Food and Agriculture Organization of the United Nations

World Health Organization

Residue Monograph prepared by the meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82nd meeting 2016

Octenyl Succinic Acid Modified Gum Arabic

This monograph was also published in: *Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 82nd meeting 2016. FAO JECFA Monographs 19*

OCTENYL SUCCINIC ACID MODIFIED GUM ARABIC

Prepared at the 82nd JECFA (2016) and published in the FAO JECFA Monographs 19 (2016), superseding tentative specifications prepared at the 79th JECFA (2014) and published in the FAO JECFA Monographs 16 (2014). An ADI "not specified" was established at the 82nd JECFA (2016)

SYNONYMS Gum arabic hydrogen octenylbutandioate; Gum arabic hydrogen octenylsuccinate; OSA modified gum arabic; OSA modified gum acacia; INS No. 423

DEFINITION Octenyl succinic acid modified gum arabic is produced by esterifying gum arabic (*Acacia seyal*), or gum arabic (*Acacia senegal*) in aqueous solution with octenyl succinic acid anhydride. The modified gum, containing not more than 3% octenyl succinate on a weight basis is subsequently spray dried.

C.A.S. number 455885-22-0

DESCRIPTION Off-white to light tan, free flowing powder

FUNCTIONAL USES Emulsifier

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Freely soluble in water; insoluble in ethanol

^{1}H-NMR spectrum The ^{1}H-NMR spectrum of the sample obtained using the procedure described in Tests under "Esterified octenyl succinic acid" corresponds to the reference ^{1}H-NMR spectrum in the Appendix.

pH (Vol. 4) 3.5 to 6.5 (5% solution)

Viscosity Not more than 30 cP (5% solution, 25°)
Add 95 ml of water to a beaker. Place a magnetic stir bar into the water and while stirring add 5 g of the sample. Stir on medium speed for 2 h. Measure viscosity on Brookfield LV viscometer, or equivalent, using spindle number 3 at 30 rpm (factor = 40).

PURITY

Esterified octenyl succinic acid Not more than 3%
See description under TESTS

Loss on drying (Vol.4) Not more than 15% (105°, 5h)

Total ash (Vol.4)	Not more than 10% (530°)
Acid-insoluble ash (Vol.4)	Not more than 0.5%
Water-insoluble matter (Vol. 4)	Not more than 1.0%
Starch or dextrin	Boil a 1 in 50 aqueous solution of the sample, add about 0.1 ml iodine TS. No bluish or reddish colour should be produced.
Tannin-bearing gums	To 10 ml of a 1 in 50 aqueous solution of the sample add about 0.1 ml ferric chloride TS. No blackish coloration or blackish precipitate should be formed.
Residual octenyl succinic acid	Not more than 0.3% See description under TESTS
Microbiological criteria (Vol. 4)	*Salmonella* species: absent in 25 g *Escherichia coli*: absent in 1 g
Lead (Vol. 4)	Not more than 2 mg/kg Determine using a method 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").

TESTS

PURITY TESTS

Esterified octenyl succinic acid

Principle: Determine using ^{1}H-NMR method to measure remaining octenyl succinic groups present in product following extraction of residual octenyl succinic acid (OSA).

Procedure

Extraction of residual OSA

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper and wash the solid residue, three times with 7 ml portions of methanol. Allow the methanol to evaporate from the filter cake in the hood and dry the filter cake in a forced air oven at 40° overnight. Grind the dried cake using mortar and pestle.

^{1}H NMR Solvent system A

Dimethyl sulfoxide-d_6 (DMSO-d_6 containing 1.88% w/w deuterated trifluoroacetic acid (TFA-d_1) and 0.42% w/w recrystallized 1,4-bis(trichloromethyl)benzene (BTCMB, recrystallized from hexane). In the solvent system, BTCMB is an internal standard.

^{1}H NMR Solvent system B

Dimethyl sulfoxide-d_6 (DMSO-d_6 containing 1.88% w/w deuterated trifluoroacetic acid (TFA-d_1) and 1.25 mg/ml OSA standard.

System validation

Dissolve 15 mg dried purified non-modified gum Arabic in total 750 µl NMR solvent system mixture A & B prepared according to the following Table. Heat the solution to fully solubilize the purified gum Arabic. Transfer the solution into a 5 mm NMR tube.

Volume A (µl)	Volume B (µl)	BTCMB (mg)	OSA (mg)	OSA % (theoritecal)
750	0	3.75	0	0
620	130	3.1	0.16	1.08
500	250	2.50	0.31	2.08
400	350	1.75	0.44	2.92

Sample preparation
Dissolve 15 mg dried purified OSA-modified gum Arabic in 750 µl ^{1}H NMR solvent system A. Heat the solution to fully solubilize the purified OSA-modified gum Arabic. Transfer the solution into a 5 mm NMR tube.

