Sunset Yellow: A Food Color for Protein Staining with SDS-PAGE

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ABSTRACT

This study shows that Sunset Yellow FCF is a fast-reacting dye for food proteins and may be used as a fast staining agent for all types of resolved proteins with PAGE, which is important because Coomassie Brilliant Blue R 250 and Amido Black 10 B take at least 24 hr for visualization of proteins bands with PAGE. Sunset Yellow FCF is highly sensitive, rapid, lasting, and provides reproducible results with a variety of proteins. It produces sharp, nondiffuseable, clear, distinct, and stable bands on gel. It requires just 1 hr for staining and destaining to visualize the known proteins separated on the acrylamide gels.

Food colors are commonly used as safe additives for cosmetic purposes. However almost no information is available about their binding to macro or micronutrients, which is responsible for uniform distribution in the products. Earlier, we reported on two novel phytostaining agents for resolving proteins by PAGE, i.e., a reddish brown dye isolated from the leaves of *Lawsonia inermis* (1,2) and an orangish brown color, PAK-RAAR from the bark of the plant ratanjot, *Onosma hispidium* (3), which have been proved to be excellent, simple, economical equivalents to Coomassie Brilliant Blue in protein visualization with PAGE. This article introduces a food color, Sunset Yellow FCF, as a rapid staining agent that may be ideal for getting immediate results in electrophoresis. Sunset Yellow FCF in food colors is identified as CI Food Yellow3, FD & C Yellow No. 6 CI (1975) No. 15985, and INS No. 110. Chemically, it is a disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate of molecular weight 452.38. It is supplied as dark orange-red powder or granules soluble in water and sparingly soluble in ethanol. Sunset Yellow FCF is approved by FDA as a food color. It is nontoxic (4) and is reduced by human intestinal anaerobes (5). The dye may be purified by a chemometric method (6) and, even in its crude form, is equally effective for protein staining on acrylamide and for use after blotting (7). Apart from Coomassie Brilliant Blue, the other standard protocols of dyes include silver and gold staining (8–10) and Amido Black 10 B (11); iodine is less commonly used (12). Although the spectrophotometric determination and estimation of Sunset Yellow FCF at 485 nm is common, capillary electrophoresis has recently been used as a sophisticated technique to estimate colors in foods, including this dye in beverages (13,14). The nanomaterials in foods, such as micronutrients and synthetic colors like Sunset Yellow FCF, may be analyzed by the above separation technique. The dye-binding assays are widely used in protein analysis. Our data show that Sunset Yellow FCF is a sensitive, reliable, and competent dye for binding proteins to make them visible with PAGE.

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MATERIALS AND METHODS

Chemicals

N,N′-methylene-bis-acrylamide was purchased from Scharlau, while Tris (hydroxymethyl) aminomethane was obtained from Research Organics. Sodium dodecyl sulfate (SDS), acrylamide, ammonium peroxodisulfate (APS), glycine, Coomassie Brilliant Blue, bovine serum albumin (BSA), *N,N,N′,N′*-tetramethylenediamine (TEMED), bromophenol blue, and trypsin were supplied from Merck, while nisin was purchased from Suzhou Hengliang Co. Ltd., Suzhou, China. The 2-mercaptoethanol and HCl were supplied from Riedel-deHaen, Seeize, Germany. Sunset Yellow FCF No. 6 was a gift from coauthor Asad Sayeed (National Foods Ltd., Karachi, Pakistan). Glycerol and takadiastase from *Aspergillus oryzae* were purchased from Fluka, Steinhein, Denmark.

