Aroma Constituents and Alkylamides of Red and Green Huajiao (*Zanthoxylum bungeanum* and *Zanthoxylum schinifolium*)

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Huajiao denotes the fruits of various species of *Zanthoxylum* in the plant family Rutaceare used for cooking. The two most commercially popular species are *bungeanum* (red huajiao) and *schinifolium* (green huajiao). Fresh huajiao has a very high content of essential oil, up to 11%, which is described as having fresh, spicy, floral, cooling, and green aroma notes. A comprehensive analysis of the essential oils by GC-MS using advanced peak deconvolution and data processing software, revealed many overlapping components. A total of 120 aroma compounds for each species has been found. In the essential oils, linalyl acetate (15%), linalool (13%), and limonene (12%) are the major components of red huajiao, whereas linalool (29%), limonene (14%), and sabinene (13%) are the major components of green huajiao. For estimation of the aroma contribution of individual components, a new concept, "aroma character impact value" (ACI), is introduced as the percentage of the ratio of the concentration of an aroma component to its odor threshold value. Despite the differences in major components, both species have six common compounds of top aroma character impact: linalool, α-terpineol, myrcene, 1,8-cineole, limonene, and geraniol. The tingling sensation of huajiao is caused mainly by the alkylamide hydroxy-α-sanshool. The tingling compound decomposes easily under hydrolytic conditions or under UV light.

KEYWORDS: Huajiao; Sichuan pepper; *Zanthoxylum bungeanum* Maxim.; *Zanthoxylum schinifolium* Sieb. et Zucc.; spice; tingling; aroma character impact; (ACI); hydroxy-α-sanshool

INTRODUCTION

The Chinese province Sichuan, known as the "land of plenty" and "land of fish and rice," is the origin for a rich and diverse cuisine. The Sichuanese cuisine is well-known for its tasteful spiciness and use of a wide range of native ingredients. When tasting a typical Sichuanese dish, visitors are often surprised by a unique taste attribute known as "ma" − the feeling of tingling in the mouth. Indeed, "ma la" (tingling and chili) is the major characteristic sensory sensation of Sichuanese cuisine, such as "Ma La Huo Guo" (tingling and chili hot pot). The chili taste comes from chili pepper, and the "ma" effect is produced from a spice known as huajiao, dried fruits of various local species of *Zanthoxulum* in the plant family Rutaceae.

The genus *Zanthoxylum* has more than 200 species. These plants are aromatic trees and shrubs, native to warm temperate and subtropical areas worldwide. In Asia, this genus is often found in the Himalaya region and additionally in Central, South, Southeast, and East Asia (*1, 2*). The fruits of these species have a distinctive aroma that can be generally described as fresh, floral, spicy, and green. The chemical composition and sensory profile of the fruits vary among the species; however, many of them have a distinctive tingling taste. Because of their unique

aroma and taste, the fruits of many species of *Zanthoxylum* have been used as spice in local cuisines in the Asian region. To the best of the author's knowledge, American and African *Zanthoxylum* species have not yet been used for culinary purposes. *Zanthoxylum* is a complicated genus with many different and not well-defined species. The literature often gives contradictory information about the local species used as spice.

Although the culinary application of the fruits of *Zanthoxylum* species is rather widespread in Asia, the Sichuanese probably have the longest history and the most intensive use in cookery. The *Zanthoxylum* species used as spice in Sichuan is well-known as "Sichuan pepper." This name sometimes also refers to all related species of *Zanthoxylum* used as spice. This may be due to the rich culinary culture and aroma and taste quality of the local species. There are many different names for the *Zanthoxylum* genus in China, the most popular name being "huajiao" (flower pepper), which refers to the group of related species.

The fruits of *Zanthoxylum bungeanum* Maxim are the most popular huajiao commercial product, called "da hong pao" (big red robe). This species is native to southwestern China in the provinces Sichuan, Yunnan, Guizhou, Tibet, Guanxi, and Guandong. The area Hanyuan in the province of Sichuan is well-known for the production of the best quality of da hong pao.

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"Green huajiao" is the other popular and widely used spice in Sichuan, which is the fruit of *Zanthoxylum schinifolium* Sieb. et Zucc (*1*).

In addition to culinary applications, many species of *Zanthoxylum* have also been used as drugs in traditional Chinese medicine for epigastric pain (*2*). The essential oil of huajiao was found to have antioxidant activity (*3, 4*). Some components showed significant biological activities such as antimicrobial and anti-inflammation (*5–8*). Huajiao is also used as an ingredient in skin care products (*9*). The North American species, *Z. americanum*, is known as "toothache tree". Due to the anesthetic power of its alkylamide constituents, the plant is effective in suppressing toothache temporarily when the unripe fruit or the wood of young branches is chewed.

In developing huajiao as a flavoring ingredient, we separated huajiao into two fractions. The nonpolar fraction mainly represents the aroma of huajiao, whereas the polar fraction contains the taste components. The huajiao extracts have great potential for applications in savory and sweet goods and beverages. Therefore, it is of great interest to investigate the compositions of essential oils and extracts. In this paper, we report comprehensive analytical results of aroma constituents and alkylamides present in the red and green huajiao. The concept of aroma character impact (ACI) is introduced to compare the aroma contribution of individual components in a mixture.

MATERIALS AND METHODS

Raw Material. Red huajiao (da hong pao) and green huajiao were obtained as commercial products in the market of Chongqing, China.

Reference Material. Reference materials of mass spectra and GC retention indices were obtained from commercial sources (see **Table 1** for details).

Essential Oil Preparation. Huajiao essential oil was obtained by hydrodistillation. Fifty grams of huajiao was ground in liquid nitrogen. The distillation was then carried out in a 1000 mL round-bottom flask with 250 mL of water for 2 h. The collected essential oil was finally dried over Na_2SO_4.

