Characterization of the Chemical Composition of a Byproduct from Siam Benzoin Gum

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The mixture of bark and gum obtained after size-grading of Siam benzoin gum was studied to establish its potential application as a valuable new grade of the balsamic resin. An analysis of its volatile constituents by means of static headspace and SPME led to the identification of 26 and 50 compounds, respectively. Significant differences were observed in both the headspace composition and olfactory properties of the byproduct as compared to those of Siam benzoin gum. This prompted the further analysis of its volatile extract and its resinoid by GC techniques, resulting in the identification of 60 (99.5%) and 16 (89.1%) components, respectively. To examine the influence of bark pieces, different extracts obtained from the raw material and from a sorted sample were analyzed by GC and HPLC techniques. The chemical compositions and the yields determined for the two resinoids lead to the conclusion that this harvesting byproduct is a new grade of Siam benzoin gum, providing interesting olfactory notes that differ from those of other grades.

KEYWORDS: Siam benzoin gum byproduct; static headspace; solid-phase microextraction; gas chromatography; HPLC

INTRODUCTION

Siam benzoin gum is a balsam obtained from *Styrax tonkinensis* that is produced mainly in Laos (*1*, *2*). It is extensively used in the flavor and fragrance industry owing to its sweet balsamic odor with a distinct note of vanilla (*3*). The gum does not exude naturally from the tree, but is rather a pathological product resulting from incisions made through the bark. After the gum flows out and hardens upon exposure to air, it is harvested by scraping the trunk. This resin is then sorted and graded according to the size of the pieces (*1*, *3*). Five different grades providing slightly different olfactory properties can be found in the trade, from grade 1 (largest pieces) to grade 5, which contains dust and small amounts of bark. The product remaining after sorting is a mixture of bark and gum and often represents >10% of the total harvested benzoin gum by weight. To the best of our knowledge, this harvesting byproduct has not yet been exploited, and its chemical composition has never before been reported.

Siam benzoin gum has previously been described as containing coniferyl benzoate (65–75%), *p*-coumaryl benzoate (10–15%), cinnamyl cinnamate (0.5–6%), benzoic acid (12%), vanillin (0.3%), and siaresinolic acid (6%) (*4*–*6*). We recently reported the chemical composition of Siam benzoin gum as established by SPME and direct GC analysis of its volatile extracts (*7*). This work was complemented by an analytical study of two grades of Siam benzoin gum (grades 3 and 5) using various headspace sampling methods, which allowed the identification of 42 volatile and semivolatile compounds (*8*).

The aim of this study was to determine whether this gum byproduct could be considered as a new grade of Siam benzoin gum or as a new raw material. For this purpose, both chemical and olfactory properties of the byproduct were examined.

MATERIALS AND METHODS

Materials. The gum byproduct was provided by AGROFOREX Co. (Vientiane, Laos). It was obtained from the grading process of Siam benzoin gum harvested on *Styrax tonkinensis* trees in northern Laos at the end of 2002.

Headspace Solid-Phase Microextraction. A manual SPME device and divinylbenzene/carboxen/polydimethylsiloxane fibers (DVB/CAR/PDMS; 50/30 μm) were purchased from Supelco. The fiber was conditioned as recommended by the manufacturer prior to use. Ten grams of crushed byproduct was placed in a 40 mL amber vial closed by a PTFE/silicone septum (Supelco). Before extraction, the headspace in the vial was equilibrated overnight at room temperature. Extraction was performed at room temperature with a sampling time of 40 min. After exposure, the fiber was thermally desorbed into the GC injection port (equipped with a 0.75 mm i.d. inlet liner) for 4 min. The injector was set at 250 °C and used in the splitless mode. Before any other sampling, the fiber was reconditioned for 5 min in the GC injection port at 250 °C.

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Chemical Composition of Siam Benzoin Gum Byproduct

J. Agric. Food Chem., Vol. 54, No. 23, 2006 **8849**

Static Headspace. The byproduct was extracted using a Hewlett-Packard 7694 static headspace autosampler (Agilent Technologies). The headspace sampler was operated as follows: oven temperature, 110 °C; loop temperature, 130 °C; transfer line temperature, 150 °C; sample equilibration time, 120 min. Eight grams of crushed byproduct was placed in a 20 mL vial (Agilent Technologies) that was tightly closed with a PTFE/white silicone septum and a cap. The injection loop (volume, 3 mL) was filled by depressurizing the headspace for 0.3 min, and the loop was swept with the carrier gas (loop equilibration, 0.05 min; sample injection, 0.5 min) to inject the volatile components into the chromatograph via a transfer line (splitless mode).

Toluene and Styrene Quantification. Toluene and styrene quantification was carried out by external calibration using static headspace GC. One gram of benzyl alcohol (Aldrich, 108006) was added to 1 g of crushed raw material in a 10 mL vial (Agilent Technologies) that was sealed with a PTFE/white silicone septum and a cap. A Combi PAL autosampler equipped with an incubator oven was used. The extraction conditions were as follows: oven temperature, 70 °C; sample equilibration time, 20 min. A 1 mL gas syringe heated at 140 °C was used for headspace extraction, and the volatile components were injected into the injection port of an Agilent 6890N gas chromatograph equipped with a flame ionization detector. The GC was equipped with a HP1 fused-silica capillary column (polydimethylsiloxane, 50 m × 0.2 mm i.d.; film thickness, 0.33 μm) and used under the following operating conditions: carrier gas, helium; constant flow, 1 mL/min; injector and detector temperatures, 250 °C; split ratio, 1/10; temperature program, 60 °C (4 min) to 110 °C at 2 °C/min then 110 to 250 °C at 20 °C/min.

