Characterization of Tequila Flavor by Instrumental and Sensory Analysis

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Tequila, the fermented and twice-distilled juice of *Agave tequilana*, was extracted using dichloromethane. The extract obtained, which represented approximately 0.03% v/v of the original product, was analyzed by gas chromatography (GC), employing both flame ionization detection (FID) and sulfur chemiluminescence detection, as well as by gas chromatography−mass spectrometry (GC−MS). More than 175 components were identified in the extract, accounting for more than 99% of the total GC FID peak area. The extract was also subjected to sensory analysis employing the technique of GC with odor port evaluation/aroma extract dilution analysis. More than 60 odorants were detected, at least 30 of which could be correlated with specific GC peaks arising from components found in the extract. On the basis of their detection in the most dilute extracts analyzed, five constituents were determined to be the most powerful odorants of tequila; these were isovaleraldehyde, isoamyl alcohol, β-damascenone, 2-phenylethanol, and vanillin. Efforts at reconstituting tequila flavor from its component parts were not successful, however, indicating that further significant contributors to tequila flavor remain to be identified.

Keywords: *Agave tequilana; aroma extract dilution; gas chromatography−mass spectrometry; flavor; tequila; volatile components*

INTRODUCTION

There are more than 300 species of *Agave* L. native to the Americas. Aside from their use as house plants and in landscaping, some are cultivated for fiber (e.g. henequen and sisal), while others are grown for the sap, which in Mexico is fermented (for pulque) and then distilled (for mescal and tequila) (*Hortus Third*, 1976; Gentry, 1982; Bluhm, 1983).

Tequila is an alcoholic beverage made from the juice of cultivated variants of *Agave tequilana*, by processes that date back more than three centuries (Valenzuela-Zapata, 1985). The United States Bureau of Alcohol, Tobacco and Firearms (BATF) amended federal regulations in 1973 to define a standard of identity for tequila as an alcoholic distillate from a fermented mash derived principally from the *A. tequilana* Weber ("blue" variety) with or without additional fermentable substances, distilled in such a manner that the distillate possesses the taste, aroma, and characteristics generally attributed to tequila and bottled at greater than or equal to 80 proof. BATF also stated that tequila is a distinctive product of, and is manufactured in, Mexico (*Federal Register*, 1973). This manufacturing process involves harvesting the "piña", the stem of the agave plant with leaves removed, followed by cooking in an oven to convert polysaccharides (inulins) to a mixture principally of fructose and glucose. Sugars are extracted by milling and pressing and are then fermented with yeast, typically *Saccharomyces cerevisiae*, in vats (often with up to 49% sugar added from sources such as cane and sorghum). The fermented mash is then doubly distilled, and the finished product is diluted to give an alcohol content usually in the range of 40−50% (80−100 proof). The final product is colorless, though many of the premium tequilas are aged in oak vats, which results in their acquiring a pale to golden yellow color.

In recent years, increasing federal excise tax rates on alcoholic beverages, together with rising consumer awareness of alcohol and other health-related issues, have resulted in declining liquor sales in the United States [e.g. Hollingsworth (1994)]. In contrast, sales of tequila during this period have increased, primarily, it appears, due to increased consumer interest in Mexican-style and so-called Tex−Mex food and tequila-based cocktails and other mixed drinks (Anon., 1993).

Incitti *et al*. (1980) used packed-column gas chromatography (GC) to determine 25 volatiles (mostly fusel alcohols and esters) and 6 unidentified constituents in 10 tequilas. Previously, Manjarrez and Llama (1969) had measured the levels of 9 components in 15 tequilas and 8 mescals. More recently, Bluhm (1983) reported on observed increases in individual congeners of tequila resulting from the aging process. In general, however, there has been relatively little information published on the chemical characterization of tequila flavor. The present study was therefore undertaken to gain in-depth knowledge on the chemical composition of tequila flavor and to understand how this relates to perceived sensory characteristics of the product.