Preparation of Slab Gel

The following solutions were first prepared. For solution A, acrylamide (30 g) and bis-acrylamide (0.80 g) were dissolved in 100 mL of double-distilled, deionized water and filtered through Whatman No. 1 filter paper. For solution B, Tris-HCl buffer (1.5*M*) was prepared by mixing 18.02 g of Tris dissolved in 80 mL of double-distilled, deionized water, and the pH was adjusted to 8.8 by using 1*M* HCl; the volume was made up to 100 mL. For solution C, Tris (12.114 g) was added to 80 mL of double-distilled, deionized water; the pH was adjusted to 6.8 with 1*M* HCl; and the volume was made up to 100 mL with double-distilled, deionized water. For solution D, SDS (10 g) was dissolved in double-distilled, deionized water, and the volume was made up to 100 mL. For solution E, APS (1 g) was dissolved in double-distilled, deionized water, and the volume was made up to 10 mL, while TEMED was used as supplied. The 12.5% slab gel (thickness 0.75 mm) was prepared by using 2.083 mL of solution A, 0.666 mL of solution B, 0.500 mL of solution D, and 0.250 mL of solution E. The mixture was degassed, and 1.66 µL of TEMED was added before use. The gel was left for 15 min to settle.

Staking Gel

Solutions A, C, D, and E were added in the ratio of 0.416:0.830:0.333:0.166, respectively. The mixture was immediately degassed, and 1.66 µL of TEMED was added before the stacking gel was poured. The wells were prewashed with reservoir buffer after the gel had settled. For the reservoir buffer, Tris (0.9 g), glycine (3.6 g),

and SDS (1 g) were dissolved in 50 mL of distilled water, and the volume was made up to 1 L.

PREPARATION OF SOLUTIONS

Staining Solutions. For Solution A, Coomassie Brilliant Blue (0.2 g) was dissolved in 7.5 mL of glacial acetic acid and 5 mL of methanol. The volume was made up to 100 mL with double-distilled, deionized water and filtered at room temperature. Solution B was prepared by dissolving 0.2 g of Sunset Yellow FCF in 7.5 mL of glacial acetic acid and 5 mL of methanol. The volume was made up to 100 mL with double-distilled, deionized water at room temperature. Solution C, Sunset Yellow FCF (acidic), was made by adjusting staining solution B (25 mL) to pH 2.0 by using 2*N* HCl at room temperature. Solution D, Sunset Yellow FCF (basic), contained 25 mL of staining solution B adjusted to pH 10.0 by using 5.0*N* NaOH at room temperature.

Destaining Solution. The solution was prepared by mixing 10 mL of glacial acetic acid and 30 mL of methanol. The volume was made up to 100 mL with double-distilled, deionized water at room temperature.

All the solutions, except those made at room temperature, were stored at 4°C in the dark to prevent autooxidation.

PREPARATION OF SAMPLES

Peanut Proteins. The protein-solubilizing solution (PSS) (5 mL) was specially prepared by mixing 1.2 mL each of aqueous solutions of SDS (2.5%), bromophenol blue (0.0025%), and glycerol (20%), to which 1 mL of Tris-HCl buffer and 0.2 mL of 2-mercaptoethanol were added. A dehulled mass of peanuts (*Arachis hypogaea*) was ground to a fine powder of mesh size 60, and 50 mg of this sample was separately added to 1.5 mL of PSS. The mixture, after having been left overnight, was continuously shaken in a boiling water bath for 30 min. It was centrifuged at 2,000 rpm for 15 min. The clear supernatant was separated by using a pipette and used as peanut sample.

Mixture of Proteins. The takadiastase, nisin, and BSA were dissolved in double-distilled, deionized water to a final concentration of 20 mg/mL and boiled for 2 min. Twenty microliters of this sample was mixed with 20 µL of PSS. The sample was centrifuged at 2,000 rpm for 15 min, and the clear supernatant was collected as the mixed sample.

Rice Bran Proteins. The rice bran proteins were prepared in the same way as the peanut proteins by taking 50 mg of fresh defatted and dried rice bran of mesh size 60, which was dissolved in 1.5 mL of PSS.

Electrophoretic Resolution

A small quantity (50 µL) of the supernatant of each sample was resolved using the reservoir buffer for the upper and lower chambers at a constant voltage of 100 V for 4 hr.

