PRESENCE OF YELLOW 6, AN ARTIFICIAL COLOUR ADDITIVE IN ORANGE JUICE

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Abstract

Soft drinks and orange juices are very popular all over the world including Bangladesh. Yellow 6 (Disodium 6-hydroxy-5-(4-sulfophenyl) azo-2-naphthalenesulfonate) was used in food and drinks as color additives earlier but due to its adverse effects on human health, yellow 6 has been banned in many countries. However, this harmful color additive yellow 6 is being used in commercial orange drinks in Bangladesh. Ten commercial brand yellow colored soft drinks and juices were analyzed to find out the presence of yellow 6. Juice prepared from fresh fruits was used as control. The UV-visible absorption maxima of yellow 6 is 429 nm. The amount of yellow 6 in commercial brands and fresh orange juices was determined by UV-visible spectrophotometer. Out of ten samples, seven juice samples were found to contain yellow 6 in the range of 0.83-1.66 mg/mL. Fresh juice did not show any absorption at 429 nm. This is an easy and cost effective method to assess the presence of yellow 6 in orange juice and the method can be used for other food stuffs.

Introduction

Soft drinks and orange juices are very popular all over the world including Bangladesh. The climate of the country is changing due to various factors and consumption of soft drinks is being increased in the country because of hot and humid weather. As a result, more soft drink companies are built up to serve the people. Globally, the popularity of orange juice and drinks increased with the development of the commercial juice industry in the late 1920s. Artificial color and synthetic sweetener are added in the commercial juice in addition to natural fruit juice to make the juice more attractive, tasty and this helps the commercial producer to make more profit. Some of the additives are beneficial for health and some are hazardous. Yellow 6 (Disodium 6-hydroxy-5-(4-sulfophenyl) azo-2-naphthalenesulfonate), known as sunset yellow or Sudan I, is such an artificial color additive which causes adverse effect on human health. Yellow 6 is assumed to be responsible for causing an allergic reaction in people with an aspirin intolerances³ resulting in various symptoms, including diarrhea, vomiting, gastric upset, nettle rash (urticaria), swelling of the skin (angioedema) and migraines.⁴ Yellow 6 and other artificial colorings were found to affect children behavior especially hyperactivity in UK. Due to its adverse effect, the Food Standard Agency (FSA) urged the UK government to ban and phase out artificial colorings found in many products such as sweets, confectionary, processed food and takeaways during 2008-2009.⁵ Yogurts and sweets

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containing yellow 6 were banned in Norway and Finland.⁶ At present food safety is a major problem in Bangladesh. Food adulteration and use of hazardous chemicals in food stuffs is published in our daily newspaper now and then. In continuation of our work on contaminants in food and food stuffs, ten locally made orange juices and drinks were analyzed for the presence of yellow 6. To the best of our knowledge monitoring of yellow 6 in orange juices is the first study in Bangladesh. Therefore, the objective of the present work is to find out an easy and cost effective method to assess the presence of yellow 6 in orange juice and soft drinks.

Structure of yellow 6

Experimental

Instruments

UV-visible spectrum of all samples was recorded on Shimadzu UV 160A. Microprocessor pH meter (Model: HANNA pH 211) was used for determination of pH of juice and soft drinks.

Reagents and Materials

Extra pure D-(+)-glucose was purchased from Aldrich Chemical Co. Ltd. and yellow 6 was purchased from Germany. Sulfuric acid (98%, w/w, BDH, U.K.) and phenol (Merck, Mumbai, India) were of analytical grade.

Sample Collection

Ten commercial brand orange juice samples (Code No. J_{01} - J_{10}) were collected from different markets of Dhaka city during December 2009 and July 2011. Orange juice was made from fresh fruits by squeezing followed by filtration & repeated centrifugation and clear juice was obtained to use as control (FJ₁ and FJ₂).