MATERIALS AND METHODS

Materials. Tequila was purchased from a local liquor store. Silica gel (70−230 mesh, 60 Å) was of chromatography grade from Aldrich Chemical Co. (Milwaukee, WI). Solvents were as follows: dichloromethane, OmniSolv HR-GC grade (EM Science, Gibbstown, NJ); diethyl ether, absolute/ACS reagent grade (Aldrich); methanol, HPLC grade (J. T. Baker, Phillipsburg, NJ); and *n*-pentane, OmniSolv Spec/Chrom grade (EM Science). Authentic flavor compounds and GC retention index standards (straight-chain ethyl esters) were from various suppliers and were used without further purification. All chemicals used in these studies should be handled with care; with dichloromethane in particular, precautions should be

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taken to avoid skin contact or inhalation of fumes (by employing standard engineering controls and personal protective equipment).

Isolation of Flavor Volatiles from Tequila. Tequila (1750 mL) was diluted to 5000 mL with deionized water and separated into three portions, and each portion was extracted with dichloromethane (4 × 100 mL). The extracts were combined, washed with deionized water (2 × 100 mL), and dried over anhydrous sodium sulfate. The sample was reduced in volume to *ca*. 50 mL by evaporation of solvent using a Kuderna-Danish apparatus; the volume was then reduced further to <1 mL by gradual evaporation at room temperature in a fume hood. The extract obtained, 0.55 mL, or *ca*. 0.03% v/v, was stored at −10 °C prior to instrumental and aroma extract dilution (AED) analysis. Dichloromethane emissions were condensed and disposed of in an approved manner.

Fractionation of Isolated Tequila Flavor. This was carried out on silica gel, using solvents and solvent mixtures of gradually increasing polarity, according to the method of Peppard (1992).

Instrumental and Aroma Extract Dilution Analysis. Tequila and tequila extracts were analyzed by gas chromatography employing various methods of detection: (i) mass spectrometry (MS); (ii) flame ionization detection (FID); (iii) sulfur chemiluminescence detection (SCD); and (iv) simultaneous FID and odor port evaluation using the technique of aroma extract dilution (AED) analysis (Grosch, 1994; Boelens *et al*., 1995).

Gas Chromatography−Mass Spectrometry. GC−MS was carried out on a Varian 3400 gas chromatograph interfaced with a Finnigan INCOS XL mass spectrometer. Separations were performed using a 50 m × 0.25 mm (i.d.) capillary column, coated with a 0.25 μm film of DB-Wax stationary phase (J&W Scientific, Folsom, CA). Helium was used as the carrier gas, with a column head pressure of 20 psig. For analysis of the whole extract, the oven temperature program used was 40−230 °C at a rate of 2.5 °C/min, with an initial temperature hold of 4 min and a final temperature hold of 30 min, resulting in a total run time of 110 min. For analysis of the extract fractions, the oven temperature program used was 50−230 °C at a rate of 4 °C/min, with an initial temperature hold of 2 min and a final temperature hold of 23 min, resulting in a run time of 70 min. Injections were made in split mode, with a split ratio of 60:1. Injection volume ranged from 0.2 to 1.0 μL, depending on sample. The injection port temperature was 250 °C. The mass spectrometer was operated with an ionization voltage of 70 eV, ion source temperature of 180 °C, and electron multiplier voltage of 1050 V and was scanned from *m*/*z* 34 to 400 at 0.6 s/scan. GC−MS of the whole extract was also carried out on a Hewlett-Packard 5880A gas chromatograph interfaced with a Hewlett-Packard 5970 mass selective detector. Separations were performed using a 30 m × 0.25 mm (i.d.) capillary column, coated with a 1.0 μm film of DB-1 stationary phase (J&W Scientific). Helium was used as the carrier gas, with a column head pressure of 15 psig. The oven temperature program used was 70−250 °C at a rate of 4 °C/min. A final temperature hold of 15 min resulted in a total run time of 60 min. The injector and transfer line temperatures were held at 250 °C. Injection volume was 0.2 μL, with a split ratio of 75:1. The mass spectrometer was operated with an ionization voltage of 70 eV and electron multiplier voltage of 2200 V and was scanned from *m*/*z* 35 to 400 at 1 s/scan.

