Detection of Fluorescent Brightening Agents in Food Matrices using UPLC with PDA/FLR Detectors

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Abstract

A method was developed and validated to detect Fluorescent Brightening Agents (FBAs) including Tinopal CBS-X and FB28 in food matrices, based on published extraction and analysis methods. FBAs are prohibited color additives also called Optical Brighteners because they are designed to make materials look whiter by absorbing light at ultraviolet (UV) wavelengths and emitting light in the visible region. Published papers in the peer-reviewed literature have reported these FBAs in foods such as shrimp and rice noodles, and are thought to be used to conceal poor product quality or spoilage by maintaining a white appearance. The analytical method uses UPLC (Ultra-Performance Liquid Chromatography) with tandem fluorescence (FLR) and photodiode array (PDA) detection in a qualitative screen, since there are no permitted levels for these prohibited color additives with uncharacterized health risks. The method performed reliably down to an estimated Method Detection Limit (MDL) of 50 ppb for Tinopal CBS-X and 260 ppb for FB28, well below the effective concentration needed for their intended effects.

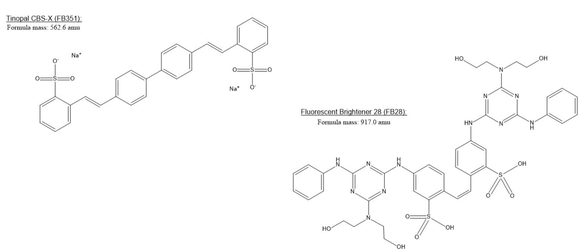

Figure 1. Laundry "Whiteners" frequently contain Optical Brightener compounds such as Tinopal CBS-X or Fluorescent Brightener 28, two members of the stilbene chemical class that have been reported as adulterants in shrimp and rice products to make them appear "whiter", and potentially disguise quality issues.

Introduction

Reports in the scientific literature describe the adulteration of foods and other products with Optical Brighteners to enhance their appearance. FBAs are legally used in products such as bleach-free laundry brighteners, but are not permitted in food matrices in the United States or many countries throughout the world. They are typically synthetic molecules designed to make textiles and other materials appear whiter and brighter, and psychologically, cleaner. There are nearly 400 Fluorescent Brightener compound designations currently listed in the International Colour Index, utilizing a variety of different chemical mechanisms. The majority of FBAs are fluorescent compounds that absorb energy (excite) at ultraviolet (UV) (non-visible) wavelengths but emit light in the visible region. This results in a brightness perceived by the human eye from non-visible illumination. It is most noticeable when an FBA-treated material is illuminated with UV ("black") light, resulting in a bright white or colored appearance compared to untreated materials.

Two commonly used FBAs are Tinopal CBS-X [FB351, CAS# 27344-41-8, 4,4'-bis(2-sulfonatostyryl)biphenyl disodium] and Fluorescent Brightener 28 [FB28, CAS# 4404-43-7, 4,4'-bis(6-anilino-[4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino)stilbene-2,2'-disulfonate disodium], as they both demonstrate good stability and are highly water-soluble (Figure 1).

Various methods have been developed to detect FBAs in consumer products such as rice papers and noodles using Liquid Chromatography (LC) separation, including Ko et al. (2014) and Liao (2016). A method to detect FBAs was developed based on these research papers, specifically looking for Tinopal CBS-X and FB28 in shrimp, rice flour, and rice noodles, three food products reported to have been adulterated with these or similar Optical Brightener compounds in some parts of the world. The method uses a simple extraction with UPLC for fast, efficient separation and tandem fluorescence and photodiode array detectors for highly sensitive and specific detection.

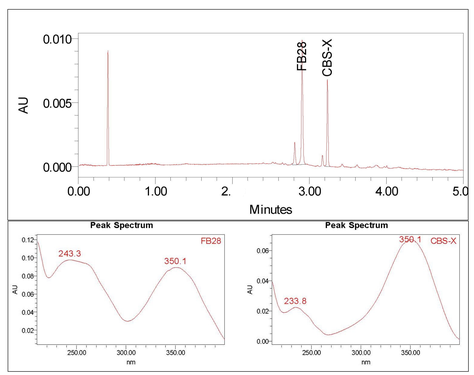

Figure 2. Representative chromatogram (top) showing primary FB28 and Tinopal CBS-X peaks. UV Spectra of FB28 (bottom left) and Tinopal CBS-X (bottom right) from the validation runs (5 ppm CBS-X, 25 ppm FB28).

Materials and Methods

One whole shrimp or approx. 3 g of rice product or other material is incubated for 30 min in 20 mL 75% methanol at 80 °C, since FBAs are highly soluble and are typically applied to the surface for effect. Tubes are vortexed and centrifuged at 6,000 rpm for 5 min at room temperature and syringe filtered with PTFE prior to UPLC injection.

