

DETERMINATION OF SUNSET YELLOW IN DIFFERENT BRANDS OF ORANGE JELLIES OF BANGLADESH BY HPLC

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ABSTRACT

Synthetic colorants may have adverse health effects, therefore, it is necessary to ensure controlled use of these colorants in various alimentary products. In this study, 54 samples of six brands of orange jellies were analyzed for the determination of 'Sunset Yellow' color by High Performance Liquid Chromatography. The results showed that the value of Sunset Yellow in one brand of orange jelly exceeded the Bangladesh Standard and Testing Institute (BSTI) value, the values in four other brands were within the range of the BSTI value, and it was absent in the last one. One brand yielded more than twice the maximum BSTI value and four times the maximum value from the European Union. These results indicated that there is a need to monitor the amounts of synthetic colorants used in food products to protect the public health from serious adverse effects related to such chemicals and to create awareness to the consumers as well as policy makers.

Keywords: HPLC, orange jellies, sunset yellow-FCF, synthetic food color, BSTI

1. INTRODUCTION

Sunset yellow (SY) is a common synthetic food color, appearing reddish-orange upon application, that is added to food to improve the color, texture, and overall appearance (KUCHARSKA and GRABKA, 2010; ABBEY *et al.*, 2014). Dairy products, cereals, candies, jellies, ice-cream, soft drinks, yogurts, fillings, liqueurs, and powdered juices are the most common food items in which this dye is added (MEINICKE and JORGE, 2008; YUAN *et al.*, 2016). Overuse of synthetic colorants is a major source of food intoxication (KOUTSOGEORGOPOULOU *et al.*, 1998). There are various adverse health effects associated with ingesting excess amounts of synthetic colorants, such as cancer, genetic diseases, etc. (TSUDA *et al.*, 2001; DAS and MUKHERJEE, 2004), asthma, abortions, weakened immune systems, and allergic reactions (GEOFFREY and FELIX, 1991; HINTON, 2000; BHATTACHARJEE, 2014). Additionally, synthetic colorants have been associated with behavioral effects in children, such as hyperactivity, decreased IQ scores, and boosted aggression (GEOFFREY and FELIX, 1991; HINTON, 2000; BHATTACHARJEE, 2014). Synthetic SY specifically has been linked with anaphylactic reactions and cardiovascular complications (angioedema, vasculitis, and thromboxane synthesis inhibition) in individuals who present a sensitivity to the compound (SARDI *et al.*, 2010). Studies have shown that most synthetic colorants bind directly to DNA and cause structural and numerical incongruities (HAMDY *et al.*, 2000; MPOUNTOUKAS *et al.*, 2010). In addition, it has been found that semi-toxic doses of sunset yellow leads to changes in total lipid storage of the body when exposed to animal models. As lipids have structural functions in biological membranes of the body, this might stimulate their metabolism and may cause potential liver injuries such as necrosis (MATHUR *et al.*, 2005). Similar to most other developing countries, it is a common scenario in Bangladesh that industrial and non-industrial sectors are involved in food production and processing activities. Food industry uses various synthetic colorants, which are the most interesting groups of food additives as the colorful food products attract consumers (KUCHARSKA and GRABKA, 2010). However, their range of use and amounts are restricted across the world (SUN *et al.*, 2013). The non-industrial sector produces two- to three-times the amount of food items compared to the industrial sector. Unfortunately, quality control systems are lacking in the non-industrial sectors, leading to high production of sub-standard food products potentially compromising the health of consumers and thus, indirectly leading to a financial burden for the nation. Based on the results of the International Research and Recommendation of Codex Committee on Food Additives and Contaminants (CCFAC), the Acceptable Daily Intake (ADI) value of food colorants is set across the world (BESSONOV *et al.*, 2011; GANESAN *et al.*, 2011). The ADI of permitted food colorants varies from 0.1 mg/kg body weight (Erythrosine red) to 25mg/kg body weight (Fast Green FCF) (SWAROOP *et al.*, 2011). However, there is warranted concern over the amount of synthetic colorants in food products as certain companies exceed the upper limit recommended by the ADI. Therefore, it is necessary to monitor the total daily intake of all food colorants to ensure the ADI is not exceeded (JOINT, on FOOD ADDITIVES, ORGANIZATION and OTHERS, 1965, 1991).