Gas Chromatography−FID. Quantitation was performed using GC with flame ionization detection, on a Perkin-Elmer Sigma 2000 gas chromatograph. Separations were performed using a 30 m × 0.32 mm (i.d.) capillary column coated with a 0.25 μm film of DB-Wax stationary phase. Helium was used as the carrier gas, with a column head pressure of 12 psig. The oven temperature program used was from 50 to 230 °C at a rate of 4 °C/min, with an initial temperature hold of 4 min and a final temperature hold of 23 min, for a total run time of 70 min. Split mode injections were made, with a split ratio of 50:1. The injection volume ranged from 0.2 to 1.0 μL, depending on sample. The injection port temperature was 230 °C, and the detector temperature was 280 °C.

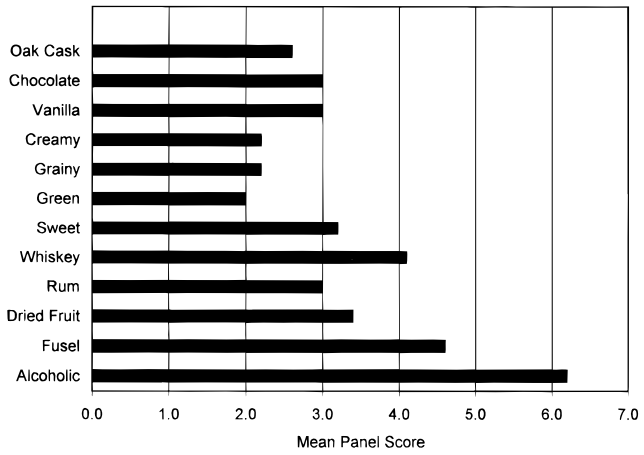

Figure 1. Sensory evaluation of tequila.

Gas Chromatography−SCD. GC with sulfur chemiluminescence detection was carried out using a Hewlett-Packard 5890 Series II gas chromatograph coupled to a Sievers Model 350 SCD (Sievers Research Co., Boulder, CO). Separations were performed using a 30 m × 0.53 mm (i.d.) capillary column, coated with a 1.0 μm film of DB-Wax stationary phase. The carrier gas was helium, with a column head pressure of 4 psig. The oven temperature program used was 50−230 °C at a rate of 4 °C/min, with a final temperature hold of 15 min, resulting in a run time of 60 min. The injection port temperature was 250 °C, and the detector temperature was 250 °C. Injections were made in splitless mode, with an injection volume of 1.0 μL. The SCD integration time was set at 0.01 s.

Gas Chromatography−FID/Odor Port Evaluation. GC with simultaneous flame ionization detection and odor port evaluation was carried out using a Hewlett-Packard 5890 Series II gas chromatograph. Separations were performed using a 30 m × 0.53 mm (i.d.) capillary column, coated with a 1 μm film of DB-Wax stationary phase. The carrier gas was helium, with a column head pressure of 6 psig. The column effluent was split 1:1 with a "Y" press fit type splitter (Restek Corp., Bellefonte, PA), with flow going to the FID and odor port via 20 cm lengths of 0.32 mm (i.d.) uncoated fused silica tubing. The oven temperature program used was 50−230 °C at 3 °C/min, with an initial temperature hold of 2 min and a final temperature hold of 20 min, resulting in a run time of 82 min. Odor port evaluation was carried out for the first 65 min of the run. Injections were made in cool on-column mode; the injector temperature was held at 3 °C above oven temperature. Injection volume was 1.0 μL. The FID temperature was 250 °C. The whole extract at 200× dilution was also analyzed using a 30 m × 0.53 mm (i.d.) capillary column, coated with a 1.5 μm film of DB-1 stationary phase, with other parameters kept the same. Odor port evaluation was carried out using a specially constructed apparatus consisting of a glass nosepiece attached to a heated insulated box, through which the outlet of the fused silica tubing was passed. The temperature of the odor port apparatus was maintained at 250 °C.

