

## Investigation On The Presence of Sunset Yellow and Tartrazine In Commercial Beverages and Quantitation Using Ion-Pair Formation and Extraction

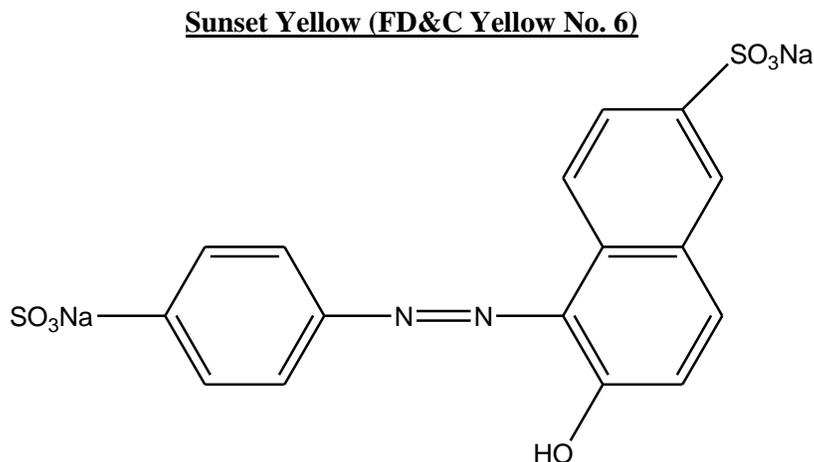
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*In this work, 18 different commercial beverages were analyzed for the detection of Sunset Yellow and Tartrazine by paper chromatography. Quantitation of samples containing only one added colour proceeded using the ion pair formation and extraction method. The results obtained showed that all the commercial beverages tested contain synthetic colours permitted for use based on the Malaysian Food Act 1983. Quantitation of soft drinks which contained only one added colour complied with the U.K. standard, although one sample showed a slight excess compared to the set limit of 50 mg/L.*

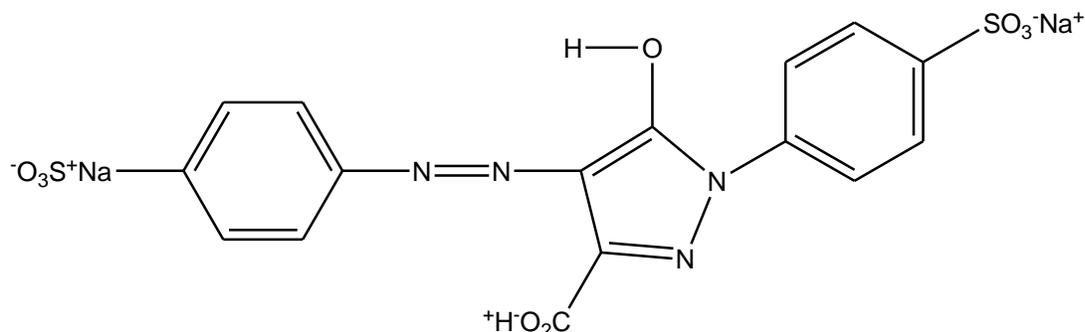
### Introduction

Sunset Yellow (otherwise known as E110 or FD&C Yellow No. 6) and Tartrazine (also known as E102 or FD&C Yellow No. 5) are synthetic dyes (Fig. 1). They are commercially used as additives in pharmaceuticals and cosmetics, with the advantages that they can be easily mixed to achieve ideal colours and because of their low price compared to the natural dyes. Synthetic colourants normally contain azo functional groups and aromatic ring structures, so they are harmful to human health (6). For this reason, the controlled use and the accurate analysis of their contents in alimentary products is important.

**Fig. 1: Structures of Sunset Yellow and Tartrazine**



## Tartrazine



Sunset Yellow is an azo food dye that was first used in 1929. It is an incredibly strong dye that gives a reddish-yellow, or orange colour to the food and drugs to which it is added. Sunset Yellow is found commonly in beverages, confections, ice-cream, dessert powders, cereals, baked goods and snack foods. The maximum daily intake recommended by the U.S. FDA is only 225 mg per day for a 132 lb person (13).