Determination of pH value

pH of each of the juice was measured by a pH meter which was calibrated using two different standard buffers (pH 7.0 and 5.0).

Determination of total solid material

All the samples (100 mL in each case) were evaporated by a rotary vacuum evaporator (20 mL) and then freeze dried.

Determination of total carbohydrate

Standard D-(+)-glucose (100 mg) was dissolved in distilled water (100 mL) and then diluted to the required concentration of 0.1 mg/mL. The solution was serially diluted to 4 different concentrations, 0.05, 0.025, 0.0125, and 0.0062 mg/mL and distilled water was taken as blank. Glucose solution (500 μ l) was taken in the test tube and 80% aqueous phenol (5 μ L) was added to it and vortexed. Conc. H₂SO₄ (3 mL) was added to the mixture. The absorbance of each of the solutions was measured at 489 nm by a UV-visible spectrophotometer. A calibration curve was made by plotting absorbance against glucose concentration and a straight line was obtained (Figure 1). Solid materials of each orange juice (10 mg) were dissolved in 100 mL water to get a solution of 0.1 mg/mL. Total carbohydrate in juice solutions were determined performing phenol sulphuric acid test⁷⁻⁹ and using the standard calibration curve made for glucose.

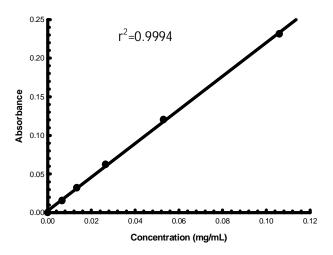


Fig. 1. Standard calibration curve of D-glucose (absorbance measured at 489 nm)

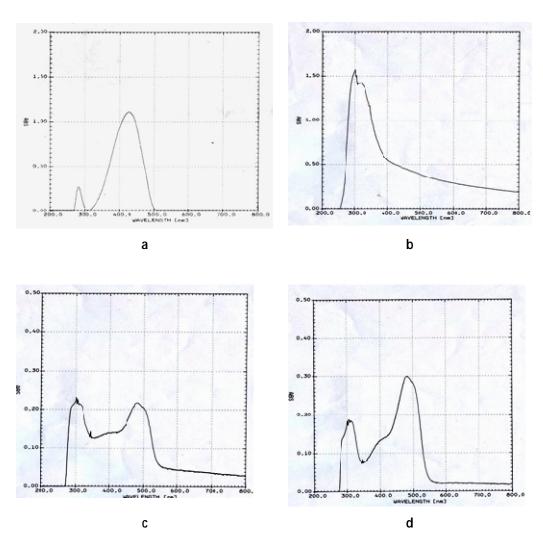


Fig. 2. UV-visible absorption spectrum of yellow 6 (a), fresh juice (b) and commercial brand orange juices (c and d)

Standard calibration curve of yellow 6

UV-visible spectrum of yellow 6 was recorded and absorption maxima (λ_{max}) was found to be at 429 nm (Figure 2). A stock solution of standard yellow 6 (1 mg/mL) was made and the solution was gradually diluted to obtain 8 different concentrations, 0.04, 0.05, 0.06, 0.08, 0.09, 0.10, 0.12 and 0.15 mg/mL. The absorbance of each diluted yellow 6

solution was measured at 429 nm by a UV-visible spectrophotometer. A calibration curve was made by plotting absorbance against concentrations (Figure 3).

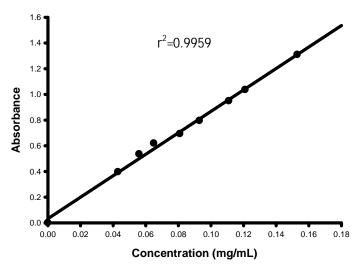


Fig. 3: Standard calibration curve of yellow 6 (absorbance recorded at 429 nm)

Determination of Yellow 6 in orange juice

The UV-visible absorption spectrum of each of the juice sample was recorded. Dried solid sample of each commercial and fresh made orange juice (1 g) was dissolved in water (100 mL) and the UV-visible absorption absorbance spectrum of each solution was recorded. Using the standard calibration curve of yellow 6, the amount of yellow 6 in commercial brands and fresh orange juices was determined.