Due to the wide industrial use of food dyes to color foods it is important to determine the amounts of synthetic colorants commonly added to foods in Bangladesh. Current analytical detection methods are costly and require substantial resources that are not available in Bangladesh. Therefore, the present study was conducted to determine the color range of SY in different brands of orange jellies collected from different shops of Tangail City, Bangladesh, using a simple and cost effective high-performance liquid chromatography (HPLC) method. The analytical determination of this particular color

may aid in establishing a method for detecting synthetic colorants and contribute to overall quality assurance and consumer safety.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents

Sodium acetate 97%, HPLC-grade chloroform, n-hexane, and acetonitrile were obtained from Merck (Darmstadt, Germany). Glacial acetic acid was procured from Sigma Chemical Co. (Darmstadt, Germany). Diethyl acetate from RCI Lifescan Ltd. was used. Deionised water (18.2 M Ω) used for chromatography processing was procured from a Barnstead Nanopure water purification system (Barnstead, USA). Sunset Yellow FCF (E110) was purchased from Rayner, Co. Ltd. (London, England).

2.2. Samples

A total of 54 samples from six brands of orange jellies (research raw material) were purchased from three stores in Tangail city, Tangail, Bangladesh after getting verbal consent from the shopkeepers. All samples were considered valid based on their expiry dates. The weights of the samples were 200 gm to 500 gm. The collected samples were preserved in refrigerator at 4 °C in the laboratory of the Department of Food Technology and Nutritional Science, Mawlana Bhashani Science and Technology University (MBSTU). Ethical approval was taken from the research cell of MBSTU.

2.3. Mobile phase

The mobile phase was prepared using the modified method of PYLYPIW and GREETHER (2000). It consisted of a mixture of 3.0 mM acetate buffer (pH ~4.00) and HPLC-grade acetonitrile with a ratio of 17:3. Acetate buffer was prepared by mixing of 1.0 ml of glacial acetic acid and 1.0 g of sodium acetate trihydrate in 1.0 L de-ionized water and mixed well. The mixture was filtered with a filter membrane (Pore size 0.2 μ m).

2.4. Preparation of standard solution

Approximately 100 mg of anhydrous sunset yellow was taken in a 25 ml volumetric flask. Ten ml 85% aqueous acetonitrile was added to the volumetric flask and was shaken well. Finally, 85% aqueous acetonitrile was added up to mark. The solution was filtered with syringe filter. The standard stock solution-1 was labeled as 4 mg/ml. Approximately 5 ml of stock solution-1 was taken in 50 ml volumetric flask and mobile phase was added up to the mark. The standard solution-2 was labeled as 0.4 mg/ml standard solution. From the stock solutions, working standard solutions 0.0, 1.0, 5.0, 10.0, 20.0, 40.0 μ g/ml were prepared by dilution of aliquots. The solution was filtered through sample filters (Pore size 0.2 μ m) prior to inject into the column.

2.5. Preparation of sample solution

Approximately 1.0 gm of orange jelly was weighted accurately and placed in a conical flask and it was made to 10 ml by adding aqueous 85% acetonitrile solution and mixed well by vigorous shaking for 10 minutes. About 2.0 ml of the solution was filtered through sample filter (Pore size 0.2 μ m) and the filtrate was then diluted 5 times and placed in an

ependorff tube. Finally, 20 μ l was injected into the HPLC column. The concentration of injected sample solution was 20 μ g/ml.

Calculation of sample concentration:

$$\frac{1 \text{ gm} \times 200 \text{ ml}}{10 \text{ ml} \times 1000 \text{ ml}} = 0.02 \text{ mg/ml} = 20 \mu\text{g/ml}$$

2.6. Sample solution preparation for Spiked/Recovery assay

Approximately 2.0 gm of orange jelly and 1.0 mg of SY was weighted accurately and placed in a conical flask and 85% aqueous acetonitrile solution was added to it to make 20 ml solution and mixed well by vigorous shaking for 10 minutes. About 2.0 ml of the solution was filtered through sample filter (Pore size 0.2 μ m) and the filtrate was then diluted 5 times and placed in an eppendorff tube. Finally, 20 μ l was injected into the HPLC column. The concentration of injected sample solution was 20 μ g/ml.