Volatile Extracts. Volatile extracts were obtained by hydrodistillation using a Clevenger type apparatus for 4 h with 5 mL of pentane as a recovery solvent, which was evaporated under a slight nitrogen stream after collection. The yields obtained were 0.01% for volatile extract 1 (made from the raw byproduct) and 0.03% for volatile extract 2 (made from bark manually isolated from the byproduct).

Resinoids. Resinoids 1 and 2 were obtained from the byproduct and bark, respectively. One hundred grams of each material was refluxed in 500 mL of ethanol for 4 h. After filtration and solvent evaporation, the resinoids were obtained in 63% (resinoid 1) and 14% (resinoid 2) yields.

Siam benzoin gum (grade 3) resinoid was supplied by CHARABOT S.A. (Grasse, France). This commercial extract was industrially made under conditions similar to those described for the above-mentioned resinoids.

Chemical Analysis. *Headspace Analyses.* GC-FID and GC-MS analyses using headspace methods were carried out with an Agilent 6890N gas chromatograph equipped with a flame ionization detector and coupled to an Agilent 5973N mass spectrometer. The GC was equipped with a HP1 fused-silica capillary column (polydimethylsiloxane, 50 m × 0.2 mm i.d.; film thickness, 0.33 μm) and used under the following operating conditions: carrier gas, helium; constant pressure, 282 kPa; initial flow, 1.35 mL/min; injector and detector temperatures, 250 °C; temperature program, 60 °C (4 min) to 250 °C at 2 °C/min then held isothermal (30 min); ion source temperature, 230 °C; transfer line temperature, 280 °C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range of 35−400 amu.

Extract Analyses. Volatile extracts were analyzed by GC-MS using a Hewlett-Packard 5890/5970A system, equipped with a HP1 fused-silica capillary column HP1 (polydimethylsiloxane, 50 m × 0.20 mm; film thickness, 0.5 μm) and used under the following operating conditions: carrier gas, helium; injector temperature, 230 °C; split ratio, 1/100; temperature program, 60 to 250 °C at 2 °C/min then held isothermal (120 min); ion source temperature, 230 °C; transfer line temperature, 280 °C; ionization energy, 70 eV; electron ionization mass spectra were acquired over the mass range of 35−400 amu. GC-FID analyses were carried out using an Agilent 6890N gas chromatograph equipped with a HP1 fused-silica capillary column (polydimethylsiloxane, 50 m × 0.20 mm; film thickness, 0.5 μm) and used under the following operating conditions: carrier gas, helium; constant flow, 1 mL/min; injector and detector temperatures, 250 °C; split ratio, 1/10; temperature program, 60 to 230 °C at 2 °C/min then held isothermal

(120 min). The resinoids (10% ethanolic solutions) were analyzed using the same conditions in splitless mode.

HPLC-UV. HPLC analyses were performed on a Varian ProStar system equipped with a ProStar 320 UV−vis detector. The column was a 250 × 4.0 mm HyPurity C18 (5 μm) (Thermo Electron Corp.) with a 10 × 4.0 mm HyPurity C18 precolumn and was used at room temperature. Analyses were performed at 254 nm. The column was eluted with a flow rate of 0.55 mL/min, and the composition of the mobile phase consisted of water (A) and methanol (B), both containing 0.1% formic acid. The gradient conditions were as follows: 0−10 min, 50% B; 10−11 min, 50−70% B; 11−21 min, 70% B; 21−22 min, 70−75% B; 22−32 min, 75% B; 32−33 min, 75−80% B; 33−38 min, 80% B; 38−39 min, 80−85% B; 39−44 min, 85% B; 44−45 min, 85−90% B; 45−55 min, 90% B; 55−56 min, 90−100% B; 56−66 min, 100% B. Samples (concentration, 2 mg/mL) were prepared in methanol, and the injection volume was 20 μL.

Component Identification. Identification of the constituents was based on computer matching against commercial libraries (Wiley, MassFinder 2.1 Library, NIST98) and a homemade mass spectra library built using pure substances and MS literature data (9−14). Several structures were also confirmed by standard compound injection.

Identification of the components was also based on their GC retention indices (RI) determined on an apolar HP1 column using a homologous series of *n*-alkanes (C_5−C_{28}) (extraction times used for headspace experiments: Static-HS, 30 s at 110 °C; HS-SPME, 20 s at 40 °C).

Chemicals. Most of the standard compounds were purchased from chemical supply companies. Ethyl benzoate, allyl benzoate, propyl benzoate, isobutyl benzoate, isoamyl benzoate, 3-methylbut-3-enyl benzoate, prenyl benzoate, hexyl benzoate, benzyl benzoate, and cinnamyl benzoate were obtained by reaction of benzoyl chloride with the corresponding alcohols. α-Terpinyl acetate and neryl acetate were obtained by reaction between acetyl chloride and the corresponding alcohols. Benzyl formate was obtained by reaction between formic acid and benzyl alcohol. Standard procedures were used (15, 16), and products were characterized by GC-MS (electronic ionization, 70 eV).

