BENZOE TONKINENSIS

Prepared at the 79th JECFA (2014), published in FAO JECFA Monographs 16 (2014) replacing the tentative specifications prepared at the 74th JECFA (2011), published in FAO JECFA Monographs 11 (2011). No safety concern at current estimated dietary exposures.

SYNONYMS Siam Benzoin gum; Siam Benzoin Laos; Styrax tonkinensis

DEFINITION Benzoe tonkinensis is a natural complex balsamic resin obtained from a native tree in Lao PDR: *Styrax tonkinensis* Pierre. It is collected directly from the tree, cleaned and sorted into four grades according to size. All grades have similar chemical composition. The resin is composed mainly of benzoic acid and coniferyl benzoate. The resin also contains minor amounts of, vanilllin and benzyl benzoate. Other compounds namely *p*-coumaryl benzoate, siaresinolic acid 3-oxo-siaresinolic acid, sumaresinolic acid and 3-oxo-sumaresinolic acid are also identified.

These specifications do not cover Sumatra benzoin (resin obtained from *Styrax benzoi*n Dryander and *Styrax paralleloneurum*), which contains cinnamic acid.

C.A.S. number 9000-72-0

DESCRIPTION White-yellow to reddish splits of flattened almond-like grains with a strong vanilla smell.

FUNCTIONAL USES Flavouring agent

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Insoluble in water; soluble in ethanol

Benzoic acid, vanillin and benzyl benzoate The sample contains benzoic acid, vanillin and benzyl benzoate identified by their characteristic peaks in the gas-chromatogram.
See description under TESTS

Coniferyl benzoate The sample contains coniferyl benzoate identified by its characteristic peak in the HPLC-chromatogram.
See description under TESTS

Cinnamic acid Not present
See description under TESTS

PURITY

Loss on drying (Vol. 4) Not more than 5.0% (105°, 4 h). Test 2 g of sample.

Total ash	Not more than 2.0% Evenly distribute 1.0 g of the powdered sample in a crucible. Dry at 100-105° for 1 h and ignite to constant mass in a muffle furnace at 600 ± 25°.
Alcohol-insoluble matter	Not more than 5%. To 2.0 g of the powdered sample add 25 ml of ethanol 90% v/v. Boil until almost completely dissolved. Filter through a previously tared sintered-glass filter and wash with three 5 ml portions of boiling ethanol 90% v/v. Dry the residue at 100-105° for 2 h and weigh after cooling.
Acid value (Vol. 4)	Between 160 and 206
Benzoic acid	Between 15 and 45% See description under TESTS
Coniferyl benzoate	Between 15 and 60% See description under TESTS
Lead (Vol. 4)	Not more than 2 mg/kg Triturate the sample in a centrifugal grinder to a particle size < 200 μm. Determine using an AAS (Electrothermal atomization technique) appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4 (under “General Methods, Metallic Impurities”).
Microbiological criteria (Vol. 4)	*Salmonella spp.:* absent in 25 g *Escherichia coli:* absent in 1 g Yeast and moulds: less than 20 CFU/g

TESTS

IDENTIFICATION AND PURITY

Benzoic acid, vanillin, benzyl benzoate and cinnamic acid

Benzoic acid, vanillin and benzyl benzoate are identified and benzoic acid is quantified by gas chromatography. The absence of cinnamic acid is confirmed.

Reagents

Ethanol 96%
Reference standard of benzoic acid (purity >99%)
Reference standard of vanillin (purity > 99%)
Reference standard of benzyl benzoate (purity > 99%)
Reference standard of cinnamic acid (purity > 99%)

Gas Chromatographic system

Detector: Flame ionization detector
Column: HP-1 (30 m x 0.2 mm I.D., 0.33 μm-film) or equivalent
Carrier gas: helium
Flow rate: 1 ml/min
Temperatures:
-Injector: 250°
-Oven: 5 min at 80°; to 300° at 8°/min; then hold for 40 min
-Detector: 300°
Injection mode: split 1/10
Injection volume: 1 μL

Benzoic acid elutes at 11.7 min. Peaks at 16.1 min, 14.8 min and 20.4 min corresponds to cinnamic acid, vanillin and benzyl benzoate, respectively.

Benzoic acid standard stock solution (20 mg/ml): Weigh accurately 0.4 g (±0.1 mg) of benzoic acid reference standard, transfer to a 20 ml volumetric flask and bring to volume with ethanol.

Benzoic acid standard solutions: Prepare five solutions from the standard stock solutions within the concentration range of 0.25 to 20 mg/ml.

