SMOKE FLAVOURINGS

Prepared at the 57th JECFA (2001) and published in FNP 52 Add. 9 (2001), superseding tentative specifications prepared at the 55th JECFA (2000) and published in FNP 52 Add. 8 (2000). An ADI of "Provisional acceptance" was established at the 31st JECFA (1987).

SYNONYMS Wood smoke flavour, Smoke condensate

DEFINITION Complex mixtures of components of smoke obtained by subjecting untreated hardwoods to (a) pyrolysis in a limited and controlled amount of air, (b) dry distillation between 200° and 800°, or (c) superheated steam between 300° and 500°. Source materials must not contain detectable amounts of pesticides, wood preservatives, or other extraneous matter that may result in hazardous constituents in the wood smoke. The major flavouring principles of Smoke Flavourings are carboxylic acids, compounds with carbonyl groups and phenolic compounds.

During manufacture, wood smoke is subjected to an aqueous extraction system or to distillation, condensation, and separation for collection of the aqueous phase, which serves to remove hazardous constituents, such as polycyclic aromatic hydrocarbons. The aqueous smoke fraction containing water-soluble constituents may be diluted with water or be extracted with an edible vegetable oil to produce a smoke flavouring with higher levels of non-polar constituents that may be further extracted using food-grade substances, such as propylene glycol or aqueous solutions of polysorbates. Commercial products may also contain additives such as emulsifiers, antifoaming agents, and gums. Smoke flavourings may also be prepared in dry form with the addition of carriers, such as yeasts, flours, salt, phosphates, carbohydrates, and anti-caking agents.

These specifications apply only to the water-soluble distillates of condensed wood smoke, their aqueous/vegetable oil or polysorbate extracts and concentrates of these. These specifications do not apply to products derived from the water-insoluble tars, to commercial products(as described in the previous paragraph), nor to pyroligneous acid, a by-product of the manufacture of charcoal by carbonation of wood in the absence of air.

Assay
Acidity: 2 to 20% (as acetic acid)
Carbonyls: 2 to 25% (as heptaldehyde)
Phenols: 0.1 to 16% (as 2,6-dimethoxyphenol)

DESCRIPTION Light brown to dark amber liquids; smoky odour

FUNCTIONAL USES Flavouring agent, colour

CHARACTERISTICS

IDENTIFICATION

Appearance and odour Characteristic

PURITY

Benzo(a)pyrene — Not more than 2 µg/kg
See description under TESTS

Lead (Vol. 4) — Not more than 2 mg/kg
Determine using an atomic absorption 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, “Instrumental Methods.”

TESTS

PURITY TESTS

Benzo(a)pyrene

Caution:
Benzo(a)pyrene and other polycyclic aromatic hydrocarbons are known carcinogens. The analyst must exercise appropriate care in handling them.

Principle:
Benzo(a)pyrene (BaP) is one of several polycyclic aromatic hydrocarbons (PAHs) that may occur in liquid smoke flavourings. Its quantitation is used as an indicator for levels of PAHs in liquid smoke flavourings. BaP, as well as other PAHs are isolated from the sample by digestion and liquid extraction, and purified by solid phase extraction. Quantitation is accomplished by high performance liquid chromatography with fluorescence detection (Volume 4).

Apparatus:
Liquid chromatograph with fluorescence detector and reverse-phase column, such as Vydac 201TP54, 4.6 x 250 mm, thermostated at 30E
Digestion apparatus consisting of 250 ml round-bottom flask, 250 ml heating mantle, reflux condenser, and variable transformer
Rotary evaporator, equipped with water bath set at 40E
Pear-shaped flasks, 50 ml and 100 ml
30 ml Buchner funnels with coarse-porosity fritted disks
Silica solid phase extraction (SPE) column, 6 ml capacity, 1000 mg packing, such as Bakerbond
C18 SPE column, 6 ml capacity, 1000 mg packing, such as Bakerbond
Stopcocks with Luer fitting (for SPE columns)
PTFE syringe filter units, 13 mm diam., 0.45 micrometer porosity

Reagents:
UV- or HPLC-grade hexane, methylene chloride, acetonitrile, and water
Anhydrous ethanol, distilled before use
Sodium sulfate, anhydrous, granular

Solutions:
0.8 g/ml aqueous potassium hydroxide
Commercial PAH Standard Solution in acetonitrile, such as NIST Standard Reference Material 1647 (National Institute of Standards and Technology, Gaithersburg, MD 20899, USA)

PAH working standards: prepare at least 4 working standards in 50% acetonitrile/50% water (in the range of 0.5 to 10 µg/l) from the commercial PAH Standard Solution.