2.7. Chromatographic analysis

The chromatographic system consisted of a Shimadzu isocratic pump, a degasser, column, oven, a UV-Vis detector, and a LC Workstation Class-VP for data acquisition and analysis. Each of orange jelly samples of 1.0 g was diluted 1:10 with mobile phase and then the sample was again diluted 1:5 with mobile phase. After that the solution was transferred into dry eppendorff tube. The clear aqueous solution was filtered through a PTFE syringe filter. Then the solution was transferred to the dry HPLC vials. 20 μ l of the sample was injected into the injector. For the chromatographic analysis, a Luna 5 μ C18 (2) 100A column (250 \times 4.6 mm) was used and the column temperature was set at 33 $^{\circ}$ C. The sunset yellow analysis was performed with isocratic solvent system using sodium acetate and acetic acid buffer (pH \sim 4.0)/acetonitrile- 17:3 with a flow rate of 1.0 ml/min.

2.8. SY identification and quantification

Optimum absorption wavelength for SY color was evaluated before and using standard solutions with UV-spectrophotometer. The determined wavelength for the analysis of SY was 480 nm. Several runs were made to determine the retention time for the analysis. The retention time for this color was used for the identification of the color present in different brands of orange jellies.

Quantification of the studied colors was done by external standard calibration. Five level analytical curves (0.00, 1.0, 5.0, 10.0, 20.0, 40.0 μ g/ml) were used and the mean of 3 injections of each standard was used to represent each calibration point. By plotting analytes (y) against the concentration (μ g/ml) of the color the peak areas were measured. To determine the slope, y-intercept and the correlation coefficients of the standards plots, least square linear regression analysis was used. Limit of detection (DL) and limit of quantification (QL) were determined by considering 3 and 10 times the signal to noise ratios respectively estimated by the regression lines as mentioned in the previous report (MACDOUGALL *et al.* 1980).

For HPLC method validation the performance parameters, i.e., precision, linearity, limit of detection, limit of quantification, the expanded uncertainty were calculated. By spiking known amounts of the studied colors to the unprocessed sample and comparing the output with the same sample without spiking, recovery evaluations were carried out.

Recoveries were calculated by differences of concentrations and were expressed as percentages.

2.9. Statistical analysis

Each test was performed in triplicate. The descriptive analyses (means, median, standard errors coefficient of variation) were summarized. Data were expressed as mean±standard error (SE). One-way analysis of variance (ANOVA) was carried out using SPSS software version 20 at a significance level of 5%. The Least Significant Difference (LSD) test was used to detect differences in means.

3. RESULTS AND DISCUSSION

3.1. Analysis of chromatogram

Numerous analytical methods have been developed for analyzing synthetic colorants in response to the concern regarding their adverse effects. The most preferred technique for quantitative determination of the colorants is using HPLC, as it is relatively simple, cost effective, and has less environmental impact due to fewer hazardous chemicals used. In this study, an efficient and accurate HPLC analytical method was used for the determination of sunset yellow in different brands of orange jellies collected from local markets of Tangail city, Bangladesh. This method was simple to use, had good operational stability, and gave reliable and reproducible results.

The developed HPLC method was applied to analyze the SY color range in orange jellies. The retention time of the sunset yellow standard was 5.62 ± 0.2 minutes and approximate time was set at 10 minutes. (Figs. 1 and 2).

The calibration curve (Fig. 3) for SY was obtained by plotting the peak areas of different concentrations of the working standard solutions (0.0, 1, 5, 10, 20 and 40 $\mu\text{g}/\text{ml}$). The recovery range was 96-131%.

Table 1 describes the analytical characteristics of the HPLC method. There was a strong linear relationship ($R^2 = 0.999$) obtained between the concentration of SY and the peak area within the HPLC chromatogram at 480 nm (Fig. 3). The detection and quantification limits were calculated as 1.14 mg/100 ml and 3.46 mg/100 ml, respectively.