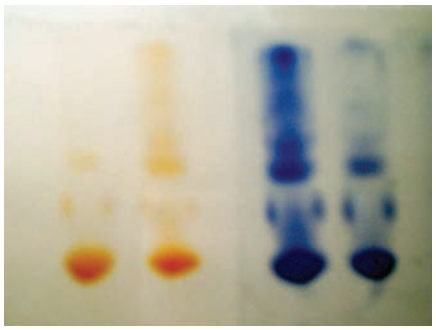

Fig. 1. Gels stained with Sunset Yellow FCF (left) and Coomassie Brilliant Blue (right) overnight and destained.

Staining and Destaining of the Gel

Procedure A. The proteins mentioned above were resolved as described earlier in duplicates on the same gel, and the gel was cut vertically into two identical halves. For Sunset Yellow, half of the gel was stained overnight in the staining solution and was washed twice with the destaining solution, with 15 min between washings, to produce clear yellowish orange bands on colorless gel. The other half of the gel was stained overnight with Coomassie Brilliant Blue and destained by washing several times with the same solution, as shown in Figure 1. The procedures of staining and destaining with the two dyes are compared in Table I.

Procedure B. The same procedure for resolution of proteins was followed, and half of the gel was stained by heating the gel in Sunset Yellow FCF solution for 15 min at 60°C. The other half was stained in Coomassie Brilliant Blue; both were destained in the same way.

Procedure C. To explore the effect of acidic or basic pH on the extent of binding of the dye with the protein bands, the three samples were resolved in the same way as described above. The gel was cut into two pieces, and the pieces were stained separately with Sunset Yellow FCF staining solutions C and D overnight and then destained.

Procedure D. To determine the impact of heating on the method of staining at acidic and basic pH, the samples were resolved in the same way. The gel was run and stained with solutions C and D; the only difference was that they were heated at 60°C for 15 min. All the gels were destained with same destaining solution.

RESULTS AND DISCUSSION

Study of Sunset Yellow FCF, used as a food cosmetic, has illustrated that it is linked to a single or multiple components of food systems like beverages, jams, or jellies. The results given in Figure 1 show that the protein-binding capacity of Sunset Yellow FCF is quite strong, as samples A, B, and C are well stained when left overnight at room temperature. The staining and destaining was much quicker than for Coomassie Brilliant Blue, suggesting that staining for protein with Sunset Yellow FCF is useful in obtaining the quick results usually desirable in research.

The interactions of Sunset Yellow FCF and proteins are rapid, but the color fades on destaining. It can be retained for few days if binding hours are prolonged (such as staining overnight). The effect of heating clearly shows that dye binding is rapid and stable, as heating is necessary to preserve the protein-stained bands for a period of six month or more. Sustainable results may be obtained only by heating the staining solution containing the gel, which shows that the electrostatic binding of the dye at the $NaSO_3$ group becomes stronger, and it is also possible that some other binding sites are created in view of the unfolding of the protein molecule due to the heat denaturation.

The resolved proteins were also stained with Sunset Yellow FCF at pH 2 and 12, and the very light color bands in both cases show that extremes in pH are not suitable for forming the linkages. The optimum pH for binding Sunset Yellow FCF to protein is 4. A temperature of 60°C makes the bands visible even at pH 2, which shows that heating is essential for durable dye binding and that the linkage site is available to protein.

Sunset Yellow FCF belongs to the azo group of dyes. However it is similar in structure to Coomassie Brilliant Blue, as both the dyes have two SO_3^{-2} groups attached to two benzene rings quite apart from each other, which attract the positive charges on the protein molecules. The structure also confirms its suitability for staining at pH 4.

CONCLUSION

The present studies indicate that using Sunset Yellow FCF No. 6 as a dye for resolved proteins with PAGE is an excellent method for quickly achieving results comparable to those from Coomassie Brilliant Blue staining. The colored bands are durable and suitable for preserving records. The intensity of the color is retained for at least three months.

Table I. Comparison of staining and destaining procedures used for Sunset Yellow FCF and Coomassie Brilliant Blue

Methodology	Sunset Yellow FCF Red No. 40	Coomassie Brilliant Blue R-250
Concentration of dye used	0.2% solution	0.2% solution
Staining time	15 min	Overnight
Destaining time	30 min	48 hr
Stability of bands	More than 6 months	Less than 2 months
Result available	Within 45 min	After 2 days

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