GC-MS Analysis. GC-MS data were obtained with an Agilent 6890 GC equipped with 5973 mass selective detector and a 60 m (0.32 mm i.d., 0.25 μm film) DB-5 ms capillary column (Agilent, Wilmington, DE). Helium was used as carrier gas with a flow rate of 30 cm/s. A sample of 0.02 μL of essential oil was analyzed in the GC-MS system with a cooled on-column injection. The column temperature was held at 50 °C for 2 min and then programmed to 270 at 3 °C/min. The mass selective detector was operated in positive EI mode with a mass scan range from *m/z* 30 to 350 at 70 eV. GC-MS data processing was achieved using the following software programs: automated mass spectral deconvolution and identification system (AMDIS, version 2.65, National Institute of Standards and Technology, Gaithersburg, MD), NIST mass spectral search program (version 2.0 d, National Institute of Standards and Technology), and an in-house Microsoft Excel-based data analysis program for GC-MS data processing, which identifies GC peaks based on mass spectrum and retention index match.

LC-MS Analysis. LC-MS analysis was performed using an Agilent 1200 HPLC equipped with a binary pump, a degasser, an autosampler, and a PDA detector. The HPLC was interfaced with a Waters micro Q-tof mass spectrometer fitted with an ESI source (Waters, Milford, MA). Full-scan mode from *m/z* 100 to 1000 was performed with a capillary voltage of 3 kV, a collision energy of 5 eV, a sample cone voltage of 30 V, a desolvation temperature of 120 °C, a source temperature of 250 °C, and a desolvation gas flow rate of 400 L/h.

An HPLC column (Luna 3μ C18, 150 × 2 mm i.d., Phenonemex, Torrance, CA) was used for the analysis. Solvent A was water with 0.1% formic acid; solvent B was acetonitrile with 0.1% formic acid. Solvents were delivered at a total flow rate of 0.4 mL/min. The solvent

gradient was from 30% B to 70% B linearly in 15 min, a linear increase to 100% B at 1 min, followed by 15 min isocratic, and then a return to 30% B at 30 min.

Extraction and Isolation. One hundred grams of dried huajiao was placed into a 1 L jar, and 200 mL of pentane was added. Huajiao and the solvent were mixed in an Omni mixer/homogenizer with blades for 2 min. During the process, huajiao was cut into small particles. The mixture was filtered. The extraction procedure was repeated three times. The pentane extracts were combined. The solvent was removed using a TurboVap 500 (Zymark Corp., Hopkinton). The extraction yielded 5 g of fraction A.

The residue from the extraction of fraction A was extracted again with 200 mL of hexane/ethyl acetate (1:4 by volume) three times. The organic phase was combined. The solvents were removed using a Turbo Vap 500 and yielded 6 g of fraction B. The residue after extraction did not have any significant taste.

Fraction B (1 g) was fractionated using the Flash system (Flash 40M, KP-Sil, 32–63 μm, Biotage, Charlottesville, VA). The solvent system used for the elution was pentane/DCM (1:1, 400 mL), DCM (400 mL), MTBE (400 mL), and MTBE with 2% methanol (400 mL). A total of 14 fractions was afforded. After solvents were removed, the fractions were tasted in aqueous solution and analyzed by HPLC. The taste screening was performed by the laboratory staff (four or five panelists). Fractions having tingling taste were further separated using semi-preparative HPLC, which was performed using an Agilent 1200 HPLC. An HPLC column (Luna 3μ C18, 250 × 10 mm i.d., Phenonemex) was used for the separation.

NMR Analysis. NMR experiments (^{1}H, ^{13}C, HMQC, HMBC, and NOESY) were performed for the structure elucidation using a Bruker DRX 500 spectrometer equipped with a 5 mm inverse probe. The four compounds were identified as hydroxy-α-sanshool, hydroxy-β-sanshool, hydroxy-γ-sanshool, and hydroxy-ε-sanshool.

Preliminary Study on the Stability of Hydroxy-α-sanshool. Pure hydroxyl-α-sanshool was dissolved in an 80% ethanol solution (0.02 mg/mL). The solution was divided into two containers. One was stored at room temperature, and the other was under UV light (λ = 254 nm). Aliquots were taken at six different times (2, 4, 9, 12, 24, and 72 h). The concentration of hydroxyl-α-sanshool was analyzed by HPLC-UV.

RESULTS AND DISCUSSION

Composition of Essential Oils. Huajiao flavor is not stable. Its aroma and taste deteriorate greatly just one year after storage at room temperature. The best quality available (grade 1, the same year crop) was chosen for flavor evaluation and analysis. The essential oil of *Zanthoxylum* species is most abundant in its pericarp, although essential oils have also been found in its leaves, bark, and roots. In contrast to most results reported in the literature of about 3–4% yield of essential oil for the dried huajiao pericarp (*10–12*), we obtained 8–11% yield via hydro-distillation of the fresh dried huajiao samples (from the same year crop). The aroma of the essential oils was described as floral, pungent, cooling, green, and refreshing. Although the red and green huajiao essential oils have many similarities in the main aroma characteristics, the green huajiao essential oil has more refreshing and green notes. Interestingly, flavorists tend to favor the aroma of red huajiao, whereas perfumers largely prefer the aroma of green huajiao.

The composition of the essential oils of red and green huajiao was analyzed by GC-MS. A total of 120 components, accounting for >98% of total peak area, was detected for each oil. The components are listed in **Table 1**. The reported GC peak areas were obtained by a peak deconvolution algorithm so that overlapping peaks such as limonene and 1,8-cineole could be differentiated. For green huajiao, linalool (29%) is the major aroma component, followed by limonene (14%) and sabinene (13%), whereas linalyl acetate (15%), linalool (13%), and limonene (12%) are the main components in red huajiao

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Table 1. Chemical Composition of Huajiao Essential Oils