Determine percent of esterified OSA in OSA-modified gum Arabic by ^{1}H-NMR using a 400 MHz NMR spectrometer.
Experimental conditions:
Temperature: 85°.
17.8 µs 90° pulse, 32 second relaxation delay, 1.37 second acquisition time, 8192 data points, 5,973.8 Hz sweep width, 0.5 Hz exponential apodization line broadening, 16 scans.

Calculation

Use the OSA methyl proton peak at 0.8-0.89 ppm and BTCMB peak at 8.1 ppm to calculate the percent of esterified OSA in the sample.

$$\%\ \text{OSA} = \frac{I_{\text{OSA-Me}}}{I_{\text{BTCMB}}} \times \frac{4}{3} \times \frac{210.27}{312.84} \times \frac{W_{\text{BTCMB}}}{W_{\text{MGA}}} \times 100$$

where:

$I_{\text{OSA-Me}}$ is integrated peak area of the OSA methyl proton peak;
I_{BTCMB} is integrated peak area of the BTCMB internal standard proton peak;
W_{BTCMB} is the weight of BTCMB internal standard in mg (4.2 mg/ml x 0.750 ml) and W_{MGA} is the weight of modified gum Arabic present in solution in the NMR experiment tube in mg.

NOTE:

1: The signal intensity for OSA-Me comes from 3 protons/molecule, therefore, $I_{\text{OSA-Me}}/3$ corresponds to the number of molecules of OSA.

The signal intensity for BTCMB comes from 4 protons/molecule and so $I_{BTCMB}/4$ corresponds to the number of molecules of BTCMB. 210.27 and 312.84 are the molecular weights of OSA and BTCMB, respectively.

2. Plot the % OSA theoretically calculated in the Table above vs the % OSA calculated by the NMR measurement, using the weight of the unmodified gum arabic in the place of W_{MGA}. (A linear correlation should be obtained with correlation coefficient >0.99, slope close to 1 and low intercept).

3. Use the correlation slope and intercept to correct the calculated amount in the sample.

Residual octenyl succinic acid

Determine by HPLC on the 2-bromoacetophenone-derivatised methanolic extract of the sample.

Extraction and Preparation of Sample Solution

Accurately weigh 500 mg (to nearest 0.1 mg) of the sample in a 25 ml Erlenmeyer flask, add 15 ml of methanol, stopper the flask and shake it on a shaker overnight. Filter the extract using a filter paper, wash the residue, three times with 7 ml portions of methanol and combine the filtrate (about 80% of the OSA residues is extracted by this procedure). Add 1 ml of 0.16 N KOH in methanol to the combined filtrate. Dry the extract using a flash evaporator at 30° and dissolve the residue in 2 ml of methanol. Pipette 0.5 ml of this solution into a reaction vial, add 0.5 ml of derivatisation reagent [2.8 g of 2-p-dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml CH_3CN]. Add 2 ml CH_3CN to the reaction vial, cap the vial and heat at 80° for 30 min. Allow the vial to reach room temperature and analyse the reaction product by HPLC within 24 h.

HPLC Conditions:

Column: µ-Bondapack C18 or equivalent
Mobile Phase: Methanol and Water with gradient elution: 70% to 80% of methanol in water in 5 min
Flow rate: 1.5 ml/min
Detector: UV at 254 nm
Injection volume: 5 µl

Preparation of Standard Curve

Prepare a 105.14 mg/ml solution of octenylsuccinic acid anhydride (available from Milliken Chemicals) in methanol (Solution A). Using a syringe draw 0.25 ml of Solution A, transfer into a 25-ml volumetric flask and dilute to mark with methanol (Solution B).

Prepare three working standard (Solution C1, C2 and C3) by transferring 0.5, 1 and 2 ml each of Solution B into three 50-ml round bottom flasks, add 1 ml of 0.16 N KOH in methanol to each flask, dry the solution using a flash evaporator at 30° and dissolve the residue in 2.0 ml of methanol. To 0.5 ml each of these solutions in reaction vials, add 0.5 ml each of derivatisation reagent [2.8 g of 2-p-

dibromoacetophenone and 0.28 g of 1,4,7,10,13,16-hexaoxacyclooctadecane (18-Crown-6) in 50 ml of CH_3CN]. Add 2 ml of CH_3CN to each vial, cap the vials and heat for 30 min at 80°. Allow the vials to reach room temperature and analyze by HPLC immediately. The amount of octenyl succinic acid in each 5-µl injection is as follows:

Solution C1: 0.2375 µg
Solution C2: 0.4750 µg
Solution C3: 0.9500 µg

Construct the standard curve using peak area against the amount of standard in the injected volume.

Inject 5-µl of prepared sample solution and read the amount of octenyl succinic acid in the injection from the standard curve.

Calculation

$$\% \text{ Residual octenyl succinic acid} = \frac{300 \times V}{W}$$

where

V is the amount of OSA (µg) in the injected volume; and
W is the weight of the sample (mg).

NOTE: The formula is corrected to 100% recovery by dividing with 0.80, so that 240/0.80 = 300.

Appendix Representative ^{1}H NMR spectrum of OSA modified gum Arabic