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Characterization and properties of *Acacia senegal* (L.) Willd. var. *senegal* with enhanced properties (Acacia (*sen*) SUPER GUM™): Part 2—Mechanism of the maturation process

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Abstract

The molecular changes which accompany the maturation process to produce Acacia (*sen*) SUPER GUM™ are further described and demonstrate that *Acacia senegal* can be produced with precisely structured molecular dimensions. The controlling feature is the agglomeration of the proteinaceous components within the molecularly disperse system that is naturally occurring *A. senegal* gum to increase the amount of arabinogalactan protein (AGP) component. The new structural unit is investigated by enzymatic treatment of a number of Acacia (*sen*) SUPER GUM™ samples, which demonstrates that the AGP so formed is hydrolyzed by the enzyme protease in exactly the same way as for the AGP in control *A. senegal* which has not been matured. The rheological features of the matured *A. senegal* gum reflect the increase in molecular dimensions and indicate that the AGP formed is highly cross-linked. A model for the aggregation of the proteinaceous components is proposed.

Keywords: Gum arabic; *Acacia senegal*; SUPER GUM™; Maturation; Molecular weight; Enzyme digestion; GPC-MALLS

1. Introduction

Previously in Part 1 of this series (Al-Assaf, Phillips, Aoki, & Sasaki, 2006), we reported the maturation process to produce a new series of standardized Acacia gums (designated generally Acacia (*sen*) SUPER GUM™ when derived from *Acacia senegal* and as also Acacia (*sey*) SUPER GUM™ when derived by the same process from *Acacia seyal*) which accelerates and enhances this same natural aggregation process, under strictly controlled conditions, which were worked out first at laboratory level, then pilot scale and finally at plant level (Hayashi, 2002). Such an aggregation process occurs when the tree grows older up to about 15 years (Idris, Williams, & Phillips, 1998). The naturally occurring association of the smaller molecular weight arabinogalactan (AG) and glycoprotein (GP) units into larger units is taken a further stage to give larger molecular weight arabinogalactan protein (AGP) aggregates. By monitoring the molecular architecture of the gum at all stages, specific new products have been characterized. In all aspects this specially matured gum is chemically and molecularly identical to the base gum, but because of the difference in distribution of smaller units into larger aggregates, the physical and functional performance is greatly enhanced.

In Part 1 of this series (Al-Assaf et al., 2006) the functional emphasis was placed on emulsification, since this property has proved the most high value application of commercial gum arabic and the most difficult to reproduce consistently. We also demonstrated that there is no restriction upon the particular molecular characteristics and protein distribution, which can be produced by the maturation process. Here, we have selected representative

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samples with complete solubility for large-scale production with weight average molecular weights of 1.0 to ca. 2.5×10^6 g/mole.

This paper further describes the molecular maturation changes which can be effected to enhance the performance of natural gum arabic, without introducing any new chemical groups and ensure that the material is of constant properties and performance.

2. Material and methods

2.1. Materials

Gum arabic (*Acacia senegal* var. *senegal*) samples Lot No. FR-2876 (hand picked selected, grade in lump form) were provided by San Ei Gen F.F.I., Inc. (Osaka, Japan) and were originally obtained from the Gum Arabic Company in Sudan. The sample was mechanically kibbled and three matured gum arabic samples were prepared, according to the method reported in Part 1 of this series (Al-Assaf et al., 2006). The matured samples were labeled FR-2877, FR-2878, and FR-2979 and were spray dried following maturation. Additionally two commercial Acacia (*sen*) SUPER GUM™ samples (EM1 and EM2), produced in ton quantities were also used. Sodium chloride (99.9 > % for analysis), Tris(hydroxymethyl) amino-methane (99.9 > %), and hydrochloric acid (35.5–37.5%) were purchased from Fisher Scientific UK. Protease (Type XIV: Bacterial from *Streptomyces griseus*; Product No. P5147) was obtained from Sigma. Sulfuric acid, hydrochloric acid and 2-mercapt ethanol were purchased from Wako Pure Chemical Industry (Osaka, Japan). Distilled water (Bibby Merit W4000) was used for all experiments. Pullulan standard (P-50, molecular weight 4.73×10^4 g/mole) was obtained from Shodex, Japan (Tokyo, Japan).

2.2. Loss on drying

The loss of drying was measured to determine water content of spray-dried samples. Around 1.5 g of test material was accurately weighed into a weighted vial and dried (105 °C, 4 h, SANYO convection oven MOV-212F). Then, the sample was moved to a desiccator to cool at room temperature. The loss of weight was used to calculate the solid content of the samples.

2.3. Specific rotation

The sample was dissolved in distilled water and measured by a Jasco P-1020 Polarimeter.

2.4. Sugar analysis

The sample was hydrolyzed with 2-N sulfuric acid at 100 °C for 2 h and neutralized with sodium hydroxide. HPLC analysis was performed using a Dionex Carbo Pac PA-1 with a 0.01 M sodium hydroxide mobile phase. A pulsed amperometoric detector (ED40 Electrochemical Detector, Dionex) was used.

2.5. Amino acids analysis

The sample was hydrolyzed with 6 N hydrochloric acid containing 2-mercapt ethanol at 110 °C for 24 h. The hydrolyzed sample was analyzed by HPLC using an AApak Na II-S2 column (Jasco, Japan) with Amino buffer-II as mobile phase. Post-column *o*-phthalaldehyde (OPA)-fluorescent detection (excitation 345 nm, emission 455 nm) was used.

2.6. Intrinsic viscosity

In all, 30 mg/ml gum arabic solution (as solid content) in 1.0 M and 0.2 M NaCl aqueous solution was prepared. The precise concentration of the sample was calculated based on a dry solid weight basis. The solution was filtered through 0.8 μm cellulose acetate membrane (Naglene, 25 mm).

A calibrated Ubbelohde viscometer (Cannon Ubbelohde Semi-Micro Calib 75) was used for measuring in efflux time (measured in seconds) to calculate the relative viscosity based on the efflux time of the solvents at 25.0 °C. The efflux time was measured three times by allowing the sample solution to flow freely through bulb.

Firstly, the efflux time of solvents was measured and that of the test solution (30 mg/ml, 2 ml) was determined. Then, the test solution in the viscometer was successively diluted by adding 0.4, 0.6, 1, 2 and the 4 ml of solvents and the efflux time of the diluted solution was measured.

2.7. Gel permeation chromatography—multi-angle laser light scattering (GPC-MALLS) analysis

The procedures used in this investigation are extensions of the basic technique described previously (Al-Assaf, Katayama, Phillips, Sasaki, & Williams, 2003). However, 1.0–4.0 mg/ml of test solution was used, after taking into account the loss on drying. The solution was agitated on a tube roller mixer (SRT-2, Jencons Scientific, Inc., UK) for at least 5 h to ensure that the sample fully dissolves and hydrated. The test solution was filtered using 0.45-μm nylon filter (Whatman, 13 mm).

Astra for Windows software (version 4.90.07, Wyatt Technology Corporation) was used in the instrument control and data acquisition by an Agilent 1100 series G1314A UV detector (214 nm, Agilent Technologies), a DAWN EOS multi-angle laser light scattering detector ($\lambda_0 = 690$ nm, Wyatt Technology Corporation) and an Optilab® rEX refractometer (Wyatt Technology Corporation). DAWN EOS was calibrated using toluene (SPECTRANAL 99.9%, Riedel-de Haën). The Pullulan standard (molecular weight 47,300 g/mole) was used to normalize the detectors and for the determination of the delay volume. The delay volumes of the equipment were