Sensory Evaluation. Descriptive sensory evaluation was carried out by a panel of six trained judges, during three replicate tasting sessions. Each of the sensory attributes shown in Figure 1 was assessed on a scale of 0−7 (0 = none, 3 = slight, 5 = moderate, 7 = high, etc.), by comparison to the following series of reference materials: alcoholic, 25% neutral grain spirits in water; fusel, 0.002% Fusel Oil Wine Pure American (Citrus & Allied Essences Ltd., Floral Park, NY) in water; dried fruit, prunes; rum, Bacardi Gold Reserve; whiskey, Cutty Sark; sweet, 12% sucrose in water; green, 0.002% leaf alcohol (*cis*-3-hexenol) in water; grainy, bran flakes/Kix/Cheerios; creamy, heavy cream; vanilla, 0.4% vanilla extract 2-fold in water; chocolate, Nestle semisweet milk chocolate morsels; oak cask, solid extract oak chips.

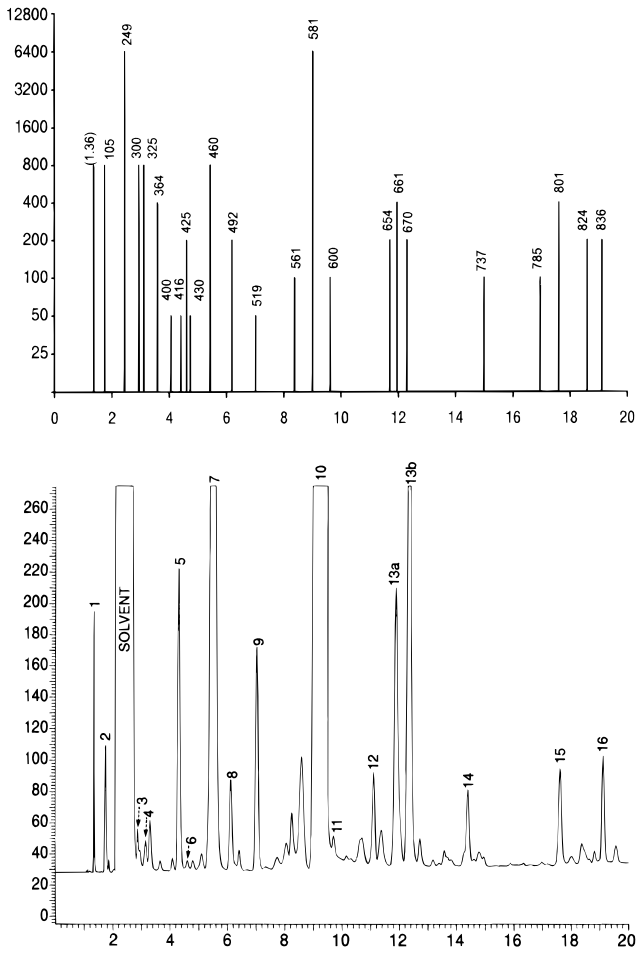

Figure 2. Analysis of tequila extract by (a) GC−FID (lower trace) and (b) GC−AED (upper trace), retention time 0−20 min. *x*-axis is time (minutes); *y*-axis is (a) FID response or (b) aroma extract dilution. Numbers on peaks in AED trace are GC retention indices; those in parentheses refer to retention time in minutes. For identities of peaks in FID trace (where given) see Table 1.