Results and Discussion

The total solid in fresh juice and commercial brand samples were found to be in the range of 64.80-80.60 and 74.60-167.70 g/L, respectively (Table 1). High value of solid in commercial sample was due to the additional additives in comparison to low value of fresh juice. Carbohydrate content of two fresh orange juices were found to be 36.13 and 40.54 g/L, whereas the commercial brand samples contained in the range of 83.30-124.50 g/L. High carbohydrates contain juice causes obesity to the consumers. ¹⁰

Table 1. pH and total solid & carbohydrate contents of orange Juice

Sample code	pН	Total solid (g/L)	Carbohydrate (g/L)
J_{01}	2.80	131.10	116.96
J_{02}	2.81	142.40	122.09
J_{03}	2.92	137.50	113.31
J_{04}	3.34	167.70	124.50
J_{05}	3.95	97.40	83.30
J_{06}	4.01	74.60	88.86
J_{07}	2.86	138.40	112.41
J_{08}	2.79	155.10	116.89
J_{09}	2.90	131.60	115.08
J_{10}	2.81	147.80	119.11
FJ_1	4.81	80.60	36.13
FJ_2	4.01	64.80	40.54

Due to the presence of naturally occurring organic acid, orange juices usually are acidic and have pH around $3.5.^{11}$ pH of commercial brand and fresh orange juices were found to be in the range of 2.79-4.01 and 4.01-4.81, respectively. A lower pH of commercially made juice might be due to the presence of acidic additives which can cause acidity in the stomach and erosion of the tooth enamel. 12

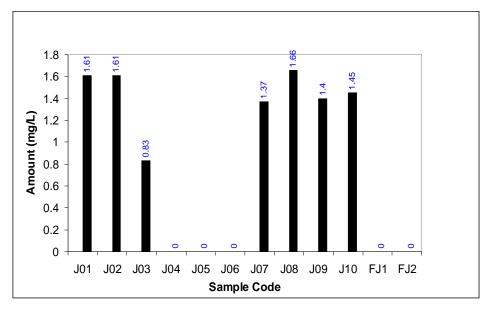


Fig. 4. Amount of yellow 6 in orange juice samples

The absorption maxima for fresh juice samples were found to be 282 nm but all the juices except J_{04} , J_{05} and J_{06} gave absorption at 282 and 429 nm. The first absorption maxima was due to the naturally occurring compounds which would be beneficially for human health. The second absorption at 429 nm is similar to the absorption maxima of yellow 6 which would be harmful to health. As fresh juice did not show any absorption at 429 nm, these samples did not contain yellow 6. Out of ten samples, 7 juice samples were found to contain yellow 6 in the range of 0.83-1.66 mg/mL (Figure 4). These results indicate that yellow 6 is being added in the commercial orange juices and soft drinks as additives. This is an easy and cost effective method to assess the presence of yellow 6 in orange juice and the method can be used for other food stuffs. Bangladesh is one of the malnourished countries in the world. By random use of color additives in juices and other food stuffs may effect human health by allergic reactions and increase hyperactivity of children. Further study is required to monitor the uses of yellow 6 in commercial orange juices and drinks.

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 $J_{01} \quad J_{02} \quad J_{03} \quad J_{04} \quad J_{05} \quad J_{06} \quad J_{07} \quad J_{08} \quad J_{09} \quad J_{10} \quad F_{J1} \quad F_{J2}$

 $J_{01} \quad J_{02} \quad J_{03} \quad J_{04} \quad J_{05} \quad J_{06} \quad J_{07} \quad J_{08} \quad J_{09} \quad J_{10} \quad F_{J1} \quad F_{J2}$