Synthesis of (*E***)-Coniferyl Ethyl Ether.** Thionyl chloride (15 mmol) was added to an ice-cooled solution of 10 mmol of ferulic acid in absolute ethanol (20 mL). The mixture was refluxed overnight and the solvent evaporated. A saturated potassium carbonate solution was added until neutral pH was reached. The aqueous layer was extracted with diethyl ether; the organic phases were collected and dried over magnesium sulfate. The solvent was evaporated under vacuum, and the crude yellow oil was then purified on silica gel to afford crystallized ethyl ferulate in 90% yield (characterized by ^{1}H, ^{13}C NMR, and GC-MS). Ethyl ferulate (10 mmol) was then reduced by diisobutylaluminum hydride (4.2 equiv) (17), and the coniferyl alcohol obtained was crystallized from a dichloromethane/petroleum ether mixture (97% yield). Five milliliters of ethanol was added to 1 mmol of coniferyl alcohol, and the mixture was refluxed for 5 days to reach a sufficient conversion; (*E*)-coniferyl ethyl ether was obtained after purification on a silica gel column (70/30 petroleum ether/diethyl ether) in 10% yield: ^{1}H NMR (200 MHz; CDCl$_3$) δ 1.25 (t, 3H, *J* = 7 Hz, CH$_2$C$\underline{H}_3$), 3.55 (q, 2H, *J* = 7 Hz, C$\underline{H}_2$CH$_3$), 3.88 (s, 3H, OCH$_3$), 4.12 (dd, 2$\underline{H}$, *J* = 1.3, 6.2 Hz, CH=CH−C$\underline{H}_2$), 5.73 (s, 1H, OH), 6.15 (dt, 1H, *J* = 6.2, 15.8 Hz, CH=C$\underline{H}$−CH$_2$), 6.52 (d, 1H, *J* = 15.8 Hz, C$\underline{H}$=CH−CH$_2$), 6.8−7.0 (m, 3H$_{arom}$); ^{13}C NMR (50 MHz; CDCl$_3$) δ 15.2̄1, 55.83, 65.59, 71.32, 108.27, 114.36, 120.35, 123.94, 129.36, 132.31, 145.51, 146.58; EI-MS, *m/z* (%) 77 (26), 91 (84), 103 (54), 119 (89), 131 (100), 147 (27), 151 (37), 163 (24), 179 (26), 208 (M$^{\bullet+}$, 87).

RESULTS AND DISCUSSION

Headspace Sampling Methods. In a previous study (*8*), HS-SPME using a DVB/CAR/PDMS fiber allowed us to identify a large number of volatile compounds in Siam benzoin gum. However, because the volatile constituents have different affinities toward the fiber coating, this technique does not readily allow a quantitative comparison of different samples (*18*). In contrast, the use of the S-HS method has enabled us to compare a large variety of samples on the basis of their respective headspace compositions.

In the present work, these two headspace sampling methods optimized elsewhere (*8*) (fiber, temperature, exposure time) were used to qualitatively compare the byproduct and commercial Siam benzoin gum (grade 3). For each headspace sampling method, experiments were replicated three times. For both the byproduct and Siam benzoin gum (*8*), compounds were identified by GC-RI, GC-MS, and injection of standard compounds. Relative percentages obtained by direct integration and retention indices are listed in **Table 1**.

The S-HS method was used to compare the headspace composition of the byproduct to that of the gum and led to the identification of 26 constituents in the byproduct headspace that represented 99.7% of the total GC-FID area. Among these components, we observed six monoterpene hydrocarbons (∼11.1%), one sesquiterpene hydrocarbon (0.3%), seven esters (∼23.9%), four aldehydes (∼41.5%), and eight other compounds (∼23.9%). The main constituents that characterized the byproduct were benzaldehyde and α-pinene (32.7%; coeluted), methyl benzoate (16.7%), and toluene (13.5%). These compounds were also identified as major volatile components in Siam benzoin gum. In contrast, the headspace of the byproduct also contained hexanal (6.9%), furfural (1.9%), β-myrcene (3.0%), and terpinolene (2.8%), none of which were identified in the resin.

SPME-GC-MS and SPME-GC-FID analyses allowed us to identify 50 compounds in the byproduct headspace representing 99.4% of the total FID area. Thirteen monoterpene hydrocarbons (∼23.8%), 2 oxygenated monoterpenes (9.6%), 8 sesquiterpene hydrocarbons (5.0%), 6 esters (23.3%), 6 aldehydes (∼17.7%), and 15 other constituents (∼20.0%) were observed. The main components were linalyl acetate (17.9%), benzaldehyde and α-pinene (14.4%), linalool (8.5%), limonene and β-phellandrene (8.3%), β-myrcene (5.6%), benzyl alcohol (5.2%), and toluene (3.9%).

Athough a quantitative comparison of the samples by SPME analysis is not relevant, this technique allowed us to easily distinguish the byproduct from Siam benzoin gum. The presence of acetic acid and β-myrcene is representative of the byproduct. It is reasonable to assume that these two compounds are precursors of numerous monoterpene hydrocarbons [e.g., (*Z*)- and (*E*)-β-ocimene] and monoterpene acetates (linalyl, neryl, and geranyl acetate) or alcohols (linalool) not previously identified in commercial Siam benzoin gum.

In summary, the combination of these two headspace sampling methods resulted in the identification of 58 volatile compounds that allowed us to easily distinguish the byproduct from Siam benzoin gum. Accordingly, it is assumed that the numerous monoterpene compounds and aldehydes identified came directly from the bark.

We also detected large amounts of toluene and styrene in both the byproduct headspace and that of Siam benzoin gum (13.5 and 2.6%, respectively) and that of Siam benzoin gum (5.2 and 1.0%). This observation led us to quantify these compounds in the raw materials. The quantification was carried out by means of static headspace GC using an external calibration, with benzyl alcohol as the sample dilution solvent. Toluene concentrations of 3 and 1 ppm were measured in the byproduct and Siam benzoin gum, respectively. Only trace levels of styrene (<1 ppm) were detected in the raw materials. The amount of toluene in both the byproduct and the gum were below the level authorized for pharmaceutical products (*19*). As a result, the concentrations determined for both compounds do not preclude any further application of the byproduct in the field of flavor and fragrance.