RI	compound	green huajiao (area %)[a]	red huajiao (area %)[a]	ID[b]	reference source[c]
768	acetaldehyde	0.005	0.002	RI, MS	Givaudan
968	3-buten-2-one	0.001	0.003	RI, MS	Aldrich
982	diacetyl	0.002	nd	RI, MS	Givaudan
1000	decane	0.003	nd	RI, MS	Aldrich
1023	isobutyl acetate	0.028	0.266	RI, MS	Givaudan
1033	α-pinene	1.56	0.347	RI, MS	Aldrich
1030	α-thujene	0.517	0.760	RI, MS	other
1044	2-methyl-3-buten-2-ol	0.012	0.012	RI, MS	Acros
1055	toluene	0.002	0.001	RI, MS	Aldrich
1056	crotonaldehyde	0.001	nd	RI, MS	Acros
1078	camphene	0.015	0.008	RI, MS	Givaudan
1094	isobutanol	0.021	0.027	RI, MS	Givaudan
1095	hexanal	0.003	0.010	RI, MS	Givaudan
1121	β-pinene	1.36	0.345	RI, MS	Givaudan
1132	sabinene	13.1	5.55	RI, MS	Bedoukian
1151	1-butanol	0.001	nd	RI, MS	Givaudan
1152	methyl 4-methylvalerate	0.026	0.005	RI, MS	Givaudan
1164	δ-3-carene	0.001	nd	RI, MS	Acros
1169	butyl butyrate	0.003	nd	RI, MS	Givaudan
1173	myrcene	6.02	5.40	RI, MS	Givaudan
1176	α-phellandrene	0.187	0.113	RI, MS	Givaudan
1196	α-terpinene	1.21	2.96	RI, MS	Givaudan
1201	isoamyl alcohol	0.001	0.001	RI, MS	Givaudan
1201	2,3-dehydro-1,8-cineole	nd	0.003	MS	other
1217	limonene	14.3	12.3	RI, MS	Givaudan
1226	1,8-cineole	1.32	5.60	RI, MS	Givaudan
1227	β-phellandrene	3.40	2.30	RI, MS	other
1244	cis-β-ocimene	0.430	1.49	RI, MS	Givaudan
1250	linalool oxide dehydrate	nd	0.012	RI, MS	Bedoukian
1257	6-methylheptanal	0.037	nd	RI, MS	other
1261	γ-terpinene	2.38	4.86	RI, MS	Givaudan
1262	p-mentha-1(7),2,8-trien	nd	0.008	MS	other
1270	2,6-dimethylheptanal	0.015	nd	MS	other
1281	hexyl acetate	0.001	0.013	RI, MS	Givaudan
1282	p-cymene	0.317	0.273	RI, MS	Givaudan
1299	terpinolene	0.677	1.46	RI, MS	Givaudan
1296	octanal	0.007	0.005	RI, MS	Givaudan
1317	4-methyl-1-pentanol	0.006	nd	RI, MS	Aldrich
1322	prenol	nd	0.003	RI, MS	Givaudan
1328	2-propenal	0.001	nd	RI, MS	Aldrich
1344	6-methyl-5-hepten-2-one	nd	0.003	RI, MS	Givaudan
1354	1-hexanol	0.006	nd	RI, MS	Givaudan
1359	3-nonanone	nd	0.001	RI, MS	Givaudan
1361	ethyl pyruvate	nd	trace	RI, MS	Givaudan
1372	heptyl acetate	0.004	0.013	RI, MS	Givaudan
1376	trans-allo-ocimene	0.001	0.005	RI, MS	Aldrich
1400	tetradecane	0.126	0.005	RI, MS	Aldrich
1423	nonanal	0.087	0.019	RI, MS	Givaudan
1425	rosefuran	nd	0.007	RI, MS	other
1438	myrcene epoxide	0.009	0.005	MS	other
1438	2,4-hexadienal	nd	0.030	RI, MS	Givaudan
1442	2,6-dimethyl-2-heptenal	0.022	nd	RI, MS	Pfaltz & Bauer
1444	perillene	0.023	0.045	RI, MS	other
1460	α-thujone	1.48	0.038	RI, MS	Aldrich
1469	trans-linalool oxide	0.314	0.207	RI, MS	Givaudan
1470	p,α-dimethylstyrene	0.007	0.013	RI, MS	Givaudan
1474	1-heptanol	0.018	nd	RI, MS	Givaudan
1475	p-mentha-1,3,8-triene	0.004	0.008	RI, MS	other
1476	bois de rose ketone	nd	0.001	RI, MS	other
1479	isodecanal	0.067	nd	MS	other
1480	β-thujone	0.962	0.035	RI, MS	other
1484	α-cubebene	nd	0.007	RI, MS	Aldrich
1487	trans-sabinene hydrate	1.12	0.292	RI, MS	other
1494	trans-sabinene hydrate acetate	nd	0.434	MS	other
1496	trans-limonene oxide	0.018	nd	RI, MS	Aldrich
1498	cis-linalool oxide	0.269	0.109	RI, MS	Givaudan
1499	nerol oxide	nd	0.014	RI, MS	other
1500	pentadecane	0.134	nd	RI, MS	Fluka
1507	citronellal	0.067	0.009	RI, MS	Givaudan
1514	trans-ocimene epoxide	nd	0.005	RI, MS	other
1525	α-copaene	nd	0.032	RI, MS	Fluka
1527	decanal	0.047	nd	RI, MS	Givaudan
1530	6-methyl-1-heptanol	0.074	nd	MS	other
1531	2,4-heptadienal	nd	0.004	RI, MS	Givaudan

