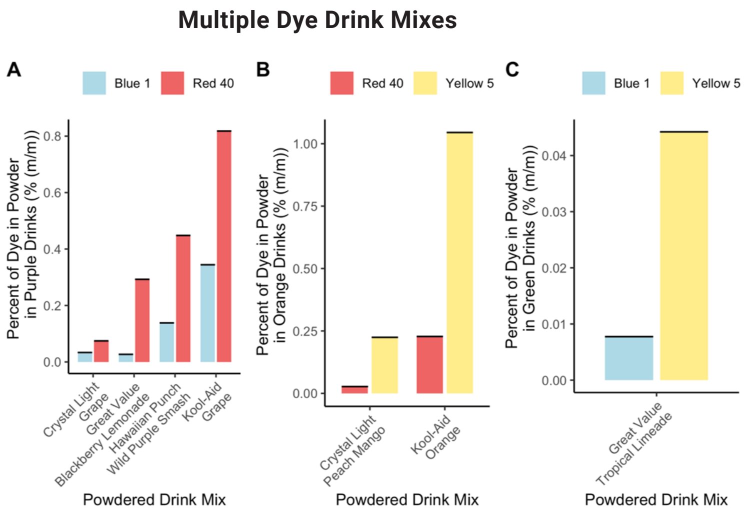

Figure 4: Concentrations of Red 40, Blue 1, and/or Yellow 5 in powdered drink mixes containing two food dyes: purple (A), orange (B) and green (C). For each color evaluated with multiple food dyes, there was no detectable difference in percent mass of the individual component dyes.

Despite noticeable trends in the data, there were no statistical differences between component dyes in multi-dye drink mixes (H (2) = 0.249). In mixes containing multiple dyes, our data suggests purple drink mixes have higher concentrations of Red 40 over Blue 1 (Figure 4A). Similarly in orange drinks mixes, data suggests a higher concentration of Yellow 5 compared to Red 40 (Figure 4B), and for the green drink mix the concentration of Yellow 5 was larger than Blue 1 (Figure 4C).

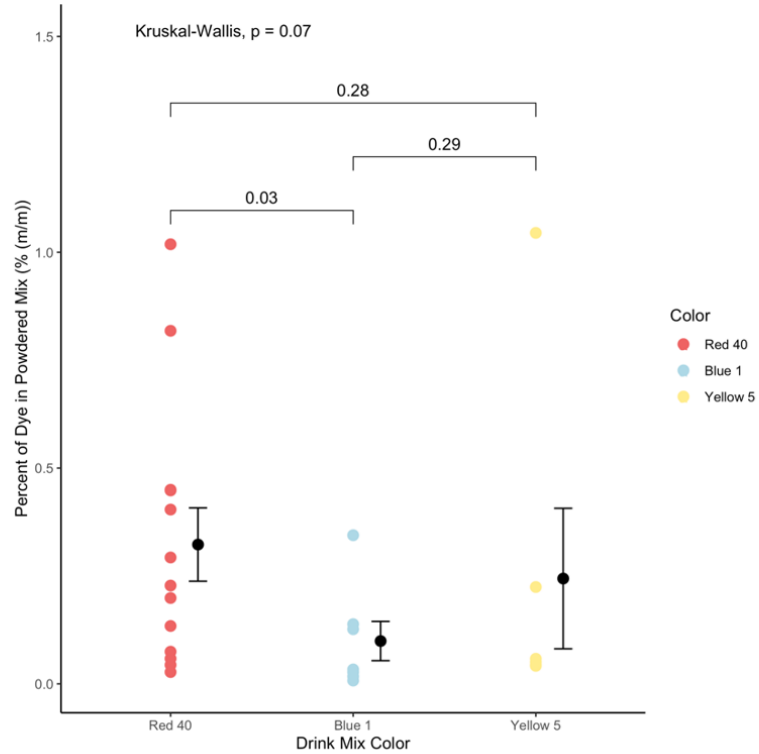

Figure 5: Combined data from single dye drinks and component dye measurements from multi-dye drinks. Percent mass of dyes varies between Red 40, Blue 1, and Yellow 5 (H (2) = 0.07). Combining data suggests Red 40 percent mass (Mdn = 0.228, 95% CI [0.0585, 0.4498]) is larger compared to Blue 1 (Mdn = 0.033 95% CI [0.0077, 0.3443], BH adjusted p = 0.03), but not compared to Yellow 5 (Mdn = 0.054, 95% CI [0.0417, 1.0449], BH adjusted p = 0.28). There is no difference in percent mass between Blue 1 and Yellow 5 (BH adjusted p = 0.29).

Combined Results

To obtain the dye component in multi-dye drinks, percent masses of dyes in single-dye drinks were combined with percent mass values (Figure 5). Results from single-dye drink mixes supported a difference in percent mass between Red 40 and Yellow 5 (Figure 3A). When data for multi-dye drink mixes are included, the only statistically relevant differences are between percent masses of Red 40 and Blue 1 (Figure 5, BH adjusted p = 0.03). Taken together, our data suggests that while yellow drink mixes have low percent masses of Yellow 5, orange drink mixes contain a larger percent mass of Yellow 5. The percent mass of Blue 1 is statistically lower compared to Red 40 when both single dye and multi-dye drink mixes are considered (BH adjusted p = 0.03).

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Discussion

In total, we provided percent mass calculations for twelve single dye drink mixes, and seven multi-dye drink mixes. The overall trend from our data supports previous findings that Red 40 has the highest average percent mass among Red 40, Blue 1, and Yellow 5 (Lehmkuhler et al. 4; Sigmann and Wheeler 1477). When comparing single-dye drink mixes, Red 40 percent mass is larger than Yellow 5, but this trend disappears when combined with data from multi-dye drink mixes.

The elevated concentration of Yellow 5 in orange drink mixes removes any statistical differences between percent mass of Red 40 and Yellow 5 in the combined data. Our data suggests that the Kool-Aid orange drink mix is more vibrant when compared to Crystal Light Peach Mango. Our data supports that manufacturers vary in the percent mass of dye used in their drink mixes, and some products are likely more vibrant. One limitation is that due to local product availability we were only able to analyze two orange drinks, and one green which is the only other multi-dye combination that contains Yellow 5.

One additional limitation of the study is that water was used as a blank for the powdered drink mixes. It is possible that there are additional additives in the drink mixes that could influence the absorbance values, resulting in a slightly higher concentration of dye. Further studies could investigate the other additives in powdered drinks to determine if they influence the absorbance values.

Conclusion

In this study, UV-Vis spectrometry was used to determine the amount of food dyes in Kool-Aid as well Crystal Light, Great Value, Hawaiian Punch, Starburst, Sunkist, Hi-C, and Gatorade Zero powdered drink mixes. Similar to previous studies, single-dye drinks with Red 40 statistically had a higher concentration of food dye when compared to drinks containing Yellow 5. In multiple dye drink mixes, the concentration of Red 40 statistically differed in concentration of food dye when compared to Blue 1 in purple drink mixes. Since the amount of dye in food products is proprietary, periodic studies determining the concentration of dye in food are valuable.

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