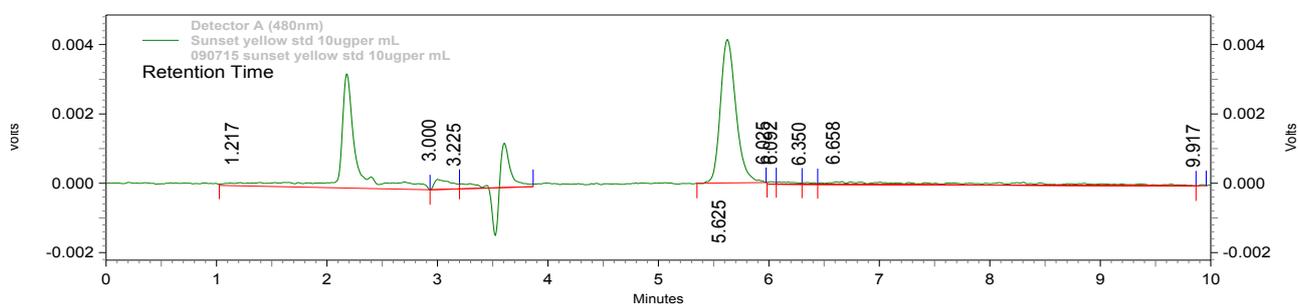


Figure 1. HPLC chromatogram of 10 $\mu\text{g}/\text{ml}$ sunset yellow standard solution with a retention time of 5.625 min.

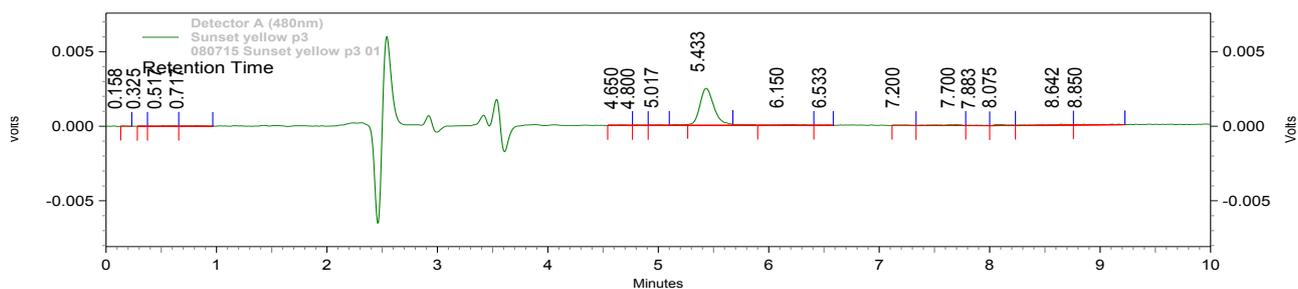


Figure 2. HPLC Chromatogram of sunset yellow present in orange jelly brand-1 (obtained from shop 1) with a retention time of 5.433 min.

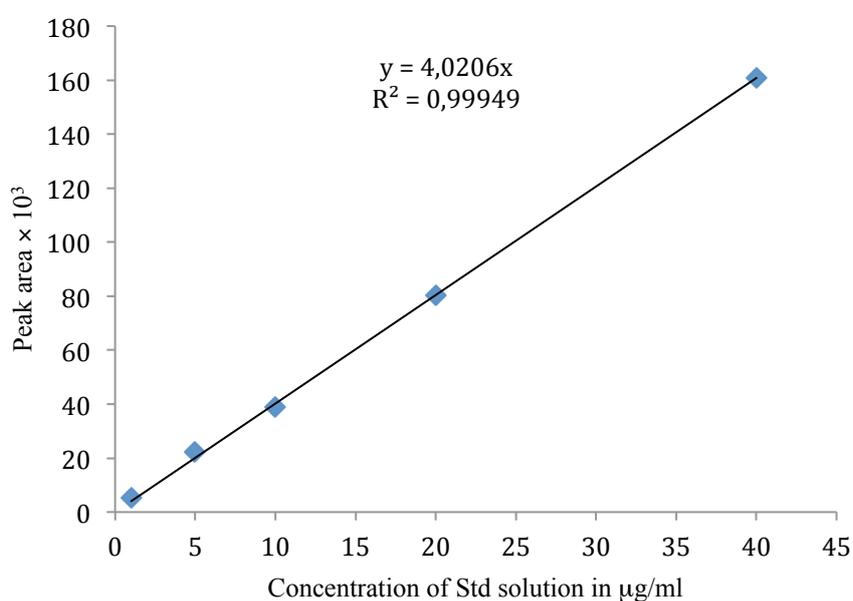


Figure 3. Calibration curve for the sunset yellow standard.