Table 1. Continued

RI	compound	green huajiao (area %)[a]	red huajiao (area %)[a]	ID[b]	reference source[c]
1537	4-methyl-1-heptanol	0.007	nd	MS	other
1566	linalool	29.3	13.2	RI, MS	Givaudan
1570	β-cubebene	nd	0.042	RI, MS	other
1578	cis-sabinene hydrate	0.469	nd	MS	other
1581	linalyl acetate	3.99	15.2	RI, MS	Givaudan
1588	methyl citronellate	0.003	nd	RI, MS	other
1593	isopulegol	nd	0.003	RI, MS	Alfa Aesar
1594	1-terpineol	0.337	0.792	RI, MS	other
1600	hexadecane	0.261	nd	RI, MS	Aldrich
1601	lilac aldehyde	nd	0.036	RI, MS	other
1601	linalyl formate	0.012	0.017	RI, MS	Aldrich
1606	pinocarvone	0.070	0.003	RI, MS	other
1607	α-bergamotene	nd	0.010	RI, MS	other
1610	bornyl acetate	0.035	0.046	RI, MS	Givaudan
1611	β-elemene	nd	0.013	RI, MS	other
1615	hotrienol	0.037	0.076	RI, MS	other
1615	linalyl propionate	nd	0.007	RI, MS	Givaudan
1618	neoiso-isopulegol	0.005	nd	RI, MS	other
1619	4-terpinenol	3.96	7.79	RI, MS	Givaudan
1620	β-caryophyllene	1.38	0.300	RI, MS	Givaudan
1629	4-terpinenyl acetate	0.021	0.285	RI, MS	other
1631	3-methylpentadecane	0.066	nd	MS	Pfaltz & Bauer
1634	dihydrocarvone	0.007	0.017	RI, MS	other
1636	cis-p-2-menthen-1-ol	0.227	0.545	MS	other
1638	cis-p-mentha-2,8-dien-1-ol	n.d.	0.006	RI, MS	other
1644	p-menth-1-en-9-al	trace	0.018	MS	other
1653	allo-aromadendrene	nd	0.008	RI, MS	Fluka
1658	1-nonanol	0.029	nd	RI, MS	Givaudan
1660	myrtenal	0.295	0.004	RI, MS	Givaudan
1662	citronellyl acetate	nd	0.026	RI, MS	Givaudan
1663	trans-β-farnesene	nd	0.059	RI, MS	Fluka
1669	γ-gurjunene	nd	0.021	RI, MS	Fluka
1675	trans-pinocarveol	0.016	0.003	RI, MS	Fluka
1681	trans-p-mentha-2,8-dien-1-ol	nd	0.038	RI, MS	other
1684	verbenol	0.022	nd	RI, MS	Bedoukian
1694	α-humulene	0.680	0.416	RI, MS	Fluka
1703	p-mentha-1,8-dien-4-ol	0.005	0.064	RI, MS	other
1706	2,4-diethylheptan-1-ol	0.021	nd	RI, MS	other
1705	cryptone	nd	0.169	RI, MS	other
1712	α-terpineol	1.37	3.93	RI, MS	Givaudan
1715	α-terpinyl acetate	0.185	2.28	RI, MS	Givaudan
1723	borneol	0.037	nd	RI, MS	Aldrich
1727	3,6-α-farnesene	0.011	0.057	RI, MS	Fluka
1733	neryl acetate	0.113	1.32	RI, MS	Givaudan
1733	germacrene D	2.70	0.532	RI, MS	other
1746	γ-cadinene	0.029	0.124	RI, MS	other
1746	α-muurolene	0.001	nd	RI, MS	other
1742	2-oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, 6-acetate	0.008	0.111	RI, MS	other
1752	α-selinene	0.005	nd	RI, MS	other
1747	α-farnesene	nd	0.110	RI, MS	Fluka
1754	cis-linalool oxide	0.003	0.010	RI, MS	other
1755	trans-piperitol	nd	0.279	RI, MS	other
1764	bicyclogermacrene	0.730	nd	RI, MS	other
1758	geranyl acetate	nd	2.19	RI, MS	Givaudan
1769	carvone	0.074	0.217	MS	other
1770	citronellol	0.020	nd	RI, MS	Givaudan
1781	δ-cadinene	0.103	0.530	RI, MS	Fluka
1789	benzeneacetic acid, methyl ester	0.068	nd	MS	other
1782	cis-carveyl acetate	nd	0.011	RI, MS	Givaudan
1812	nerol	0.099	0.806	RI, MS	Givaudan
1817	myrtenol	0.220	0.018	RI, MS	Acros
1820	2-tridecanone	0.011	nd	RI, MS	Givaudan
1811	2,4-dimethylacetophenone	0.049	0.064	RI, MS	Aldrich
1834	2-phenylethyl acetate	0.065	0.089	MS	other
1842	trans-carveol	0.062	0.111	RI, MS	other
1857	trans-myrtanol	0.074	nd	RI, MS	other
1857	geraniol	0.250	1.78	RI, MS	Givaudan
1855	germacrene B	nd	0.081	MS	other
1871	p-cymen-8-ol	0.049	0.088	MS	other
1868	isopiperitenone	nd	0.014	RI, MS	other
1872	cis-carveol	0.031	0.085	RI, MS	other
1910	p-1,8-menthadienyl-7 acetate	nd	0.027	RI, MS	other
1931	α-calacorene	nd	0.001	RI, MS	other
1986	p-mentha-1,8(10)-dien-9-ol	nd	0.009	RI, MS	other
1995	caryophyllene oxide	0.090	0.087	RI, MS	Givaudan

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Table 1. Continued

RI	compound	green huajiao (area %)[a]	red huajiao (area %)[a]	ID[b]	reference source[c]
2004	*trans*-nerolidol	0.050	0.139	RI,MS	Fluka
2031	germacrene D-4-ol	0.047	0.007	RI,MS	other
2038	humulene oxide	nd	0.044	RI,MS	other
2096	α-elemol	0.054	nd	RI,MS	other
2066	*p*-isopropylbenzyl alcohol	nd	0.030	RI,MS	Aldrich
2076	10-epi-γ-eudesmol	0.042	0.010	RI,MS	other
2111	2-propenoic acid, 3-phenyl-, methyl ester	0.036	nd	RI,MS	Givaudan
2144	spathulenol	0.087	nd	RI,MS	other
2180	epi-α-cadinol	0.044	0.094	RI,MS	other
2201	δ-cadinol	0.006	nd	RI,MS	other
2229	α-cadinol	0.045	nd	RI,MS	other

[a] nd, not detected; trace, trace amount (<0.001%). [b] RI, match with reference GC retention index; MS, matched with reference mass spectrum. [c] Reference materials were obtained from the following commercial sources: Givaudan, Givaudan Corp., Cincinnati, OH; Aldrich, Sigma-Aldrich, St. Louis, MO; Acros, Acros Organics, Morris Plains, NJ; Bedoukian, Pfaltz & Bauer, Waterbury, CT; Fluka, Sigma-Aldrich, St. Louis, MO; Alfa Aesar, Alfa Aesar Ward Hill, MA; other, reference mass spectrum and retention index were measured outside the author's laboratory (the identification is therefore considered to be tentative).

essential oil. To determine aroma character impact components, aroma dilution analysis with gas chromatography−olfactometry (GCO) is one of the often used techniques (*13, 14*). The other method for evaluating the aroma impact of a known composition is based on the components' odor threshold value and their concentration in the aroma. The odor activity value is thus defined as the ratio of the concentration of an aroma component to its odor threshold value. We often use aroma character impact (ACI) to compare aroma contributions of the components in a mixture:

$$ACI_i\% = \frac{C_i/T_i}{\sum\limits_k C_k/T_k} \times 100 \qquad (1)$$

where C_i is the headspace concentration and T_i is the threshold value of vapor of the ith component.