- Waters Acquity H-Class UPLC running Empower 3
- Photodiode Array (PDA) Detector (210-400 nm range)
- Fluorescence (FLR) Detector (350 nm Excitation, 430 nm Emission)
- Waters Acquity BEH Shield RP18 ULPC column (2.1 x 50 mm, 1.7 μm)
- Column Temp = 40 °C, Sample Temp = 25 °C
- Mobile Phases: (A) 10 mM amm. acetate in water; (B) Acetonitrile
- Gradient: (t=0 min) 90% A; 10% B; (t=8 min) 100% B w/4 min hold; (t=13 min) 90% A w/4 min hold.
- Flow rate 0.4 mL/min with overall run time of 17 min.
- Routine spikes were made at ~5 ppm. (Tinopal CBS-X is highly effective at ~1-10 ppm whereas FB28 is ~5x less fluorescent).

Results and Discussion

The FBAs tested (Tinopal CBS-X and FB28) had primary chromatographic peaks that were well-resolved and gave unique UV spectra, corresponding well with published data. Analyte detection proved to be significantly (≥10-fold) more sensitive using the fluorescence detector compared to the PDA, but the PDA spectra were useful for confirming identity. The instrument level of detection (i-LOD) was estimated to be 1 ppb for CBS-X and 5 ppb for FB28 for the FLR, but 50 ppb for CBS-X and 260 ppb for FB28 using the PDA. MDL estimates were determined as the upper i-LOD values of 50 ppb CBS-X / 260 ppb FB28. Even though the screen is meant to be performed qualitatively (since there is no permitted level for FBAs), examination of the LOQ in shrimp estimated it to be approx. 0.5 ppm CBS-X and 2.5 ppm FB28. When evaluated as a qualitative method, the false positive/negative rates were both estimated at <5%.

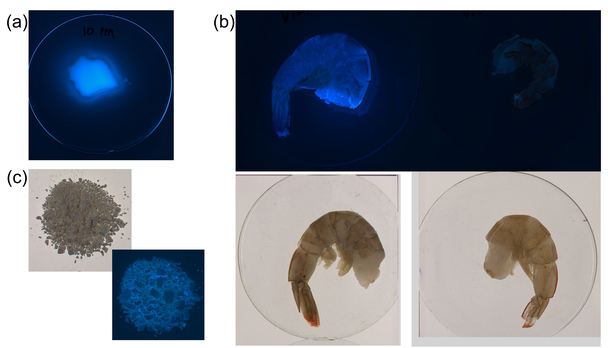

Figure 3. (a) 10 ppm Tinopal CBS-X illuminated with 366 nm UV light. (b) Shrimp soaked in 10 ppm CBS-X (left) vs control (right) at 366 nm. (c) Rice flour illuminated at 366 nm showing natural fluorescence.

Conclusion

An analytical method to detect FBAs/Optical Brighteners in food products was developed based on published methodology. These compounds can cause a low quality product to have an artificially brighter appearance, which can be an aspect of Economically-Motivated Adulteration (EMA). A Single Laboratory Validation was performed for three food matrices (shrimp, rice flour and noodles) where these FBA compounds have been reported as adulterants in the past. The method was found to perform adequately and with high selectivity as a qualitative screen, with potential to be used as a quantitative method if warranted.

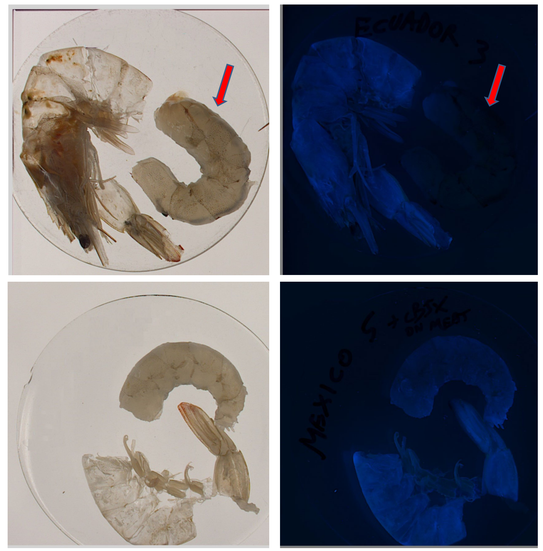

Figure 4. White (top left) and 366 nm (top right) illumination showing natural fluorescence of carapace but not flesh (arrows). Shrimp soaked in 10 ppm Tinopal CBS-X (bottom) have flesh that also fluoresces.

References

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