Tartrazine is a synthetic yellow azo dye found in foods such as fruit cordial, coloured fizzy drinks, cake mixes, custard powder, sweets, jam and ice-cream. It can also be found in the shells of medicinal capsules. It appears to cause the most allergic reaction and/or intolerance reactions of all the azo dyes, particularly amongst those with an aspirin intolerance and asthmatics (14). The usage of synthetic colours is well-regulated world wide, but the regulations differ from one country and to the next. The United States Food and Drug Administration (FDA) has classified all colour additives permitted for use in foods as 'certifiable' or 'exempt from certification' (16). Certifiable colour additives are man-made, with each batch being tested by manufacturer and FDA. Colour additives that are exempt from certification include pigments derived from natural sources such as vegetables, minerals or animals, and man-made counterparts of natural derivatives. For example, caramel colour is produced commercially by heating sugar and other carbohydrates under strictly controlled conditions for use in sauces, gravies, soft drinks, baked goods and other foods.

To ensure compliance with regulatory requirements, the colours used have to be identified and quantified according to national directives. The Malaysian Food Act 1983 however does not specify the exact permissible limit for each synthetic dye. The classical method for extraction of organic acids from an aqueous into an organic phase is based on the higher solubility of the undissociated molecules in organic solvents. The ion-pair extraction method introduced by Schill (12) consists of the formation of a complex between an ionized, more or less hydrophobic substance, and an ion of opposite charge. The complex is more hydrophobic than the ionized molecule and hence more readily extracted into organic solvents.

In the many methods proposed for detection and determination of dyes in foods the colours need to be extracted from the foods first. In this study, a simple ion-pair extract of the dye from different beverages was measured spectrophotometrically after it was determined to contain single synthetic food colour using paper chromatography. This work aims to investigate the presence of Sunset Yellow and Tartrazine in beverages and to quantify the amount of food dye present in samples containing single synthetic colour.

## **Experimental**

### **Apparatus**

A Shimadzu UV-1601 UV-visible spectrophotometer was used.

### **Reagents**

All reagents including the standard food colours were of analytical reagent grade.

### **Samples**

Beverage samples were obtained from various supermarkets and stores around Perlis, Kedah, Penang and Kuala Lumpur.

### **Method for determination of samples containing a single synthetic colour**

Synthetic dyes are isolated according to the method described in the Manual For Chemical Analysis of Food, Food Quality Control Division (Method 5.1.2.1, 1999). About 25 g of the beverage was mixed with water to make a 100 mL solution before isolation of colors. The solution was acidified using 1% (v/v) glacial acetic acid and white animal wool was added in the beaker and the contents were boiled for a few minutes. The wool took any synthetic dye if present. The wool was removed from the beaker and washed under cold running tap water. The colour from the dyed wool was recovered by boiling it in a dilute ammonia solution. The wool was discarded and the coloured solution was evaporated using steam bath to remove the ammonia solution. The colour was re-dissolved using 20 % (v/v) aqueous acetone. The extracted colours were identified using paper chromatography. Samples containing single synthetic food colour were further analyzed to quantitate the dye present.

### **Determination of the amount of food dye in soft drinks**

Spectrophotometric determination of single synthetic food colour in soft drinks was done according to the ion-pair formation and extraction method in *n*-butanol as described by Lau et al. (1995) (9). About 150 mL of the soft drink was degassed. 25 mL aliquots were pipetted into each of four separating funnels. 2 mL of phosphate buffer solution (pH 5.6) was added into each separating funnel. 0.0 mL, 0.10 mL, 0.20 mL and 0.30 mL each of 1000 ppm standard food colour were added into the separating funnels followed by addition of 10 mL 0.5 % (m/v) of the ion-pairing reagent, octadecyltrimethylammonium bromide in *n*-butanol. Each mixture was shaken and two layers formed. The aqueous phase was discarded. The organic phase was quantitatively transferred into a 25 mL volumetric flask and diluted to the mark with ethanol. All organic extracts were analyzed using a UV-visible spectrophotometer (Shimadzu UV-1601) at a wavelength of 482 nm. A standard addition curve was constructed to determine the amount of Sunset Yellow in the beverage samples.