$C_i = C_T P_i$, where C_T is the total concentration and P_i is the concentration percentage of ith component, therefore

$$ACI_i\% = \frac{C_T P_i/T_i}{\sum\limits_k C_T P_k/T_k} \times 100 = \frac{P_i/T_i}{\sum\limits_k P_k/T_k} \times 100 \qquad (2)$$

The peak area percentages of a gas chromatogram from FID or MSD response are generally good approximations for the aroma component ratio and can be used to replace P_i in the above equation. To estimate the aroma character impact components, we assume that the evaporation is nondiscriminated during smell so that we can use the threshold value of a component in air to calculate ACI.

The ACI values of the essential oils of red and green huajiao were calculated on the basis of the threshold data of 84 compounds retrieved from our sensory threshold database of flavoring material. The red and green huajiao have six common components as their major aroma character impact molecules. These components comprise 52 and 42% of total GC peak area but have 92 and 91% aroma contributions in green and red huajiao, respectively. For both huajiao spices, linalool contributes the most to their aroma character. For green huajiao, linalool is the most prominent aroma contributor (76%), whereas other components play a much less important role. Although linalool is still the major aroma contributor (39%) for red huajiao, 1,8-cineole (19%), α-terpineol (16%), and geraniol (11%) are significant contributors (**Table 2**).

The goodness of estimation of aroma character impact using ACI value for an essential oil depends on the availability and accuracy of the threshold data, as well as the completeness of

Table 2. Major Aroma Character Impact Components in Huajiao

compound	green huajiao		red huajiao	
	area %	ACI %	area %	ACI %
linalool	29.3	76	13.2	39
α-terpineol	1.37	5	3.93	16
myrcene	6.02	4	5.40	4
1,8-cineole	1.32	4	5.60	19
limonene	14.3	2	12.3	2
geraniol	0.25	1	1.78	11
total	52.56	92	42.21	91

identification of significant aroma components. This estimation serves two purposes: to provide a first glance of the important aroma contributors among the known components and a "visual smell" of the essential oils − imagining the smell when viewing the composition; and to test if any significant aroma contributors are unidentified and/or not in the sensory threshold database. Aroma reconstitution can be conducted using selected components based on ACI value to check the estimation to the actual aroma. A significant discrepancy between the reconstituted aroma and the essential oil often reveals the presence of unknown potent aroma components in the essential oil.

Aroma reconstitutions were made for both huajiao species using the six major aroma impact components. The sensory profile of the simple reconstitutions resembles that of the corresponding essential oil aromas. The results indicate that there are no unknown components with major aroma impact. As expected, however, these reconstitutions are by no means exact copies of the original aroma: they lack the complexity, richness, and depth of the aroma profile of the essential oils. As we have often observed, the minor aroma contributors, even with their concentrations below the threshold value, often collectively play a very important role in aroma perception. They provide background, richness, and roundness of the aroma as a whole.

Jiang and Kubota reported that the major components of the essential oil of dried pericap of *Z. piperitum* from Japan were limonene (29.54%), β-phellandrene (17.79%), geranyl acetate (17.71%), geraniol (8.9%), and citronellal (7.26%) (*15*). Their results of aroma dilution analysis indicated that geraniol, citronellal, and linalool were the most potent aroma contributors for the dried pericarp. We calculated ACI values on the basis of the reported composition of the essential oil composition and compared them with the GCO results. The major aroma impact components are listed in **Table 3**. The calculated ACI results largely agree with the GCO analysis: geraniol and citronellal are the major aroma impact components. However, ACI values

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Table 3. Comparison of FD (*15*) with ACI for the Major Aroma Impact Components in *Z. piperitum*

compound	area % (*15*)	ACI %	FD (*15*)
geraniol	8.9	58	4096
citronellal	7.26	16	1024
limonene	29.54	5	64
methyl cinnamate	0.83	4	256
myrcene	4.4	3	64
linalool	0.78	2	1024
β-phellandrene	17.79	2	64
geranial	0.34	2	16
piperitone	0.43	2	16
geranyl acetate	17.71	1	256
total	87.98	95	

indicate lower aroma contributions for linalool, methyl cinnamate, and geranyl acetate than GCO results. One possible reason for the different results is the variation of concentration calculated on the basis of the MSD response.

The essential oil composition of huajiao reported in the literature varies widely depending not only on species but also geographic regions, aging, and preparation methods. *Z. piperitum* from Thailand was reported to have limonene (37.99%), sabinene (13.3%), and *β*-myrcene (7.17%) in its essential oil as the major components (*16*). Iseli et al. reported the composition of essential oils of *Z. bungeanum* and *Z. schinifolium* obtained in China (*17*). They identified 20 components and found that the oils of the two species have similar composition, mainly composed of limonene (18.48–21.63%), 4-terpineol (14.5–15.98%), *γ*-terpinene (6.01–8.19%), *β*-linalool (4.32–5.18%), and *α*-terpineol acetate (5.13–5.33%). Wang et al. also found that limonene was the major component in the essential oil of *Z. bungeanum*, but the composition of the oil was very different (*18*): limonene (14.82%), myrcene (13.26%), 1,8-cineole (10.69%), sabinene (6.74%), *α*-pinene (5.51%), *α*-thujene (4.99%), linalool (4.51%), and *α*-terpineol (4.32%). Supercritical fluid extraction has also been applied to huajiao. The major components of the supercritical CO_2 extract of *Z. bungeanum* were reported as linalool (32.43%), limonene (13.89%), polyene amides (13.47%), and *β*-pinene (8.13%) (*19*). Some nonvolatile components were also found in the extract.