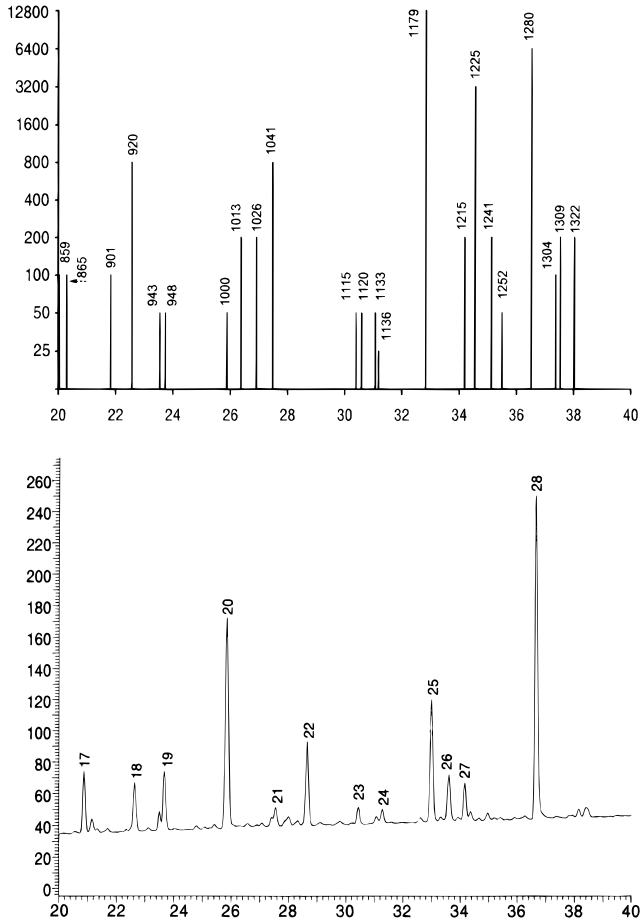

Figure 3. Analysis of tequila extract by (a) GC−FID (lower trace) and (b) GC−AED (upper trace), retention time 20−40 min. *x*-axis is time (minutes); *y*-axis is (a) FID response or (b) aroma extract dilution. Numbers on peaks in AED trace are GC retention indices. For identities of peaks in FID trace (where given) see Table 1.

RESULTS AND DISCUSSION

The particular brand of tequila selected for detailed analysis in the present study was one having a major market share in the United States. This brand is golden yellow in color, indicative of aging in wood; sensory evaluation by a panel of trained assessors revealed the descriptive flavor profile shown in Figure 1. Aside from alcoholic, fusel, and whiskey notes, the tequila was characterized by dried fruit, sweet, rum, vanilla, and several other significant odors. Analysis of the product by GC, using a direct injection technique (and comparison with external standards made up in 40% ethanol), allowed quantitation of the major components (other than ethanol) as follows: 2- and 3-methylbutanol, 491 ppm; 1-propanol, 232 ppm; 2-methylpropanol, 228 ppm; and ethyl acetate, 176 ppm. However, to be able to detect and identify the majority of components present in the tequila (many at low or below parts per billion levels), it was necessary to concentrate them, as described under Materials and Methods. Further, to obtain "cleaner" mass spectra allowing identification of minor components, especially those coeluting with other components present in the extract, the tequila extract was fractionated on silica gel using solvents and solvent mixtures of gradually increasing polarity, as also mentioned above.

Figures 2a, 3a, and 4a show sections of the gas chromatogram obtained by analysis of the whole tequila extract on DB-Wax stationary phase, employing flame ionization detection. The extract was also analyzed by GC−SCD, which revealed the presence of dimethyl disulfide, dimethyl trisulfide, and 4-methyl-5-vinylthiazole, as well as numerous other, unidentified sulfur-containing constituents (see Figure 5). Altogether, more than 175 components were identified in the extract, by comparison of mass spectra and GC retention indices with those of authentic materials, accounting for greater than 99% of the total GC−FID peak area. The compounds identified are listed in Table 1, together with their relative levels in the extract (based on GC FID peak area percentage); peak numbers (where given) correspond to those shown in Figures 2a, 3a, and 4a.

Chemical functionalities confirmed to be present among the flavor volatiles of tequila include acetals, acids, alcohols, aldehydes, esters, ethers, furans, ketones, phenols, pyrazines, sulfur compounds, and terpenes (see Table 1). In terms of the numbers of components identified, esters represent the largest group, with approximately 50 individual compounds detected in the tequila extract. Of these, many were ethyl esters, though some methyl, isoamyl, and phenylethyl esters (among others) were also detected; included within the ester category were several unsaturated, keto, and hydroxy esters. It seems likely that the majority of esters identified in this study are products