## **Results and Discussion**

The health hazard due to consumption of food colours have been reported by FAO / WHO in 1994 (2). Studies elsewhere have reported that food colours might cause health hazards such as allergic and respiratory reaction, birth defects and cancer problems. In 1979, the U.S. FDA issued the requirement that products containing FD&C Yellow No. 5, tartrazine carry a notice on the label to the consumer noting that the colour is present in

the product (1). This was due to the mounting evidence of allergic-type reactions to the colour additive. A case study involving additive allergy causing gastroenteritis due to a diet containing Sunset Yellow was also reported (Gross, P.A., et al., 1989) (7) Tartrazine has been reported to be associated with irritability, restlessness and sleep disturbance in hypertensive children aged between two and fourteen years (Rowe and Rowe, 1994) (11).

This study was carried out since there is a need to create awareness in consumers about the colorants used by food producers to give their products a fresh and natural appearance. Health authorities in some countries have begun to question and even ban the use of certain food colorants. In the U.S. the colour additives not authorized for use are Amaranth (FD&C Red No. 2), Azorubine (also called Azo Rubine and Carmoisine), Dibromofluorescein (D&C Orange No. 5), Eosine (D&C Red No. 22), Eosine B, Fast Crimson GR (also known as C.I. Acid Red 1), Ponceau 4R (C.I. Acid Red No. 18), Quinoline Yellow (C.I. Acid Yellow No. 3) (15). This list however may differ from the national food standards or consumers' organization in other countries. In Malaysia the synthetic dyes permitted to be used as colouring substances in food are listed in Table 1. (3) (Food Act 1983, Jan 1999)

**Table 1: Permitted synthetic colouring substance in Malaysia**

Common Name of Colour	Colour Index Number
Allura Red AC	16035
Amaranth	16185
Brilliant Blue FCF	42090
Carmoisine	14720
Chocolate Brown HT	20285
Erythrosine BS	45430
Fast Green FCF	42053
Green S	44090
Indigotine	73015
Ponceau 4R	16255
Quinoline Yellow	47005
Red 2G	18050
Sunset Yellow FCF	15985
Tartrazine	19140

Survey of colours in food products is necessary since consumers should be fully informed about the presence of ingredients in food. A survey of colours in sweets was conducted in the U.K. by the Food Standards Agency (FSA), where five out of 196 retail samples of packaged sweets were found to contain levels of either Sunset Yellow FCF or Carmoisine in excess of the maximum permissible limit of 50 mg/kg in confectionary (5).

Tartrazine and Sunset Yellow were analyzed because of the widely available yellow or orange coloured beverages in the market. The positive identification of individual food colours is often quite difficult (8). A large number are the sodium salts of sulfonic acids and this results in their having no precise melting point or boiling point. In addition synthesized colours usually contain subsidiary colouring matters. Identification therefore is best achieved by comparison of the observed properties of authentic commercial samples. Since the presence of subsidiary colouring matters may affect the

observed spectra, positive identification of the principal component cannot be made. Therefore it is necessary to separate the colouring matters before additional means of identification and quantitation are attempted. In this study, the determination of synthetic colours using paper chromatography showed that only two beverages contain a single added colour as shown in Table 2.

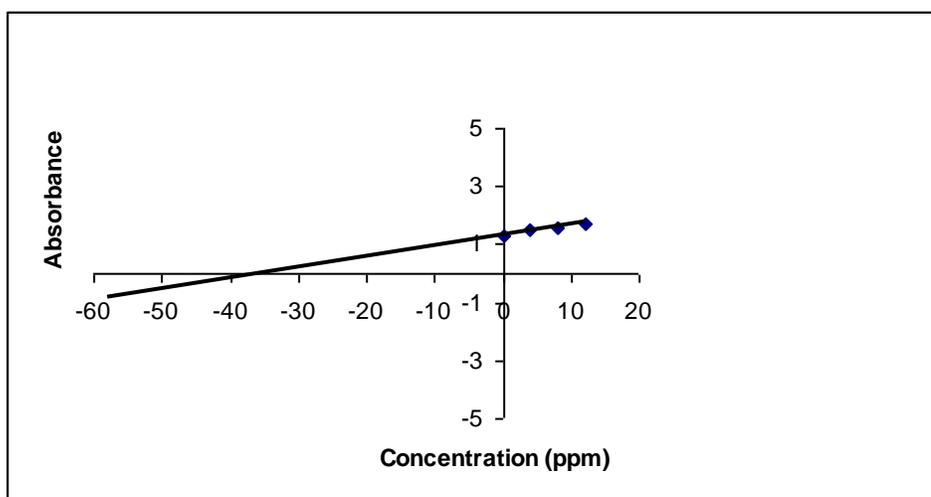
**Table 2 : Summary of food colours contained in beverage samples.**