Next, the olfactory properties of the byproduct were established and compared to those of Siam benzoin gum. The byproduct was described as providing a strong vanilla character and to be sweeter, less ambery, and less powdery than the resin due to a weaker benzoic acid note, but giving, in contrast, a pungent acetic acid note.

Natural raw materials such as resins, oleoresins, and balsams are usually used in the flavor and fragrance industry to manufacture two types of products: volatile extracts (or essential oils) and resinoids. As a result, both types of extracts were produced from this harvesting byproduct. In addition, to further investigate the influence of bark, these extracts were produced from both the raw material and pieces of bark that had been manually separated from the remaining resin.

Volatile Extracts. Volatile extracts were obtained by hydrodistillation of the raw material (VE1) and of the sorted byproduct (VE2). Due to very low yields of the volatile fractions, it was necessary to use a recovery solvent during extraction. GC-FID and GC-MS analyses led to the identification of 60 and 74 compounds in VE1 and VE2, respectively, representing 99.5 and 99.8% of the total FID area (**Table 2**). These analyses revealed that VE1 and VE2 contained 5 (∼0.3%) and 9 (∼1.1%) monoterpene hydrocarbons, 5 (∼2.1%), and 8 (7.2%) oxygenated monoterpenes, 5 (trace level) and 11 (0.2%) sesquiterpene hydrocarbons, 18 (∼80.9 and 72.9%) esters, 11 (∼13.2%), and 9 (∼13.6%) aldehydes, and 16 (∼3.0%) and 19 (∼4.8%) other compounds, respectively. The main components were determined to be benzyl benzoate (39.8 and 41.7% for VE1 and VE2, respectively), allyl benzoate (19.8 and 11.5%), methyl benzoate (17.3 and 15.4%), benzaldehyde and α-pinene (9.6 and 12.6%), linalool (1.5 and 4.4%), decen-2-(*E*)-al (2.3 and 0.1%), and ethyl benzoate (1.5 and 0.8%). The two volatile extracts presented the same five major compounds, although their overall compositions were quite different. As previously mentioned for the headspace compositions, the number and the amount of terpenoids were higher in VE2 (28 terpenoids representing about 8.5% of the total FID area) than in VE1 (15 terpenoids representing about 2.4%). In addition, the amount of benzoic esters was smaller in VE2 (70.6%) than in VE1 (80.1%). As a result, the comparison of these two volatile extracts allowed us to confirm that most of the monoterpene components came directly from the bark.

Resinoids. Resinoids were obtained by ethanol extraction from the raw material (R1) and the sorted byproduct (R2) in 63 and 14% yields, respectively. R1 and R2 were investigated by GC techniques, and the results are summarized in **Table 2**. GC-FID and GC-MS analyses led to the identification of 16 and 15 compounds in R1 and R2, respectively, representing 89.1 and 83.5% of the total FID area. The major components identified were benzoic acid (42.6 and 28.6%), coniferyl ethyl ether (19.0 and 6.1%), vanillin (10.6 and 22.1%), benzyl benzoate (4.6%), and 2-propiovanillone (4.1 and 4.7%). Several compounds presenting higher retention indices were not clearly identified, but their mass spectra corresponded to aromatic ester structures. The byproduct (R1) and sorted byproduct (R2) resinoids were closely similar in their compositions, because the same major components were identified. However, the amounts of some components, such as benzoic acid and coniferyl ethyl ether, were higher in R1 than in R2. In contrast, the content of vanillin was lower in R1 than in R2.

We assumed that the unusual presence of coniferyl ethyl ether was due to the reaction of ethanol with coniferyl derivatives during the extraction process. To confirm this, coniferyl alcohol obtained by the reduction of ethyl ferulate (*17*) was refluxed in ethanol, and we were able to observe the formation of (*E*)-coniferyl ethyl ether in the reaction mixture. This compound