Procedure:
Caution: Due to the toxicity of solvents used in this method, the use of gloves is recommended.

Sample preparation:
Weigh 10 g of liquid smoke flavouring into a 250 ml round-bottom flask. Add 20 ml ethanol, 2 ml 0.8 g/ml aqueous KOH solution, and a few boiling chips. Assemble the digestion apparatus, set the variable transformer to 50% output, and reflux the sample for 2 hr. Let cool about 15 min. Transfer the digest to a 250 ml separatory funnel; rinse the flask with 20 ml water, 10 ml ethanol, and 15 ml hexane and add to the separatory funnel; extract by shaking gently for 2 min. Drain the lower layer into a second separatory funnel and repeat the extraction with 15 ml hexane. Discard the lower layer. To each retained hexane layer add about 3 ml water and swirl. Discard the water washes. Pass each hexane layer through a Buchner funnel containing 25 g sodium sulfate (pre-washed with 15 ml hexane) into a 100 ml pear-shaped flask. Rinse the sodium sulfate with 15 ml hexane; apply gentle pressure to the top of the funnel with heel of hand to force residual solvent through the sodium sulfate. Concentrate to about 1 ml on a rotary evaporator. Attach a stopcock to the outlet of the silica SPE column and condition the column with 15 ml methylene chloride, followed by 3 ml hexane; close the stopcock. (If there is a delay between column conditioning and charging the column with the sample, close the stopcock, leaving about 1 mm hexane above the head of the column. Just before loading the sample extract onto the column, open the stopcock to drain the excess solvent; then, close the stopcock.) Transfer the concentrated sample extract from the pear-shaped flask to the column, using two 1 ml portions of hexane to rinse. Open the stopcock and allow the sample to pass onto the column using gravity flow only, discarding the eluate. Elute the sample (by gravity) with 5 ml of 25% methylene chloride/75% hexane, discarding the first milliliter; collect the eluate in a 50 ml pear-shaped flask. Use pressure from a syringe to force residual solvent through the column. Add 4 ml acetonitrile and concentrate the sample extract to < 1 ml on a rotary evaporator.

Attach a stopcock to the outlet of the C_{18} column and condition it with 15 ml of methylene chloride, followed by 5 ml acetonitrile. Close the stopcock. Transfer the concentrated sample extract to the column using two 0.5-ml portions of acetonitrile to rinse the flask. Open the stopcock to charge the column with the sample; discard the eluate. Elute the sample with 5 ml of 10% methylene chloride/90% acetonitrile, collecting the eluate in a 50 ml pear-shaped flask. Use a syringe to force residual solvent through the column. Concentrate the eluate to < 1 ml on a rotary evaporator. Transfer to a 5 ml volumetric flask, using three 0.5 ml portions of acetonitrile to rinse. Add 2.5 ml water, and dilute to 5.0 ml with acetonitrile. Filter through a PTFE syringe filter.

High Performance Liquid Chromatography:
Analyse the filtered sample extract and the PAH working standards for BaP.

Chromatographic conditions:
Solvent flow rate: 1.0 ml/min
Solvent program: 3 min hold at 50% acetonitrile/50% water; linear gradient to 100% acetonitrile in 15 min; and 8 min hold at 100% acetonitrile
fluorescence detector: excitation wavelength - 290 nm; emission wavelength - 410 nm
Injection volume: 100 µl
The retention time of BaP is approximately 24 min. Using the peak heights for BaP in the standards, prepare a calibration curve of peak height vs. concentration (µg/l), and determine the slope (S) and the intercept (I).

Calculate the concentration of BaP:

BaP (µg/kg) = V(H-I)/(S x W)

where
H = the peak height of BaP in the sample extract
W = the weight of the sample (10 g)
V = the volume of the filtered sample extract (5.0 ml).

METHOD OF ASSAY

Acidity

Accurately weigh about 1 ml of sample in a 250 ml beaker. Dilute with about 100 ml of water. Titrate with 0.1 N sodium hydroxide solution to an equivalence point of pH 8.15, as determined using a pH meter. Calculate acidity as percent by weight of acetic acid using the factor: 1 ml of 0.1 N sodium hydroxide is equivalent to 60.05 mg acetic acid.

Carbonyls

Principle:
The total carbonyls of Smoke Flavourings are determined spectrophotometrically by reaction with 2,4-dinitrophenylhydrazine (2,4-DNPH) and expressed as heptaldehyde. The carbonyls of liquid smoke are converted to hydrazone derivatives by reaction with 2,4-DNPH in acid medium at 60^{o}. A red pigment is formed on adjusting to alkaline pH and the absorbance is read at 430 nm.