For the species of rhetsa, sabinene (55–66.7%), terpinene-4-ol, and *α*-terpineol were the major components (*20–22*). The wild-growing *Z. acanthopodium* in northern Sumatera, Indonesia, has an exotic citrus-like aroma. The major aroma components are geranyl acetate (32.04%) and limonene (15.8%) (*23*).

Although hydrodistillation is a common method used to obtain essential oils, it causes the degradation of the taste components in huajiao. To produce both aroma and taste extracts for different food/beverage applications, we have developed a solvent extraction process. Extraction with hexane affords an aroma extract with an aroma similar to that of the essential oil, although a small part of the taste components was also extracted in the aroma fraction (fraction A). A subsequent extraction by ethyl acetate/acetone yields a tingling fraction (fraction B), which contains 60−80% hydroxyl-α-sanshool.
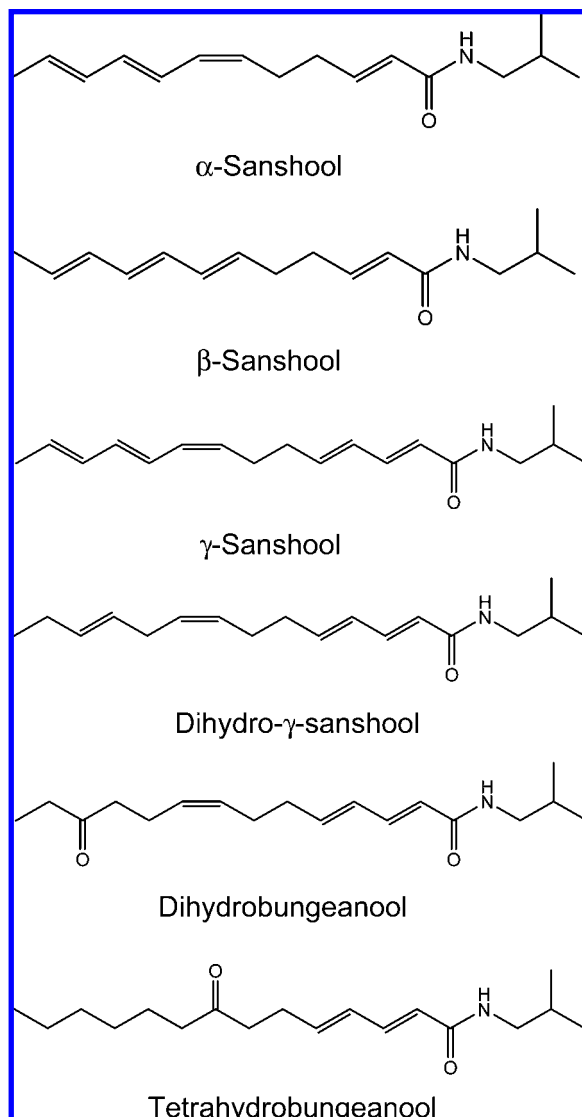
Alkylamides and the Tingling Principle. The interesting taste of huajiao is described as "ma" or tingling; a distinctive trigeminal effect on the tongue and in the mouth. It has been reported that the tingling taste of *Zanthoxylum* fruits is caused by a series of polyunsaturated alkylamides. The taste sensation of huajiao components was studied by Bryant and Mezine at Monell Chemical Senses Center (*24*). The authors examined

Scheme 1. Tingling components found in huajiao. Concentration ranges of dried huajiao are indicated in parentheses

Hydroxy-α-sanshool (3-6%)

Hydroxy-β-sanshool (0.5-2%)

Hydroxy-ε-sanshool (0.1-1%)

Hydroxy-γ-sanshool (trace)
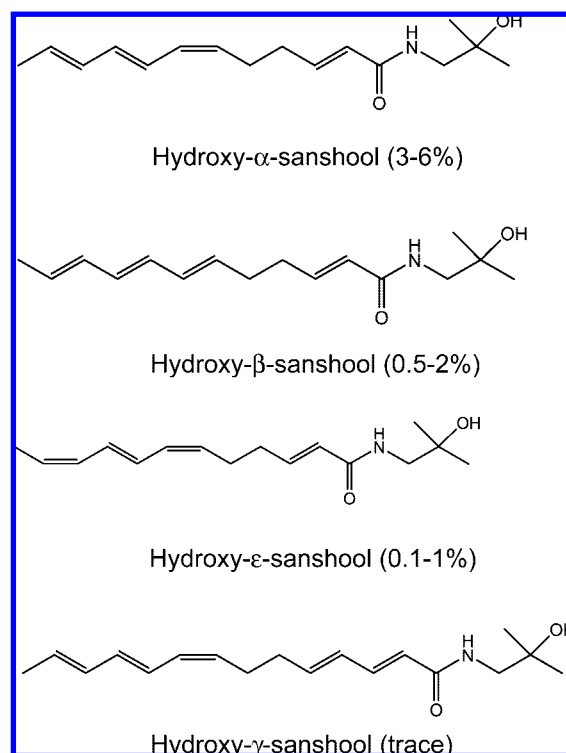
three unsaturated hydroxy-alkylamides isolated from huajiao: hydroxy-α-sanshool, hydroxy-β-sanshool, and hydroxy-ε-sanshool. Hydroxy-α-sanshool was found to be the primary pungent compound, which altered the levels of spontaneous activity in cool-sensitive fibers as well as inducing activity in tactile fibers, cold nociceptors, and silent fibers that were insensitive to innocuous thermal or tactile stimuli. The neuronal distribution of sensitivities to capsaicin and to hydroxy-α-sanshool indicates that this compound affects neurons mediating innocuous sensations. Hydroxy-ε-sanshool also showed the same tingling sensation, whereas hydroxy-β-sanshool was inactive in human tests at 100 μg.