Table 1. Headspace Studies of the Crushed Byproduct and Siam Benzoin Gum

compound[a]	RI[b]	SPME byproduct % ± SD[c]	SPME Siam 3 % ± SD[c]	static-HS byproduct % ± SD[c]	static-HS Siam 3 % ± SD[c]	identification methods[d]
ethanol		0.6 ± 0.1				MS, Std
formic acid			0.6 ± 0.2			MS, Std
acetic acid	566	3.4 ± 0.3	1.8 ± 0.1			MS, RI, Std
toluene	750	3.9 ± 0.4	1.7 ± 0.5	13.5 ± 2.4	5.2 ± 0.2	MS, RI, Std
hexanal	772	2.1 ± 0.3	0.3	6.9 ± 1.7		MS, RI, Std
furfural	800			1.9 ± 1.2		
p-xylene[e]	853	0.4 ± 0.1				MS, RI
styrene	872	0.3 ± 0.3		2.6 ± 2.6	1.0 ± 0.5	MS, RI, Std
heptanal	874	0.2 ± 0.1				MS, RI, Std
benzaldehyde	933	14.4 ± 1.6*	21.9 ± 2.2	32.7 ± 1.0*	46.8 ± 2.3*	MS, RI, Std
α-pinene			6.6 ± 0.9			
β-pinene	970	0.2 ± 0.2	0.3 ± 0.3		2.1 ± 1.3	MS, RI, Std
2-pentylfuran[e]	975	0.2 ± 0.1				MS, RI
β-myrcene	978	5.6 ± 0.1		3.0 ± 1.2		MS, RI, Std
1,3,5-trimethylbenzene[e]	979	0.3				MS, RI
α-phellandrene	997	0.5 ± 0.3				MS, RI, Std
decane	1000	0.3 ± 0.1				MS, RI, Std
benzyl alcohol	1004	5.2 ± 0.3	6.7 ± 0.4	4.8 ± 1.3	6.9 ± 0.2*,†	MS, RI, Std
α-terpinene	1009	0.8 ± 0.1				MS, RI, Std
p-cymene	1017	1.9 ± 0.1	0.7 ± 0.1			MS, RI, Std
1,8-cineole	1015		0.2			MS, RI, Std
limonene	1018	8.3 ± 3.5*	0.2 ± 0.1	2.6 ± 0.4	5.5 ± 0.3*	MS, RI, Std
β-phellandrene[e]	1021					MS, RI
(Z)-β-ocimene	1022	2.1 ± 0.1		0.7 ± 0.3		MS, RI, Std
acetophenone	1030			2.0 ± 1.6*		MS, RI, Std
(E)-β-ocimene[e]	1032	2.6 ± 0.2				MS, RI
benzyl formate	1044		1.6 ± 0.2	1.6 ± 0.2		MS, RI, Std
γ-terpinene	1049	0.3			3.7 ± 0.3*	MS, RI, Std
guaiacol	1058	1.5 ± 0.1		1.5 ± 0.1	0.6 ± 0.3	MS, RI, Std
1,4-diethylbenzene	1069	0.4				MS, RI, Std
terpinolene	1079	1.2 ± 0.1		2.8 ± 0.6		MS, RI, Std
methyl benzoate	1082	1.4 ± 0.1	27.7 ± 1.1	16.7 ± 1.6	17.5 ± 1.3	MS, RI, Std
nonanal	1085	0.2			0.5 ± 0.2	MS, RI, Std
linalool	1089	8.5 ± 0.7				MS, RI, Std
undecane	1100	0.1 ± 0.1		0.3 ± 0.1		MS, RI, Std
1,2,4,5-tetramethylbenzene	1101	0.3 ± 0.1				MS, RI, Std
1,2,3,5-tetramethylbenzene[e]	1104	0.5 ± 0.1				MS, RI
allo-ocimene[e]	1112	0.3				MS, RI
unknown 1[f]	1134	0.4 ± 0.1				
ethyl benzoate	1145	2.1 ± 0.1	2.3 ± 0.2	0.8 ± 0.3	2.6 ± 0.3	MS, RI, Std
benzoic acid	1148	2.6 ± 0.5	15.3 ± 0.7	-	0.8 ± 0.1	MS, RI, Std
unknown 2[f]	1158	0.2 ± 0.1				
α-terpineol	1169	1.1 ± 0.1				MS, RI, Std
unknown 3[f]	1226				1.0 ± 0.1	
allyl benzoate	1230			2.1 ± 0.6*		MS, RI, Std
cinnamaldehyde	1231	0.3 ± 0.1				MS, RI, Std
linalyl acetate	1243	17.9 ± 1.7		0.6 ± 0.5		MS, RI, Std
propyl benzoate	1247		0.2	0.2 ± 0.1	0.6 ± 0.1	MS, RI, Std
tridecane	1300			0.1		MS, RI, Std
isobutyl benzoate	1308		2.1 ± 0.1			MS, RI, Std
eugenol	1326				0.3	MS, RI, Std
α-terpinyl acetate	1328	0.6 ± 0.1				
neryl acetate	1336	0.5 ± 0.1				MS, RI, Std
vanillin	1350	0.5 ± 0.1	8.0 ± 1.0			MS, RI, Std
geranyl acetate	1354	0.8 ± 0.1				MS, RI, Std
unknown 4[f]	1365				0.9 ± 0.1	
α-copaene[e]	1379	1.8 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	1.2 ± 0.6	MS, RI
β-elemene[e]	1385	0.4 ± 0.1			0.4 ± 0.2	MS, RI
β-bourbonene[e]	1387	0.5 ± 0.1				MS, RI
tetradecane	1400			0.1 ± 0.1	-	MS, RI, Std
α-gurjunene[e]	1414	0.3				MS, RI
β-caryophyllene	1421	1.5 ± 0.1			0.4 ± 0.2	MS, Cary, Std
β-gurjunene[e]	1429	0.3 ± 0.1	0.1 ± 0.1			MS, RI
unknown 5[f]	1448			0.3 ±0.1	0.7 ± 0.1	
γ-muurolene[e]	1475		0.2 ± 0.1		0.3 ± 0.1	MS, RI
unknown 6[f]	1478		0.1 ± 0.1			
α-selinene[e]	1488		0.1			MS, RI
valencene	1490	0.1 ± 0.1				MS, RI, Std
α-muurolene[e]	1498		0.1			MS, RI
calamenenee	1509		0.1			MS, RI
δ-cadinene[e]	1520	0.1	0.1 ± 0.1			MS, RI
benzyl benzoate	1723		0.9 ± 0.3	1.9 ± 0.1	1.1 ± 0.3	MS, RI, Std