Cinnamic acid, vanillin and benzyl benzoate solution: Prepare a 10 mg/ml solution containing cinnamic acid, vanillin and benzyl benzoate in ethanol.

Sample solution
Accurately weigh about 5.0 g (±0.1 mg) (w_s) of the previously crushed Benzoe tonkinensis and solubilize in 20 ml of ethanol (96%). Sonicate the mixture and filter. Re-extract the residue with a second portion of 20 ml ethanol (96%), sonicate and filter. Combine the ethanol extracts and evaporate the solvent under vacuum. Weigh accurately the extracted resin (w_{ex}). Dissolve the resin with ethanol to a final concentration of 30 mg/ml.

Procedure
Inject 1 µl of each benzoic acid standard solutions and record the peak areas. Inject 1 µl of the 10 mg/ml solution containing cinnamic acid, vanillin and benzyl benzoate.
Plot a standard curve (concentration of benzoic acid (mg/ml) (X-axis) vs. peak area of benzoic acid (Y-axis)) and determine the slope (m) and the linear coefficient (a).
Inject 1 µl of the sample solution and record the peak area.
For identification of cinnamic acid, vanillin and benzyl benzoate, compare the retention times of the corresponding peaks of cinnamic acid, vanillin and benzyl benzoate in the chromatograms obtained with the standard solution and sample solution.

Calculation
Calculate the content of benzoic acid as follows:

$$\text{Benzoic acid (w/w, \%)} = \left(\frac{A_{BzA} - a}{m}\right) \times \frac{1}{30} \times \frac{w_s}{w_{ex}} \times 100$$

where
A_{BzA} is the peak area of benzoic acid in the sample;
a is the linear coefficient of the standard curve;
m is the slope of the standard curve;
w_{ex} is the weight of resin extracted with ethanol (g); and
w_s is the weight of sample Benzoe tonkinensis (g).

Coniferyl benzoate

Coniferyl benzoate is identified and quantified by high performance liquid chromatography.

Reagents
Acetonitrile, HPLC grade
Formic acid
Reference standard of coniferyl benzoate (>95%)

Chromatographic system
HPLC system with a diode array detector (DAD), auto sampler or injector.
Detector wavelength for quantitation: 300 nm
Column: Luna C18 Phenomenex (150 mm x 4.6 mm, 5 μm) or equivalent.
Mobile phase: solvent A: water added of 0.1% formic acid and solvent B: acetonitrile added of 0.1% formic acid
Gradient elution: A:B 65:35 v/v (0 to 5 min) to A:B 0:100 v/v (5 to 25 min)
Column temperature: 25°
Flow rate: 1 ml/min
Injection volume: 10 μl

Coniferyl benzoate elutes at 14.9 min.

Standard stock solution (coniferyl benzoate 8 mg/ml): Weigh accurately 0.04 g (±0.1 mg) of coniferyl benzoate reference standard and transfer to a 5 ml volumetric flask and bring to volume with ethanol.

Standard solutions: Prepare five solutions, by the dilution of the standard stock solution of coniferyl benzoate with ethanol, in the concentration range of 0.05 to 0.8 mg/ml.

Sample preparation
Accurately weigh about 5 g (±0.1 mg) (w_s) of the previously crushed Benzoe tonkinensis sample and solubilize in 20 ml of ethanol (96%). Sonicate the mixture and filter. Re-extract the residue with a second portion of 20 ml ethanol (96%), sonicate and filter. Combine the ethanol extracts and evaporate the solvent under vacuum. Weigh accurately the extracted resin (w_{ex}). Dilute the resin with ethanol to a final concentration (C_{resin}) of 1 mg/ml.

Procedure
Inject 10 µl of each standard solution and record the peak areas. Plot a standard curve (concentration of coniferyl benzoate (mg/ml) (X-axis) vs. peak area of coniferyl benzoate (Y-axis)) and determine the slope (m) and the linear coefficient (a).
Inject 10 µl of the sample and record the peak area.

Calculation
Calculate the content of coniferyl benzoate as follows:

$$\text{Coniferyl benzoate } (w/w\,,\,\%) = \left(\frac{A_{ConBz} - a}{m}\right) \times \frac{1}{C_{resin}} \times \frac{w_s}{w_{ex}} \times 100$$

where
A_{ConBz} is the peak area of coniferyl benzoate in the sample;
a is the linear coefficient of the standard curve;
m is the slope of the standard curve;
C_{resin} is the final concentration of the extracted resin diluted in ethanol;
w_{ex} is the weight of extracted resin with ethanol (g); and
w_s is the weight of sample of Benzoe tonkinensis (g).