Japan Customs Analysis Methods

No. 111

Determination of Piperine in Pork Preparations

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1. Scope

This method is applied for determining piperine in raw pork seasoned with pepper.

2. Outline of Test Method

This quantitative method is applied for determining piperine in raw pork seasoned with pepper, and the method shall be carried out according to the following procedure.

(1) Extraction of piperine, together with fat, from raw meat.
(2) Fractionation of piperine by removing fat with a silica gel column.
(3) Determination of piperine by ultraviolet absorbance method.

3. Reagents

All chemicals must be JIS special reagent grade or equivalent, unless otherwise specified.

4. Apparatus and Preparation of silica gel column

(1) Ultraviolet and visible spectrophotometer

Use one which is capable of measuring absorbance at a wavelength of 343 nm.

(2) Preparation of silica gel column

Prepare a slurry of 5 g of silica gel (100 – 200 mesh, for chromatographic use) in toluene and gently pour it into a 15 mm i.d. chromatographic column according to general slurry method (the length of the silica column [packing length] shall be in the rage of 5 – 10 cm).

4. Preparation of sample

Analytical sample should be prepared in a proper manner suitable for its original form. For instance, large lumps should be chopped into about 1 cm square pieces and mixed well. Then about 200 g of the chopped pieces are further minced for use as an analytical sample.

5. Procedure

5.1. Extraction of Piperine

Accurately weigh approximately 10 g of an analytical sample into a 200-mL brown stoppered-flask (or stoppered-flask protected from light by being covered with aluminum foil; hereafter the same shall apply). Add 40 mL of chloroform into the flask and plug. Shake the flask using a shaker for 30 min, and filter the solution quickly in a dark place through a filter paper (JIS P 3801, No. 6), by decantation.

Then, add 40 mL of chloroform to the residue in the flask and shake it vigorously by hand. Filter the solution quickly in a dark place through the filter paper previously used, by decantation. Repeat this operation three times in total.

Collect all the filtrates in a brown extraction flask and concentrate it under reduced pressure using a rotary evaporator connected with an aspirator, while heating the flask in a warm water bath at the temperature of about 45°C, until the volume decreases to about 30 mL. Transfer the concentrated liquid to a 50-mL brown volumetric flask and dilute to volume with toluene. Use the solution as piperine extraction solution.

5.2. Determination

5.2.1. Ultraviolet absorbance method

Put 10 mL of the piperine extraction solution into a brown silica gel column using a whole pipet and remove fat by leaching with 140 mL of toluene (at a flow rate of 2 mL/min). Using a 50 mL brown volumetric flask as a receiver, elute piperine from the column with 45 mL of methanol (at a flow rate of 2 mL/min) and dilute to volume with methanol. Use it as piperine solution for UV absorbance measurement.

5.2.2. Measurement Procedure

Measure the absorbance of the piperine solution obtained in 5.2.1. in a 10 mm-cuvette at 343 nm, using an effluent from a blank test[1)] as reference solution.[2)]

Note 1) After flowing 140 mL of toluene through a silica gel column for blank test (at a flow rate of 2 mL/min), followed by disposing the effluent, put 45 mL of methanol onto the column and flow in the same manner. Collect the effluent in a 50 mL brown volumetric flask and dilute to volume with methanol. Use the solution as blank test effluent.

Note 2) After obtaining the sample and blank effluents according to 5.2.1., measure the absorbance immediately.

5.2.3. Calculation of piperine content

Calculate the content of piperine from the following formula:

$$\%, \text{piperine in sample} = \frac{E \times 250}{S \times 126878} \times 100$$

Where–

E: Absorbance
250: Dilution ratio
S: Amount of sample collected (g)
126878: Extinction coefficient

Round off fractions to the third decimal place.

6. References

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