[a] Compounds are listed in order of their elution times from a HP1 column; compositional values lower than 0.1% are denoted as traces (tr). [b] RI, retention indices, determined on HP1 using the homologous series of n-alkanes. [c] Reported percentages are relative; (% ± SD) with SD = standard deviation. [d] Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; Std, by injection of an authentic sample. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI): *, coelution, ratio corresponding to the total integration of the two compounds; †, coelution with p-cymene. [f] Unknown 1 (RI 1134): 134 (51.0), 119 (100), 105 (22.0), 91 (17.0), 77 (15.7). Unknown 2 (RI 1158): 136 (40.4), 122 (23.4), 121 (46.8), 119 (40.6), 111 (34.0), 93 (100), 91 (49.0), 77 (45.6), 71 (41.4), 43 (22.7). Unknown 3 (RI 1226): 162 (5.6), 106 (8.6), 105 (100), 77 (27.2), 51 (7.3). Unknown 4 (RI 1365): 189 (100), 161 (44.5), 109 (39.2), 108 (25.9), 107 (23.9), 95 (27.7), 93 (33.1), 91 (27.0), 82 (42.1), 81 (24.0), 79 (22.8), 67 (24.5). Unknown 5 (RI 1448): 204 (M^+, 22.0), 190 (16.0), 189 (100), 163 (15.4), 133 (24.7), 119 (15.4), 107 (12.3), 105 (15.3), 91 (18.5), 81 (11.7). Unknown 6 (RI 1478): 204 (M^+, 80.0), 189 (67.0), 161 (80.2), 147 (52.0), 133 (57.7), 107 (80.2), 105 (100), 93 (83.3), 91 (67.8), 79 (58.1).

Table 2. Chemical Compositions of Volatile Extracts and Resinoids

compound[a]	RI[b]	volatile extracts		resinoids			identification methods[d]
		VE1 % ± SD[c]	VE2 % ± SD	R1 % ± SD	R2 % ± SD	SBR % ± SD	
toluene	750	0.1					MS, RI, Std
hexanal	772	0.3					MS, RI, Std
furfural	800	tr					MS, RI, Std
1-hexanol	845	0.1	0.1 ± 0.1				MS, RI, Std
p-xylene[e]	853	tr	tr				MS, RI
heptan-2-one	871	tr	tr				MS, RI, Std
styrene	872		tr				MS, RI, Std
heptanal	874	0.1	0.1				MS, RI, Std
o-xylene	882		tr				MS, RI, Std
benzaldehyde	933	9.6*	12.6 ± 0.1*				MS, RI, Std
α-pinene							MS, RI, Std
camphene	943		tr				MS, RI, Std
1-heptanol	953		0.1				MS, RI, Std
oct-1-en-3-ol	960	0.1	0.2				MS, RI, Std
2-pentylfuran[e]	975	tr	tr				MS, RI
octanal	977	0.1	0.1				MS, RI, Std
β-myrcene	978	0.1	0.3 ± 0.1				MS, RI, Std
α-phellandrene	997		tr				MS, RI, Std
benzyl alcohol	1009	0.1	0.1	0.3	3.0 ± 0.1	0.1	MS, RI, Std
p-cymene	1017	0.1	0.1				MS, RI, Std
1,8-cineole	1019		0.1 ± 0.1				MS, RI, Std
limonene	1018	0.1	0.3 ± 0.1				MS, RI, Std
(Z)-β-ocimene	1022	tr	0.1				MS, RI, Std
(E)-β-ocimene[e]	1032		0.2				MS, RI
oct-2(E)-enal	1033	0.1	0.1				MS, RI, Std
benzyl formate[e]	1044	0.1	0.9				MS, RI, Std
1-octanol	1054	0.1 ± 0.1					MS, RI, Std
(Z)-furanoid linalool oxide[e]	1055	0.1	0.4				MS, RI
(E)-furanoid linalool oxide[e]	1076		0.3 ± 0.1				MS, RI
terpinolene	1079		0.1				MS, RI, Std
methyl benzoate	1082	17.3 ± 0.1	15.4 ± 0.4	tr		0.1 ± 0.1	MS, RI, Std
nonanal	1085	0.3	0.4 ± 0.1				MS, RI, Std
linalool	1089	1.5	4.4 ± 0.2				MS, RI, Std
α-campholenic aldehyde[e]	1104		tr				MS, RI
1,2-dimethoxybenzene	1110		0.1				MS, RI, Std
camphor	1120		0.1				MS, RI, Std
benzyl acetate	1129	tr	0.2				MS, RI, Std
non-2(E)-enal	1131	0.2	0.2 ± 0.1				MS, RI, Std
p-ethylphenol[e]	1141		tr				MS, RI
ethyl benzoate	1145	1.5	0.8	0.2	0.2	0.7 ± 0.1	MS, RI, Std
1-nonanol	1158	tr					MS, RI, Std
terpinen-4-ol	1164	0.1	0.2 ± 0.1				MS, RI, Std
methyl salicylate	1168	0.4 ± 0.1*	0.1				MS, RI, Std
α-terpineol	1169		1.7 ± 0.1				MS, RI, Std
decanal	1180	0.1					MS, RI, Std
benzoic acid	1200		0.1 ± 0.1	42.6 ± 3.2	28.6 ± 1.1	41.7 ± 3.6	MS, RI, Std
unknown 1[f]	1211		0.2				
allyl benzoate	1230	19.8 ± 0.3	11.5 ± 0.3				MS, RI, Std
dec-2(E)-enal	1240	2.3 ± 0.5	0.1				MS, RI, Std
linalyl acetate	1243	0.3	0.5				MS, RI, Std
propyl benzoate	1247	0.4	0.3				MS, RI, Std
p-ethylguaiacol	1253	0.5	1.0 ± 0.1				MS, RI, Std
nonanoic acid	1261		0.2				MS, RI, Std
thymol	1264	tr	tr				MS, RI, Std
undecan-2-one[e]	1273	tr	tr				MS, RI
p-vinylguaiacol[e]	1278	tr	0.1				MS, RI
unknown 2[f]	1286			5.2 ± 0.5	7.1 ± 0.2	2.1 ± 0.1	
deca-2(E),4(E)-dienal[e]	1287		tr				MS, RI
isobutyl benzoate	1298	0.2	0.1				MS, RI, Std
4-hydroxybenzaldehyde	1323			0.1	0.1		MS, RI, Std
eugenol	1326	1.2	1.3				MS, RI, Std
α-terpinyl acetate	1328	0.1					MS, RI, Std
neryl acetate	1336	0.6*	0.4				MS, RI, Std
dihydroeugenol	1337		1.1				MS, RI, Std
vanillin	1350	0.1		10.6 ± 0.8	22.1 ± 0.3	8.8 ± 0.6	MS, RI, Std
geranyl acetate	1354	0.2					MS, RI, Std
eugenol methyl ether[e]	1368	0.1					MS, RI
α-copaene[e]	1379	tr	0.1				MS, RI
β-elemene[e]	1385	tr	tr				MS, RI
β-bourbonene[e]	1387		tr				MS, RI
unknown 3[f]	1407			0.6	0.6 ± 0.1	0.5 ± 0.1	
isoamyl benzoate	1409	0.1	0.1				MS, RI, Std
3-methyl-3-butenyl benzoate	1412	0.4	0.3				MS, RI, Std
isoeugenol	1415			0.6 ± 0.1	0.8 ± 0.2	0.2 ± 0.1	MS, RI, Std