Table 1. Flavor Constituents of Tequila Extract

peak no.	component[a]	identification[b]	relative level[c]
	acetals		
	acetaldehyde methyl ethyl acetal	MS	+
	formaldehyde diethyl acetal	MS	+
	acetaldehyde diethyl acetal	MS, RI	2.86
	acetaldehyde propylene glycol acetal	MS	+
	acetaldehyde 2,3-butanediol acetal	MS	+
	propanal diethyl acetal	MS	+
	acetaldehyde ethyl propyl acetal	MS	+
	isobutyraldehyde diethyl acetal (and propan-2-ol)	MS (MS, RI)	0.07
	acetaldehyde ethyl isobutyl acetal	MS	0.03
	butanal diethyl acetal	MS	+
	2-methylbutanal propylene glycol acetal?	MS	+
	acetaldehyde ethyl butyl acetal	MS	+
	2-methylbutanal diethyl acetal	MS	0.01
	3-methylbutanal diethyl acetal	MS	0.01
	acetaldehyde ethyl 2-methylbutyl acetal	MS	0.03
	acetaldehyde ethyl 3-methylbutyl acetal	MS	0.14
	acetaldehyde ethyl pentyl acetal	MS	+
	acetaldehyde 2-methylpropyl 2-methylbutyl acetal	MS	+
	acetaldehyde 2-methylpropyl 3-methylbutyl acetal	MS	+
	unidentified acetal	MS	+
13a	acetaldehyde diisoamyl acetal I	MS	*
13b	acetaldehyde diisoamyl acetal II	MS	*
	phenylacetaldehyde diethyl acetal	MS, RI	+
	acetaldehyde ethyl phenylethyl acetal	MS, RI	+
	acids		
	acetic acid	MS, RI	+
	2-methylpropanoic acid	MS, RI	0.03
21	2-methylbutyric acid	MS, RI	0.03
	pentanoic acid	MS, RI	+
27	hexanoic acid	MS, RI	0.04
30	octanoic acid	MS, RI	0.29
35	decanoic acid	MS, RI	0.62
38	dodecanoic acid	MS, RI	0.21
	tetradecanoic acid	MS, RI	0.02
	hexadecanoic acid	MS, RI	0.03
	hexadecenoic acid	MS, RI	0.03
	alcohols		
	ethanol	MS, RI	0.53
5	propanol	MS, RI	0.61
7	isobutyl alcohol	MS, RI	8.93
	pentan-2-ol	MS, RI	+
9	butanol	MS, RI	0.47
10	2-methylbutanol and 3-methylbutanol	MS, RI	77.07
	3-methylbut-3-en-1-ol (α-prenol)	MS, RI	0.03
	pentanol	MS, RI	+
	pent-4-en-1-ol?	MS	0.06
	3-methylpentan-1-ol	MS	+
	4-methylpentan-1-ol	MS	+
	hexanol	MS, RI	0.03
	octan-3-ol	MS, RI	+
	oct-1-en-3-ol	MS, RI	+
	heptanol	MS, RI	+
	unidentified branched C_8 saturated alcohol	MS	+
	octanol	MS, RI	+
	decanol	MS, RI	+
28	2-phenylethyl alcohol	MS, RI	0.60
	dodecanol	MS, RI	+
31	tetradecanol	MS, RI	0.06
37	hexadecanol	MS, RI	0.06
	aldehydes		
1	acetaldehyde	MS, RI	+
2	isobutyraldehyde	MS, RI	+
	2-methylbutanal and 3-methylbutanal	MS, RI	0.03
	but-2-enal	MS	+
	benzaldehyde	MS, RI	+
	β-cyclocitral	MS, RI	+
	phenylacetaldehyde	MS, RI	+
	hexadecanal	MS, RI	+
	esters		
	methyl acetate	MS, RI	+
	ethyl acetate	MS, RI	2.34
3	ethyl propionate	MS, RI	0.02
	ethyl isobutyrate	MS, RI	+
	propyl acetate	MS, RI	+
	butyl acetate	MS, RI	0.02
	ethyl butyrate	MS, RI	0.02

