Method 12.1

Piperine Content of Black and White Pepper, Their Oleoresins and Soluble Pepper Seasonings

Purpose: To determine the percent piperine content of pepper by spectrophotometric procedure. Piperine is extracted into denatured alcohol and absorbance is measured at the maximum absorbance between 342nm and 345 nm.

A. Apparatus:

1. Spectrophotometer, double beam, UV (deuterium) light source, capable of accurately measuring absorbance at 342-345 nm.

2. Cuvettes, 1 cm. square, silica.

3. Amber Erlenmeyer flask, 125 mL capacity with T.S. 24/40 ground joint.

4. Condenser, West type, with water cooled drip tip, 400 mm., T.S. 24/40 ground joint.

5. Amber volumetric flasks, 100 mL with T.S. stoppers.

6. Pipettes, volumetric type 1, 2, 3, 4, 5, and 10 mL.

7. Funnel, 65 to 75 mm. I.D. at top.

8. Filter paper, fluted, Whatman No. 2 or equivalent.

B. Reagents:

1. SDA No. 3A - Denatured Alcohol (Note 1).

2. Piperine, Sigma-Aldrich, (800)325-3010, Cat #P-4900-7 or equivalent,
   OR
   Piperine, Analytical Standard, Sigma-Aldrich, (800)325-3010, Cat # 75047, (preferred, characterized by hplc). See Note 4.

C. Preparation of Sample:

1. Method 1.0 modified to 60 mesh sieve.
2. If the sample has a larger granulation than a 60 mesh, it will require grinding. Grind subsample to pass through an U.S. Standard No. 60 sieve. It is important to grind to pass at least 99% of sample due to the wide variation in composition within a spice. Since there can be a loss of piperine with excessive heat, below are different techniques used by different laboratories to minimize loss.
   - Freeze sample before grinding with a coffee grinder.

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- Freeze spice bowls prior to grinding samples (ie. Waring ESG30 or a water cooled FOSS KN295).
- Grind sample immediately before use, avoiding generating heat.

**It has been demonstrated that not grinding to a 60 sieve and or high heat can yield lower piperine concentrations.

D. Procedure:

1. a. Weigh accurately 0.1000 gram of piperine standard into a 100 mL volumetric flask with ca.70 mL SDA No. 3A. Shake to dissolve. Make up to volume. Mix well.
   b. Pipette 10 mL aliquot into 100 mL volumetric flask and make up to volume with SDA No. 3A. Shake well.
   c. Pipette 1, 2, 3, 4, 5, and 6 mL aliquots from solution in b. and transfer into 100mL volumetric flasks and make up to volume with SDA No. 3A. These solutions represent concentrations of 1, 2, 3, 4, 5, and 6 µg/mL in the standard solutions.
   d. Zero spectrophotometer with SDA No. 3A in both cells.
   e. Determine the absorbance readings A1, A2, A3, A4, A5, A6 of corresponding solutions in item c, at absorbance maxima between 342-345 nm.
   f. Calculate average absorbance of standards A1 – A6, normalized to 1 µg/mL for each standard. See calculation E.1.

2. Procedure for Black/White Pepper:
   a. Weigh accurately 0.5000 gram of sample.
   b. Place sample in a 125 mL Erlenmeyer flask and add ca. 70 mL SDA #3A.
   c. Reflux one hour, cool to room temperature and filter quantitatively into 200 mL volumetric flask. Transfer the extracted residue to the filter. Wash thoroughly with SDA No. 3A and dilute to mark with SDA No. 3A.
   d. Pipette 4 mL of this solution into a 100 mL volumetric flask and make up to volume with SDA No. 3A. Shake well. Using SDA No. 3A as the reference solution, record absorbance reading of this solution at maxima 342-345 nm. within 15 minutes. See calculation E.2.

3. Procedure for Oleoresins:
   a. Weigh 1.000 gram of well mixed sample and transfer into 100 mL volumetric flask. Make up to volume with SDA No. 3A. Shake well until dissolved.
   b. Pipette 10 mL of solution in a. into 100 mL volumetric flask and fill to mark with SDA No. 3A. Shake well.
   c. Pipette 1 mL of solution in b. into 100 mL volumetric flask and fill to mark with SDA No. 3A. Shake well.

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d. Using SDA No. 3A as the reference solution, record absorbance of solution in c. at maxima 342-345 nm. within 15 minutes. See calculation E.2.

4. Procedure for Soluble Pepper:
   a. Weigh accurately 2.000 grams sample of soluble pepper and transfer to 100 mL volumetric flask. Add ca. 70 mL of SDA No. 3A and swirl occasionally for 20 minutes. Fill to mark with SDA No. 3A and allow to settle.
   b. Pipette 1 mL of solution in a. into 100 mL volumetric flask and fill to mark with SDA No. 3A. Shake well.
   c. Using SDA No. 3A as the reference solution record absorbance of solution in b at maxima 342-345 nm. within 15 minutes. See calculation E.2.

E. Calculation:

1.

$$A_{avg} = \left(\frac{A_1}{1} + \frac{A_2}{2} + \frac{A_3}{3} + \frac{A_4}{4} + \frac{A_5}{5} + \frac{A_6}{6}\right) \div 6$$

2.

$$\%\ \text{Piperine} = \frac{A_s}{A_{avg}} \times \frac{V}{W_s \times 10^6} \times 100$$

Where:

A_s = absorbance of sample
A_{avg} = Average of standard absorbances, each normalized to 1µg/mL
V = dilution volume, milliliters
W_s = sample weight, grams

F. Statistics:

Coefficient of Variation: Black Pepper 8%

G. Notes:

1. SDA no. 3A: 5 gallons of methyl alcohol plus 100 gallons 95% ethyl alcohol.
2. Method includes absorbance of piperine isomers and related compounds, if present, including piperettine and piperylin, which have absorbance at the wavelength used but are not as spicy as piperine. Use of amber glassware reduces formation of these compounds during analysis and prevents consequent overstatement of pungency.
3. The method does not call for a piperine standard to be used on every occasion of

analysis. Stability of response is therefore critical. A recalibration with fresh standards must be performed if any action is taken which may change instrument response. This includes preventative maintenance, replacement of the lamp, adjustment of wavelength calibration, and any other actions likely to affect measured absorbance.

4. The original standard did not have a COA, only a specification. A better documented standard is added as "preferred". Earlier standard left as permissible, for cost and availability reasons.

H. References:

1. The original standard did not have a COA, only a specification. A better documented standard is added as "preferred". Earlier standard left as permissible, for cost and availability reasons.

2. AOAC Official Methods of Analysis (1995) 43.1.17 (987.07)-replaced Ethylene Dichloride with SDA.

3. Direct link to AOAC OMA online (Membership or other access required): http://www.eoma.aoac.org/gateway/readFile.asp?id=987_07.pdf

I. Revision History

1. 04/14/2018. Corrected error in formula for piperine content of sample in original version– A left hand (open) bracket was missing.

2. 04/14/2018. Added notation for use of reciprocal of normalized absorbance instead of the "factor".

3. 09/29/2021. Added additional information for sample preparation and grinding.