Table 2. (Continued)

		volatile extracts		resinoids			
compound[a]	RI[b]	VE1 % ± SD[c]	VE2 % ± SD	R1 % ± SD	R2 % ± SD	SBR % ± SD	identification methods[d]
vanillyl ethyl ether[†]	1419			0.7 ± 0.1	1.1 ± 0.1	0.3 ± 0.1	MS
β-caryophyllene	1421	tr					MS, RI, Std
β-gurjunene[e]	1429		tr				MS, RI
acetovanillone	1437			1.5 ± 0.1	5.6 ± 0.1	0.2 ± 0.1	MS, RI, Std
unknown 4[f]	1440	0.5 ± 0.1					
prenyl benzoate	1453	0.5 ± 0.1	0.3				MS, RI, Std
benzyl tiglate[e]	1463	0.1					MS, RI
β-selinene[e]	1482	tr	0.1 ± 0.1				MS, RI
2-propiovanillone	1484			4.1 ± 0.3	4.7 ± 1.0	1.0 ± 0.5	MS, RI, Std
α-selinene[e]	1488		tr				MS, RI
α-muurolene[e]	1498		tr				MS, RI
unknown 5[f]	1502			1.3	0.1	0.7 ± 0.1	
calamenene[e]	1506		tr				MS, RI
δ-cadinene[e]	1520	tr	tr				MS, RI
unknown 6[f]	1534			0.5	1.2		
hex-3(Z)-enyl benzoate[e]	1540	0.1	tr				MS, RI
hexyl benzoate	1545		tr				MS, RI, Std
caryophyllene alcohol[e]	1551		tr				MS, RI
homovanillic acid[†]	1603			1.6 ± 0.1	1.2		MS
unknown 7[f]	1608			0.1	0.2	1.2 ± 0.1	
cadalene[e]	1645		tr				MS, RI
methyl homovanillate[e]	1662			1.2 ± 0.1	1.7 ± 0.3	1.7 ± 0.2	MS, RI
coniferaldehyde	1676			1.6 ± 0.1	2.6 ± 0.1	0.9 ± 0.1	MS, RI, Std
coniferyl alcohol	1685			0.4	1.0 ± 0.1		MS, RI, Std
benzyl benzoate	1722	39.8 ± 0.1	41.7 ± 0.8	4.6 ± 0.4	4.6 ± 0.1	7.3 ± 0.5	MS, RI, Std
coniferyl ethyl ether	1730			19.0 ± 1.0	6.1 ± 0.1	18.1 ± 1.2	MS, RI, Std
unknown 8[f]	1734					2.2 ± 0.2	
benzyl salicylate[e]	1827		0.2				MS, RI
palmitic acid[e]	1937	0.1	0.2 ± 0.1				MS, RI
unknown 9[f]	1979					0.4 ± 0.1	
cinnamyl benzoate	2024		0.1				MS, RI, Std
unknown 10[f]	2216			0.1	0.3	0.2	
unknown 11[f]	2326			0.5	0.7	0.3 ± 0.1	
unknown 12[f]	2423			1.0 ± 0.1	1.4 ± 0.1	5.2 ± 0.4	
unknown 13[f]	2527			1.0 ± 0.1	3.4 ± 0.8	4.0 ± 0.2	
unknown 14[f]	2563			0.6	1.5 ± 0.1	1.7 ± 0.2	