Table 1 (Continued)

peak no.	component[a]	identification[b]	relative level[c]
	ethyl 2-methylbutyrate	MS, RI	+
	ethyl isovalerate	MS, RI	+
	isoamyl formate	MS, RI	+
8	2-methylbutyl acetate and 3-methylbutyl acetate	MS, RI	0.25
	ethyl valerate	MS, RI	+
	amyl acetate	MS, RI	+
11	ethyl hexanoate	MS, RI	0.05
	ethyl 2,4-hexadienoate	MS	+
	ethyl pyruvate	MS	+
	ethyl heptanoate	MS, RI	+
14	ethyl lactate	MS, RI	0.15
	methyl octanoate	MS, RI	+
15	ethyl octanoate	MS, RI	0.19
	ethyl nonanoate	MS, RI	+
	ethyl 2-hydroxy-4-methylpentanoate	MS	+
	2-methylpropyl octanoate	MS, RI	+
	3-methylbutyl lactate	MS, RI	+
	methyl decanoate	MS, RI	+
20	ethyl decanoate	MS, RI	0.42
	3-methylbutyl octanoate	MS, RI	+
	diethyl succinate	MS, RI	+
	ethyl dec-9-enoate	MS	+
	methyl salicylate	MS, RI	+
	ethyl phenylacetate	MS, RI	+
	methyl dodecanoate	MS, RI	+
25	phenylethyl acetate	MS, RI	0.26
26	ethyl dodecanoate	MS, RI	0.13
	isoamyl decanoate	MS, RI	+
	phenylethyl isobutyrate	MS, RI	+
	ethyl 3-phenylpropionate	MS, RI	+
	ethyl dodecenoate	MS	+
	phenylpropyl acetate	MS, RI	+
	phenylethyl butyrate	MS, RI	+
	ethyl tetradecanoate	MS, RI	+
32	ethyl hexadecanoate	MS, RI	+
	ethyl hexadec-9-enoate	MS	+
	phenylethyl octanoate	MS	+
	ethyl oleate	MS, RI	+
	ethyl linoleate	MS, RI	+
	ethyl linolenate	MS, RI	+
	furans		
12	2-methyltetrahydrofuran-3-one	MS, RI	0.25
	furfuryl ethyl ether	MS	0.03
	5-methylfurfuryl ethyl ether	MS	+
16	furfural	MS, RI	0.27
17	2-acetylfuran	MS, RI	0.14
	furfuryl acetate	MS, RI	+
19	5-methylfurfural	MS, RI	0.11
	2-propionylfuran	MS	+
	methyl 2-furoate	MS, RI	+
	ethyl 2-furoate	MS	+
	furfuryl alcohol	MS, RI	+
	2-methyl-2-vinyl-5-octadienyltetrahydrofuran I	MS	+
	2-methyl-2-vinyl-5-octadienyltetrahydrofuran II	MS	+
39	(hydroxymethyl)furfural	MS, RI	0.05
	ketones		
4	diacetyl	MS, RI	0.07
6	acetylpropionyl	MS, RI	0.01
	pent-3-en-2-one	MS	+
	heptan-2-one	MS, RI	+
	cyclopentanone	MS	0.12
	3-methylcyclopentanone	MS	+
	6-methylhept-5-en-2-one	MS, RI	+
	4-methylcyclopent-2-en-1-one	MS	+
	cyclopent-2-en-1-one	MS	+
	nonan-2-one	MS, RI	+
	cyclohex-2-en-1-one	MS	+
	β-damascenone	MS, RI	+
	phenols		
	guaiacol	MS, RI	+
	cresol	MS, RI	+
	4-ethylguaiacol	MS, RI	+
	eugenol	MS, RI	+
	MW 122 (3-ethylphenol?)	MS	+
40	vanillin	MS, RI	0.04
	syringic aldehyde	MS	0.09
	coniferyl aldehyde	MS	+