Huajiao fraction B was further fractionated by flash chromatography. Fractions with a tingling taste were analyzed by LC-MS. Four taste active components were isolated by semipreparative HPLC. In the high resolution mass spectra, three components showed a peak at *m/z* 264.1958, corresponding to the protonated molecular ion $[C_{16}H_{25}NO_2 + H]^+$ (calculated *m/z* 264.1964), whereas component 4 showed a peak at *m/z* 290.2108, corresponding to the protonated molecular ion $[C_{18}H_{27}NO_2 + H]^+$ (calculated *m/z* 290.2120). These components were identified as hydroxy-α-sanshool, hydroxy-β-sanshool, hydroxy-γ-sanshool, and hydroxy-ε-sanshool, and their NMR spectra matched those reported in the literature (*31*). Hydroxy-ε-sanshool was not detected in green huajiao extract. The structures of these hydroxy sanshools and their contents in huajiao are shown in **Scheme 1**. On the basis of the tingling intensity and concentration of these sanshools, our taste evaluation concluded that hydroxy-α-sanshool is the principal tingling component for both red and green huajiao.

In addition to its tingling taste, huajiao also has a pungent sensation. Two pungent principles from the pericarps of *Zanthoxylum ailanthoides*, *γ*-sanshool and hydroxy-*γ*-sanshool, were reported (*25*). Etsuko et al. (*26*) conducted sensory evaluation of pungent qualities of four sanshools (*α*, *β*, *γ*, and

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Scheme 2. Other alkylamides found in other species of *Zanthoxylum*

α-Sanshool

β-Sanshool

γ-Sanshool

Dihydro-γ-sanshool

Dihydrobungeanool

Tetrahydrobungeanool

Figure 1. Stability of hydroxy-α-sanshool in 80% ethanol solution.

We further studied light effect on the stability of hydroxyl-α-sanshool. **Figure 1** shows the degradation of hydroxyl-α-sanshool in an 80% enthanol solution at a concentration of 200 ppm under different storage conditions. At room temperature, the concentration of the pure compound decreased about 70% in 24 h. When exposed to UV light, it disappeared completely in 4 h. A preliminary study indicated that hydroxyl-α-sanshool easily undergoes isomerization, hydrolysis, and oxidation under normal storage conditions. It is more stable at lower temperature and less stable in acidic solutions. However, the aroma and taste extracts of huajiao seem to be more stable than the dried pericarp. After storage at room temperature for a year, no large changes in aroma and taste were observed. More stable derivatives of sanshools have been synthesized (*32*).

ABBREVIATIONS USED

ACI, aroma character impact; SU, Scoville unit; FD, flavor dilution; GCO, gas chromatography−olfactometry; DCM, dichloromethane; MTBE, methyl *tert*-butyl ether.

δ) and two hydroxy sanshools (α and *β*). The four sanshools have Scoville unit (SU) values ranging 80000 to 110000. The SU values of hydroxy sanshools were 3–5-fold lower than those of the corresponding sanshools. They also found that these compounds have different taste profiles. Hydroxy-α-sanshool was perceived as tingling and numbing, whereas α-sanshool was burning and tingling and *γ*-sanshool had burning and fresh sensations.

Ten unsaturated alkylamides have been found in several different species of *Zanthoxylum* (*27–30*). **Scheme 2** shows the other alkylamides that were not identified in the red and green huajiao, but were reportedly present in other species.

Stability. It is well-known that the flavor of huajiao is not stable. Considerable quality degradation can be observed after approximately one year of storage at room temperature. Old products have much less aroma and less tingling taste intensity. The unsaturated alkylamides, the taste components in huajiao, are not stable in aqueous solution: After 4 weeks at 22–24 °C, an aqueous solution of hydroxyl-α-sanshool (0.2 mg/mL, pH 7.0) showed 50% degradation. These compounds are also not stable at high temperature, for example, after 1 h at 160 °C in vegetable oil, ca. 30% degradation into an inactive form was observed. They may be sensitive to oxygen due to the conjugated triene system (*24*).
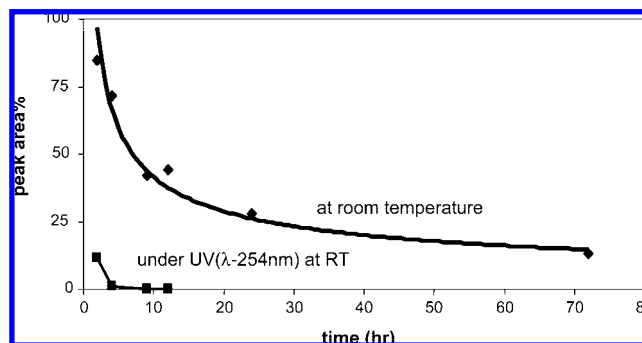
LITERATURE CITED

(1) Nanjing University of Chinese Medicine. *Dictionary of Chinese Medicine*, 2nd ed.; Shanghai Scientific & Technical Publishers: Shanghai, China, 2006; pp 1469–1473.

(2) National Pharmacopoeia Committee. *Chinese Pharmacopoeia* **2005**, *I*, 116.

(3) Yamazaki, E.; Inagaki, M.; Kurita, O.; Inoue, T. Antioxidant activity of Japanese pepper (*Zanthoxylum piperitum* DC.) fruit. *Food Chem.* **2006**, *100* (1), 171–177.

(4) Liu, X.; Zhang, Z.; Duan, Y.; Liu, Q.; Cui, J. Antioxidant activities of herbs and spices from commercial sources. *Shipin Kexue* **2002**, *23* (1), 143–145.

(5) Setzer, W. N.; Schmidt, J. M.; Eiter, L. C.; Haber, W. A. The leaf oil composition of *Zanthoxylum fagara* (L.) Sarg. from Monteverde, Costa Rica, and its biological activities. *J. Essent. Oil Res.* **2005**, *17* (3), 333–335.

(6) Mi, X.; Gong, Z.; Zhang, W.; Zhang, X. Study on the extraction, separation and antimicrobial effect of volatile oil from *Zanthoxylum bungeanum* (Maxim). *Nanjing Shida Xuebao, Ziran Kexueban* **2004**, *27* (4), 63–66.

(7) Chen, D.; Yang, G. Quinoline alkaloids isolated from *Zanthoxylum* as antiviral agents for the treatment of hepatitis B. *Faming Zhuanli Shenqing Gongkai Shuomingshu*, **2005**, *8*,CN 1698610 A 20051123.