[a] Compounds are listed in order of their elution times from a HP1 column; compositional values lower than 0.1% are denoted as traces (tr). [b] RI, retention indices, determined on HP1 using the homologous series of *n*-alkanes. [c] Reported percentages are relative; (% ± SD) with SD = standard deviation. [d] Method of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; Std, by injection of an authentic sample. [e] Compound tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (RI): [†], compound tentatively identified according to the mass spectrum (MS); [*], coelution, ratio corresponding to the total integration of the two compounds. [f] Unknown 1 (RI 1211): 136 (22.8), 121 (30.1), 93 (100), 91 (37.7), 79 (28.1), 77 (25.2), 69 (97.1), 68 (43.5), 67 (38.1), 41 (66.8). Unknown 2 (RI 1286): 136 (2.1), 135 (2.6), 122 (3.8), 106 (8.0), 105 (100), 92 (3.0), 78 (3.8), 77 (49.1), 51 (18.4), 50 (6.8). Unknown 3 (RI 1407): 166 (27.6), 138 (8.9), 137 (100), 122 (19.5), 94 (13.3), 77 (6.2), 66 (6.4), 65 (6.0), 51 (8.8), 39 (7.8). Unknown 4 (RI 1440): 204 (M$^+$, 24.6), 190 (16.3), 189 (100), 163 (14.5), 133 (20.4), 107 (12.9), 105 (17.5), 91 (26.0), 79 (12.4), 41 (19.8). Unknown 5 (RI 1505): 208 (6.6), 163 (4.1), 106 (7.9), 105 (100), 77 (32.3), 51 (11.5), 50 (4.0). Unknown 6 (RI 1537): 194 (5.8), 152 (8.9), 151 (100), 123 (19.6), 108 (10.8), 105 (9.7), 77 (8.8), 65 (8.1), 52 (11.4), 43 (9.0). Unknown 7 (RI 1613): 178 (59.3), 149 (44.3), 134 (31.2), 133 (78.4), 131 (36.3), 121 (100), 103 (44.4), 91 (30.3), 77 (51.5), 55 (29.1). Unknown 8 (RI 1734): 224 (19.0), 150 (4.5), 138 (9.1), 137 (100), 122 (8.7), 94 (8.2), 66 (3.5), 65 (3.1), 59 (8.4), 51 (3.1). Unknown 9 (RI 1979): 296 (3.4), 182 (11.1), 181 (100), 153 (19.7), 131 (3.8), 125 (7.9), 103 (3.6), 93 (17.5), 65 (8.9), 59 (5.2). Unknown 10 (RI 2216): 281 (3.6), 250 (79.4), 165 (16.2), 152 (74.0), 151 (100), 137 (54.5), 135 (36.8), 79 (14.6), 55 (15.8), 39 (37.7). Unknown 11 (RI 2326): 286 (28.3), 164 (100), 149 (31.9), 137 (37.9), 133 (26.0), 132 (17.9), 105 (50.9), 91 (12.6), 77 (66.1), 51 (17.2). Unknown 12 (RI 2423): 242 (0.3), 163 (7.5), 150 (25.7), 137 (92.0), 122 (11.5), 106 (8.4), 105 (100), 94 (12.0), 77 (36.6), 51 (12.8). Unknown 13 (RI 2527): 182 (11.0), 181 (100), 153 (16.3), 151 (5.0), 125 (6.7), 105 (19.9), 93 (20.0), 77 (16.3), 65 (9.0), 51 (4.5). Unknown 14 (RI 2563): 272 (2.6), 182 (9.2), 181 (100), 153 (17.9), 125 (6.4), 105 (18.4), 93 (20.0), 77 (15.0), 65 (9.0), 51 (4.6).

was isolated and characterized by GC-MS and ^{1}H and ^{13}C NMR. Therefore, whereas (*E*)-coniferyl ethyl ether cannot be viewed as natural, it is nevertheless characteristic of the extract. It should be mentioned that this compound was previously described in a Karo-Karunde flower absolute (*20*).

The compositions established for the byproduct resinoids were directly compared with those obtained for the commercial Siam benzoin gum resinoid (SBR) (**Table 2**). According to the results obtained, the byproduct resinoid (R1) appeared to be similar to SBR. In contrast, the sorted byproduct resinoid (R2) was different, mainly in that it had a lower content of benzoic acid and coniferyl ethyl ether and a higher content of vanillic derivatives, which were probably brought by the bark. In addition, the difference between the yields obtained for R1 (63%) and R2 (14%) clearly shows the contribution of the

remaining resin to the byproduct resinoid (R1). Therefore, this can be unambiguously related to its chemical composition, which is close to that of SBR.

We decided to further investigate the overall composition of the three resinoids by means of HPLC-UV analysis. The chromatographic profiles obtained for the byproduct resinoid (R1) and the SBR differed only by the absence of coniferyl benzoate in the SBR. This compound is assumed to react more easily with ethanol and water during the industrial manufacturing process. In fact, a laboratory-made benzoin gum resinoid produced under the same conditions as R1 showed an intense coniferyl benzoate peak, thus displaying a very close similarity to the byproduct resinoid after HPLC analysis. In contrast, the chromatographic profile of the sorted byproduct resinoid (R2) differed strongly from the two others, with the previously

observed major components nearly disappearing in R2. In view of these results, we can conclude that the resinoids obtained from grade 3 Siam benzoin gum and from the byproduct present very similar chemical compositions.

The olfactory evaluation of the three resinoids was performed by a perfumer using 10% ethanolic solutions. The byproduct resinoid presented a weak vanilla top note and a benzoic acid bottom note, providing a powdery, pungent, and balsamic effect. The Siam benzoin gum resinoid afforded more pronounced and persistent middle and bottom notes. In contrast, the sorted byproduct resinoid provided a powerful vanilla, woody, and sweety bottom note.

In conclusion, on the basis of these results showing a similarity between extracts obtained from the byproduct and Siam benzoin gum, this gum-harvesting byproduct could be regarded as a new grade of Siam benzoin gum, possibly as a grade 6. This grade, which contains a larger proportion of bark than the other grades, presents interesting olfactory properties. Finally, extracts obtained from this new grade could be considered as new benzoin gum resinoids and may offer attractive applications for the flavor and fragrance industry.

ACKNOWLEDGMENT

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