Table 1. Analytical characteristics of HPLC method.

Parameter	Value
Accuracy	107±14.3
Slope	4020
Intercept	0
Linearity range	1.32 µg/ml to 40 µg/ml
Correlation coefficient	0.999
STEYX	1390
LOD	1.14 mg/100 ml
LOQ	3.46 mg/100 ml

3.2. Calculation of % recovery of SY in spiked samples

Approximate 500 µg of SY was added to the 1.0 g of unspiked sample containing SY 4049.41 µg/g. The observed value of SY of the spiked sample was calculated 4560.60 µg/g. So the recovered added SY was 511.19 µg. Finally, the % recovery was 102.23%.

3.3. Determination of SY in samples

The results in Tables 2 and 3 show the levels of the SY from the six brands of orange jellies collected from three different shops with different batches. After HPLC analysis, majority of the orange jellies contained the same synthetic color as was mentioned on their respective product labels. Chromatogram's peak from five out of the six orange jelly brands, were identified as SY matched with the SY standard. No peak in the chromatogram of the samples of brand 6 was matched to the peak of SY standard.

Table 2. Level of SY (mg/100 g) in different brands of orange jellies.

Orange Jelly	Concentration of sunset yellow (mg/100 g)			Mean±SD (n = 3)	p-value	
	Shop	Sample 1	Sample 2			Sample 3
Brand 1	1	25.10	27.40	28.70	27.07±1.49	0.118
	2	17.30	15.20	25.80	19.43±4.58	
	3	25.70	28.20	17.90	23.93±4.39	
Brand 2	1	42.50	40.40	42.00	41.63±0.90	0.351
	2	37.33	44.30	39.20	40.28±2.95	
	3	39.20	40.50	37.90	39.20±1.06	
Brand 3	1	10.10	8.70	8.89	9.23±0.62	0.833
	2	8.89	10.79	7.49	9.06±1.35	
	3	9.02	9.25	8.07	8.78±0.51	
Brand 4	1	19.40	18.50	17.40	18.43±0.82	0.109
	2	13.8	17.00	18.70	16.50±2.03	
	3	18.00	20.3	19.70	19.33±0.97	
Brand 5	1	12.90	13.60	13.30	13.27±0.29	0.003
	2	13.10	11.30	12.10	12.17±0.74	
	3	9.95	11.20	10.10	10.42±0.59	
Brand 6	1	ND	ND	ND	ND	
	2	ND	ND	ND	ND	
	3	ND	ND	ND	ND	

ND = Not detected.

The concentrations of SY in the studied orange jellies varied between 8.78 to 41.63 mg/100 g, depending on the brand in Table 2. Among the collected samples of Brand 1, SY concentration (Mean ± SD) of two samples were higher than the BSTI value as a whole, but the difference was not statistically significant ($p = 0.118$). SY values in all samples of brand 1 exceeded the recommended value of EU. When compared to the SY concentration of brand 2, all values exceeded the BSTI and EU recommended values. No samples of brand 3 exceeded the EU and BSTI values. On the other hand, all samples of brand 4 exceeded

the EU recommended value, but did not exceed the BSTI value. The SY values in brand 5 sample were within the BSTI value, but only one was within the EU value. However, brand 6 showed no trace of SY. A discrepant finding compared to the product label from brand 6 was observed. Added synthetic color can be reduced during manufacturing process due to formation of inorganic salts as byproducts e.g. NaCl (KIRSCHBAUM *et al.*, 2003). Orange jellies from brand 2 contained the highest amount (41.63 mg/100 g) of SY in Table 3, which was 2 times more than the maximum value of BSTI and 4 times from the EU value (Fig. 4). Again, when comparing among different brands, SY showed significant difference among the brands ($p = 0.003$) in Table 3. Among the statistical parameters, the mean value, standard errors, and coefficient of variance were 19.5 mg/100 g, 13.0 and 76% respectively.

Table 3. Summary of concentration of SY (mg/100 g) in selected brands of orange jellies.