(8) Oriowo, M. A. Anti-inflammatory activity of piperonyl-4-acrylic isobutyl amide, an extract from *Zanthoxylum* zanthoxyloides. *Planta Med.* **1982**, *44* (1), 54–6.

(9) Kawasaki, K. Antioxidant compositions containing herbal drug extracts and water-soluble carbohydrates. **2007**, *7*,JP 2007119360 A 20070517.

(10) Tirillini, B.; Stoppini, A. M. Volatile constituents of the fruit secretory glands of *Zanthoxylum bungeanum* Maxim. *J. Essent. Oil Res.* **1994**, *6* (3), 249–52.

(11) Zhao, X.; Li, Z.; Chen, N.; Yan, Q. Study on chemical constituents of volatile oil of *Zanthoxylum bungeanum* Maxim. *Lanzhou Daxue Xuebao, Ziran Kexueban* **1992**, *28* (4), 74–77.

(12) Guo, Z.; Zhao, J.; Xie, Z. Study on chemical constituents of the essential oil from *Zanthoxylum bungeanum* Maxim by gas chromatography–mass spectrometry. *Sepu* **2001**, *19* (6), 567–568.

(13) Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 111–113.

(14) Acree, T. E.; Barnard, J.; Cunningham, D. G. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.* **1984**, *14*, 273–286.

(15) Jiang, L.; Kubota, K. Differences in the volatile components and their odor characteristics of green and ripe fruits and dried pericarp of Japanese pepper (*Xanthoxylum piperitum* DC.). *J. Agric. Food Chem.* **2004**, *52*, 4197–4203.

(16) Choochote, W.; Chaithong, U.; Kamsuk, K.; Jitpakdi, A.; Tippawangkosol, P.; Tuetun, B.; Champakaew, D.; Pitasawat, B. Repellent activity of selected essential oils against *Aedes aegypti*. *Fitoterapia* **2007**, *78* (5), 359–364.

(17) Iseli, V.; Potterat, O.; Hagmann, L.; Egli, J.; Hamburger, M. Characterization of the pungent principles and the essential oil of *Zanthoxylum schinifolium* pericarp. *Pharmazie* **2007**, *62*, 396–400.

(18) Wang, L.; Li, Z. Chemical constituents of essential oil from *Zanthoxylum bungeanum* growing in Hancheng, China. *Shipin Gongye Keji* **2003**, *24*, 23–20.

(19) Liu, X.; Kan, J.; Wang, H.; Chen, Z.; Nie, X. Extracting and separating *Zanthoxylum bungeanum* oleoresin with supercritical CO_2. *Zhongguo Shipin Xuebao* **2005**, *5* (4), 52–57.

(20) Rout, P. K.; Naik, S. N.; Rao, Y. R.; Jadeja, G.; Maheshwari, R. C. Extraction and composition of volatiles from *Zanthoxylum rhesta*: Comparison of subcritical CO_2 and traditional processes. *J. Supercrit. Fluids* **2007**, *42* (3), 334–341.

(21) Shafi, P.; Jose, B.; Radhamani, K. T.; Clery, R. A. Influence of pH on ssential oil composition of *Zanthoxylum rhetsa* seeds obtained by steam distillation. *Flavour Fragrance J.* **2006**, *21*, 317–318.

(22) Shankaracharya, N. B.; Puranaik, J.; Nagalakshmi, S.; Rao, L. J. Chemical composition and flavor quality of tirphal (*Zanthoxylum rhetsa*). *PAFAI J.* **1994**, *16* (1), 15–21.

(23) Wijaya, C. H.; Hadiprodjo, I. T.; Apriyantono, A. Identification of volatile compounds and key aroma compounds of andaliman fruit (*Zanthoxylum acanthopodium*). *Food Sci. Biotechnol.* **2002**, *11* (6), 680–683.

(24) Bryant, B. P.; Mezine, I. Alkylamides that produce tingling paresthesia activate tactile and thermal trigeminal neurons. *Brain Res.* **1999**, *842* (2), 452–460.

(25) Yasuda, I.; Takeya, K.; Itokawa, H. Two new pungent principles isolated from the pericarps of *Zanthoxylum ailanthoides*. *Chem. Pharm. Bull.* **1981**, *29*, 1791–1793.

(26) Sugai, E.; Morimitsu, Y.; Iwasaki, Y.; Morita, A.; Watanabe, T.; Kubota, K. Pungent qualities of sanshool-related compounds evaluated by a sensory test and activation of rat TRPV1. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 1951–1957.

(27) Mizutani, K.; Fukunaga, Y.; Tanaka, O.; Takasugi, N.; Saruwatari, Y.; Fuwa, T.; Yamauchi, T.; Wang, J.; Jia, M. R. Amides from haujiao, pericarps of *Zanthoxylum bungeanum* Maxim. *Chem. Pharm. Bull.* **1988**, *36*, 2362–2365.

(28) Xiong, Q.; Shi, D.; Yamamoto, H.; Mizuno, M. Alkylamides from pericarps of *Zanthoxylum bungeanum*. *Phytochemistry* **1997**, *46*, 1123–1126.

(29) Chen, I.-S.; Chen, T.-L.; Lin, W.-Y.; Tsai, I.-L.; Chen, Y.-C. Isobutylamides from the fruit of *Zanthoxylum integrifoliolum*. *Phytochemistry* **1999**, *52* (2), 357–360.

(30) Kashiwada, Y.; Ito, C.; Katagiri, H.; Mase, I.; Komatsu, K.; Namba, T.; Ikeshiro, Y. Amides of the fruit of *Zanthoxylum* spp. *Phytochemistry* **1997**, *44*, 1125–1127.

(31) Mizutani, K.; Fukunaga, Y.; Tanaka, O.; Takasugi, N.; Saruwatari, Y.; Fuwa, T.; Yamauchi, T.; Wang, J.; Jia, M. R. Amides from Haujiao, pericarps of *Zanthoxylum bungeanum* Maxim. *Chem. Pharm. Bull.* **1988**, *36*, 2362–5.

(32) Galopin, C.; Furrer, S.; Goeke, A. Pungent and tingling compounds in Asian cuisine. *ACS Symp. Ser.* **2004**, *No. 867*, 139–152.