No	Sample Brands	Separated dyes	Comments
1.	Nestea – Ice Rush	Caramel	Natural food colour
2.	Chill – Chrysanthemum Tea	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
3.	Season’s – Ice Lemon Tea	Caramel	Natural food colour
4.	Teh Hijau Yeo’s	Caramel	Natural food colour
5.	Rubaaly Ice Lemon Tea	Caramel	Natural food colour
6.	Super Chrysanthemum Tea	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
7.	Lipton – Ice Lemon Tea	Caramel	Natural food colour
8.	Teh Wangi Cap Kuda Terbang	Caramel	Natural food colour
9.	Rex – Lemon Tea	Caramel	Natural food colour
10.	Teh Wangi Ros	Caramel	Natural food colour
11.	Teh Wangi Ros (888)	Caramel	Natural food colour
12.	Difruit Orange	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
13.	Drinho Orange	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
14.	Delite Orange	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
15.	Voodoo Jelly Orange	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
16.	Super Oren	Sunset Yellow and Tartrazine	Contains more than one synthetic colour.
17.	Green Spot Orange	Sunset Yellow	Contains single synthetic colour.
18.	Borneo Oren	Sunset Yellow	Contains single synthetic colour.

Results from analysis using paper chromatography indicate that nine out of eleven tea-based samples tested contain caramel colour, a natural food colour permitted for use. All the fruit juices, two tea-based drinks, Chill-Chrysanthemum Tea and Super Chrysanthemum Tea, and one soft drink, Super Oren, contained both Sunset Yellow and Tartrazine. Since Green Spot Orange and Borneo Oren contained only one added synthetic colour, quantitation of Sunset Yellow contained in both soft drinks were done. The quantitation method selected was based on an ion-pair extraction method by Puttemans et al. (10). This study adapts the method developed by Lau to quantitate food colours in soft drinks containing only a single added colour.

The ion-pair extraction and formation method was used to quantify the food colour since this method is simple, rapid and accurate, which does not require the use of any complicated analytical instrument. The method is less time consuming since only a brief period of shaking is required for a good separation of layers to occur. The amount of Sunset Yellow contained in the drinks were obtained using the standard addition method as shown in Figure 2.

**Figure 2 : Standard addition curve for Green Spot Orange drink.**



Using this analysis, the average concentrations of Sunset Yellow were found to be 39 ppm and 64 ppm in Green Spot Orange and Borneo Oren, respectively, as shown in Table 3.

**Table 3 : Amount of Sunset Yellow in soft drink samples.**

Green Spot Orange		
Sample no.	Concentration (ppm)	Average concentration (ppm)
1	38.50	38.64±0.48
2	38.60	
3	38.83	
Borneo Oren		
Sample no.	Concentration (ppm)	Average concentration (ppm)
1	63.00	63.87±0.77
2	64.35	
3	64.27	

Due to the absence of maximum permitted level in Malaysia for synthetic food dyes, the results obtained are compared to the standard set by the UK Food Standards Agency (FSA) which is 50 mg/L maximum permitted limit of Sunset Yellow FCF in non-

alcoholic flavoured drinks (4). In a survey conducted by the FSA, out of 201 retail samples of soft drinks tested for the content of food colours, only four contained either Sunset Yellow FCF and Carmoisine in excess of the permitted level. This showed a strong compliance in colour labeling of the soft drink manufacturers in the U.K. In this work, the amount of Sunset Yellow in Green Spot Orange complies with the set standard but the level is slightly exceeded in Borneo Oren. The synthetic colours present in all the beverages tested indicate compliance with the Malaysian Food Act 1983 and are permitted for use. However, since food colours can cause a wide range of adverse reactions especially in sensitive individuals, the amount added in foods must also be regulated.

### **Conclusion**

This investigation showed that all the commercial beverages tested contain synthetic colours permitted for use. Quantitation of soft drinks which contained only one added colour, which was Sunset Yellow, indicate compliance with the U.K. standard, although one sample showed a slight excess compared to the set limit. It should also prove useful for the safety of consumers that not only the types of dyes which are being used are regulated but also for the public to be informed of the actual amount of dyes present in foods.

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