Sample	Concentration of sunset yellow (mg/100 g)			Mean \pm SD (mg/100 g) (n = 9)	p-value
	Shop 1	Shop 2	Shop 3		
Brand 1	27.07	19.43	23.93	23.48 \pm 3.14	$p_B = 0.079$, $p_E < 0.001$
Brand 2	41.63	40.28	39.20	40.37 \pm 0.99	$p_B < 0.001$, $p_E < 0.001$
Brand 3	9.23	9.06	8.78	9.02 \pm 0.19	$p_B < 0.001$, $p_E = 0.018$
Brand 4	18.43	16.50	19.33	18.09 \pm 1.18	$p_B = 0.018$, $p_E < 0.001$
Brand 5	13.27	12.17	10.42	11.95 \pm 1.17	$p_B < 0.001$, $p_E = 0.003$
Brand 6	ND	ND	ND	ND	

*ND= not detected; p_B - value compared with BSTI; p_E - value compared with EU.

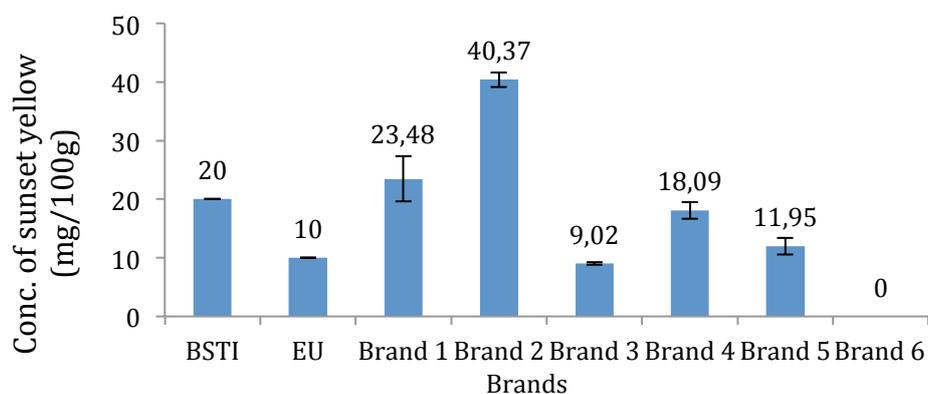


Figure 4. Comparison of SY concentration in different brands of orange jelly with BSTI and EU standard range.

ALVES *et al.* (2008) showed that the concentration of SY in one brand of mango juice powder was significantly higher compared to the maximum regulated value of 10 mg/100 g. The intakes of colors such as tartrazine, erythrocin and SY were higher in children due to the ingestion of foods containing high concentrations of colors (9.45 and 4.0 mg) (RAO and SUDERSHAN, 2008). Another study showed that the consumption of chewing gum

contains the greatest amount of tartrazine (E102), and jellies contain quinoline yellow (E104), ponceau 4R (E124) and allura red (E129) (MALCZYK *et al.*, 2015). On the other hand, the consumption of colored beverages significantly increases the adoption of SY (E110) and azorubine (E122) (MALCZYK *et al.*, 2015). The amount of tartrazine that is not secreted through urine, widely metabolized by intestinal microflora in which some metabolites are absorbed through the intestine (KHERA *et al.*, 1979; WATABE *et al.*, 1980; ELHKIM *et al.*, 2007).

BENTO *et al.* (2015) found the highest concentration containing 75.30 ± 3.85 mgL⁻¹ of INS102 colorant in one sample of milk drink among 15 samples of yogurt and milk drink. They also mentioned that INS122 was the most commonly used dye which was 33% of yogurt and milk drink samples ranging from 1.43 to 11.75 mgL⁻¹.

4. CONCLUSIONS

All but two of the samples tested in this experiment contained sunset yellow in accordance with the standard range accepted by the BSTI. Sunset yellow was absent in one brand, while the other brand substantially exceeded the amount of sunset yellow compared to the standard range accepted by the BSTI and EU. The differences in sunset yellow concentrations across the six brands of orange jellies highlight the need for improved product labeling. By providing this information to consumers and manufacturers would not only be aiding the health of consumers but they would also be assuaging the food adulteration reputation that currently taints the food product system. According to the Bangladesh Pure Food Ordinance (2005), there is prohibition of use of intoxicated food color in food. This is considered as a frightening issue which might be hazardous for public health. Therefore, further research is required to evaluate different category food products with this method to see the reproducibility of the results described here.

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