Apparatus:
Spectrophotometer with 1 cm cuvettes, water bath at 60±5^{o}, 25 ml volumetric flasks, miscellaneous glassware.

Reagents:
Toluene
Ethanol (anhydrous): Render carbonyl-free by adding 1% 2,4-DNPH, a few drops of concentrated HCl, refluxing 3 h and distilling.
2,4-DNHP: Recrystallize from carbonyl-free methanol.
Potassium hydroxide pellets (KOH)
Heptaldehyde
Trichloroacetic Acid (TCA)

Solutions:
Saturated 2,4 -DNPH: 0.05% in toluene. Shake for 1 h or prepare 1 day in advance and allow to settle overnight. Insoluble crystals of 2,4-DNPH

should be present. Filter the upper layer prior to use. Prepare fresh weekly.
KOH solution: 4% w/v in carbonyl-free ethanol. Prepare fresh daily.
TCA solution: 4% w/v in toluene. Stable at room temperature.
0.1% Smoke Flavouring, prepared as follows: 1 ml or 1 gram sample dissolved or diluted to 50 ml with carbonyl-free alcohol of which 5 ml is diluted to 100 ml with a 10% carbonyl-free ethanol - 90% toluene solution.
Heptaldehyde standard solution: 30 µg/ml in toluene.

Procedure:
Place 1 ml of diluted sample (Solution 4) in a 15 ml volumetric flask and 1 ml toluene in another flask to serve as the blank.
Add to each volumetric flask:
1 ml toluene
2 ml saturated 2,4-DNPH solution (Solution 1)
2 ml TCA solution (Solution 3)
Cover with glass stoppers and heat for 30 min at 60^{o}. Immediately cool in an ice bath. Add 5 ml of KOH solution (Solution 2) and dilute to 25 ml with carbonyl-free ethanol. Allow colour to develop exactly 10 min after addition of Solution 2, then read absorbance at 430 nm. Correct absorbance for the blank.
Construct a calibration curve using the standard Heptaldehyde solution (solution 5) in the range of 1-30 µg/ml plotting absorbance at 430 nm versus concentration.
Calculate % Carbonyls (as heptaldehyde) from the calibration curve, correcting for dilution of the sample.

Phenols

Principle:
Phenolic compounds can be determined spectrophotometrically after reacting with 2,6-Dibromo-N-chloro-p-benzoquinoneimine (BQC) in a basic buffer. The method cannot differentiate between phenolic compounds but gives reproducible results expressed as 2,6-dimethoxyphenol (2,6-DMP). A solution of sample is reacted with BQC reagent in a basic borate buffer to form indophenols, which have a blue colour. The colour developed is quantitated at 610 nm using a standard curve prepared with 2,6-DMP.

Apparatus:
Spectrophotometer with 1 cm cells
100 ml volumetric flasks
Centrifuge
Assorted glassware

Reagents:
Cupric sulfate ($CuSO_4\_5H_2O$)
Sodium borate ($Na_2B4O_7\_10H_2O$)
Sodium hydroxide
2,6-Dibromo-N-chloro-p-benzoquinoneimine
2,6-Dimethoxyphenol
1-Butanol
Methanol

Solutions:
0.05% w/v $CuSO_4\_5H_2O$ in water.
24.8 g $Na_2B_40_7\_10H_2O$ dissolved in 900 ml water. Adjust pH to 9.8 with

sodium hydroxide TS and add water to 1,000 ml volume.
40 mg BQC dissolved in 10 ml methanol. (Prepare fresh daily.)
2,6-Dimethoxyphenol in water, 20 µg/ml.
0.2% Smoke Flavourings in water.

Procedure:
Place 5 ml of diluted sample solution (Solution 5) in one test tube and 5 ml of water in another test tube to serve as the blank. Then add to each tube:
1 ml of cupric sulfate solution (Solution 1)
5 ml of borate buffer (Solution 2)
4 drops of BQC reagent (Solution 3)
Cover each tube, shake vigorously, and allow colour development in the dark for exactly 10 min. Add 10 ml of 1-butanol to each test tube, cover and invert 6 to 8 times (do not shake). Centrifuge test tubes for 5 min at 700 rpm.

Measure the absorbance of the sample at 610 nm and correct for the blank. Construct a calibration curve using the standard 2,6-DMP solution (Solution 4) in the range of 1-20 µg/ml, plotting absorbance at 610 nm versus concentration.

Calculate percent Phenols Content (as 2,6-DMP) from the calibration curve, correcting for dilution of the sample.