Method of Test for Fluorescent Whitening Agents in Foods -Test of Diaminostilbene and Its Derivatives

1. Scope

This method is applicable to the analysis of diaminostilbene and its derivatives in foods.

2. Method

- 2.1. Direct observation method
 - 2.1.1. Equipment

UV light box: with UV lamp of wavelength 365 nm.

2.1.2. Identification

Place the sample in the dark and observe under a UV lamp with wavelength of 365 nm. Conduct the dyeing method if the surface of the sample exhibits noticeable blue or purple colored fluorescent response.

- 2.2. Dyeing method
 - 2.2.1. Equipment

UV light box: as section 2.1.1.

2.2.2. Chemicals

Ammonia water (28%), reagent grade.

Hydrochloric acid, reagent grade.

Nitric acid, reagent grade.

- 2.2.3. Apparatus
 - 2.2.3.1. Beaker: 200 mL.
 - **2.2.3.2.** Filter paper: without fluorescent substances.
 - **2.2.3.3.** Gauze or cotton: without fluorescent substances.

2.2.4. Reagent

2.2.4.1. 0.1% ammonia water

Dilute 0.36 mL of ammonia water with deionized water to 100 mL.

2.2.4.2. Diluted ammonia solution

Dilute 1.5 mL of 0.1% ammonia water with deionized water to 2000 mL, adjust the pH to 7.5-9.0, prepare fresh before use.

- 2.2.4.3. Diluted hydrochloric acid Add 24 mL of hydrochloric acid into 60 mL deionized water slowly, and dilute with deionized water to 100 mL.
- 2.2.4.4. Diluted nitric acid

Dilute 5 mL of nitric acid with deionized water to 100 mL.

2.2.5. Sample solution preparation

Transfer about 10-20 g of the fine-cut sample into a beaker. Add 100 mL of diluted ammonia solution, stir occasionally, allow to stand for 30 min at room temperature. Filter with a filter paper and take the filtrate as the sample solution.

2.2.6. Identification

Transfer 50 mL of the sample solution into a beaker, add 1-2 drops of diluted hydrochloric acid to adjust the pH to 3-5. Put the gauze and heat in a boiling water bath for 30 min. Take out the gauze, wash with deionized water, squeeze out the liquid, and then dip it in diluted nitric acid. Heat in a boiling water bath for 5 min, take it out, wash with deionized water, squeeze out the liquid. Then put it in the dark and observe under a UV lamp with a wavelength of 365 nm. If the gauze exhibits noticeable blue or purple colored fluorescent response, the fluorescent whitening agent is identified. Separately, perform a blank test with 50 mL of diluted ammonia solution in the same manner.

Remark

There are many substances that produce fluorescence in nature, such as plant chlorophyll. Therefore, when local or weak fluorescence is found, the origin of the fluorescent substance must be further clarified. Taking mung bean sprouts as an example, the vessels in the root tissue and the upper stems near the cotyledon end may have natural fluorescence, and can be absorbed by the gauze of the dyeing method in section 2.2., and fluorescence can be observed. When the sample is observed under a UV lamp in Section 2.1, the distribution of the fluorescence should also be examined to confirm the presence of fluorescent whitening agent in the sample. When there is only a small part (especially the root tip) shows fluorescence, not the whole plant, it may be natural fluorescence. It should be combined with results of the on-site audit to identify the result.

Reference

The Pharmaceutical Society of Japan. 2015. Methods of Analysis in Health Science, p. 674. Kanehara & Co.Ltd, Tokyo, Japan.