Table 1 (Continued)

peak no.	component[a]	identification[b]	relative level[c]
	pyrazines		
	2,5-dimethylpyrazine	MS, RI	0.04
	2,6-dimethylpyrazine	MS, RI	+
	2-ethyl-5-methylpyrazine	MS, RI	+
	2-ethyl-3-methylpyrazine	MS, RI	+
	trimethylpyrazine	MS, RI	0.01
	sulfur compounds		
	dimethyl disulfide	MS, RI	+
	dimethyl trisulfide	MS, RI	+
	4-methyl-5-vinylthiazole	MS, RI	+
	terpenoids		
	1,4-cineole	MS, RI	+
	linalyl ethyl ether	MS	+
	cis-linalool oxide	MS, RI	0.05
	trans-linalool oxide	MS, RI	+
	geranyl ethyl ether	MS	+
	p-cymen-8-yl ethyl ether	MS	+
18	linalool	MS, RI	0.11
	terpinen-4-ol	MS, RI	+
	p-menth-1-en-9-al	MS	+
	citronellyl acetate	MS, RI	+
22	α-terpineol	MS, RI	0.18
24	citronellol	MS, RI	0.04
	nerol	MS, RI	+
	nerolidyl ethyl ether	MS	+
	geraniol	MS, RI	+
	p-cymen-8-ol	MS, RI	+
	p-cymen-9-ol?	MS	+
29	*cis*-nerolidol	MS, RI	0.09
	6(*E*)-dihydrofarnesyl acetate	MS	+
	unidentified sesquiterpene alcohol	MS	+
	thymol	MS, RI	+
	α-bisabolol	MS, RI	+
33	α-farnesyl acetate	MS	+
34	6(*E*)-dihydrofarnesol	MS	0.03
36	*trans*,*trans*-α-farnesol	MS, RI	0.14
	miscellaneous compounds		
	prenyl ethyl ether	MS	+
	2,6,6-trimethyl-2-vinyltetrahydropyran	MS, RI	+
	acetoin	MS, RI	0.03
	3-ethoxypropan-1-ol	MS	+
	3-methylbutyl phenylethyl ether?	MS	+
	unidentified components		
	bp 45, 58, 74, 101		+
	bp 43, 61, 87, 117, 89, 115		+
	MW 180, bp 137, 165, 119, 91, 41		0.04
	MW 192, bp 93, 41, 77, 121, 136, 177		+
	MW 220, bp 159, 205		+
	bp 43, 114, 111, 88, 68		+
	MW 270?, bp 43, 97, 113, 183, 255		+
	MW 240, bp 97, 153, 167		+
23	MW 184, bp 139, 83, 94, 55, 111, 125		0.05
	MW 254, bp 97, 167		+
	MW 254, bp 97, 167, 43, 71		0.04
	MW 268, bp 111, 181		+
	bp 43, 99, 127, 157, 155		+
	MW 220, bp 79, 93, 107		+

[a] MW, molecular weight; bp, base peak in mass spectrum. [b] MS, identified on basis of mass spectral data alone; MS, RI, identified on the basis of both mass spectral and GC retention index data. [c] +, present in extract at <0.01%; *, originally present at <0.01%, but level increased on storage.

of yeast metabolism or are formed subsequently during the aging process by esterification of fatty acids in the presence of ethanol at high concentration. Undoubtedly also present largely as a result of fermentation were many of the more than 20 alcohols detected in the tequila extract, several of which additionally showed up in combination with aldehydes as acetals, presumably formed predominantly during aging. In all, approximately 25 acetals were identified, with acetaldehyde representing the most prominent aldehyde moiety. More than 10 acids were detected, with fermentation in most cases again seemingly the most likely origin.

The approximately 25 terpenoid constituents detected in the tequila extract are presumably derived from the agave cactus; no terpene hydrocarbons were identified, only monoterpene and/or sesquiterpene alcohols, esters, ethers (including cyclic ethers), and one aldehyde. Whether any of these are formed by transformation during fermentation, distillation, storage, or the original cooking of the piña is not known. It seems likely, however, that several groups of compounds, such as the aldehydes, ketones (including several cyclic ketones), and furans, a number of sulfur-containing components, and possibly some of the phenols, are formed during the

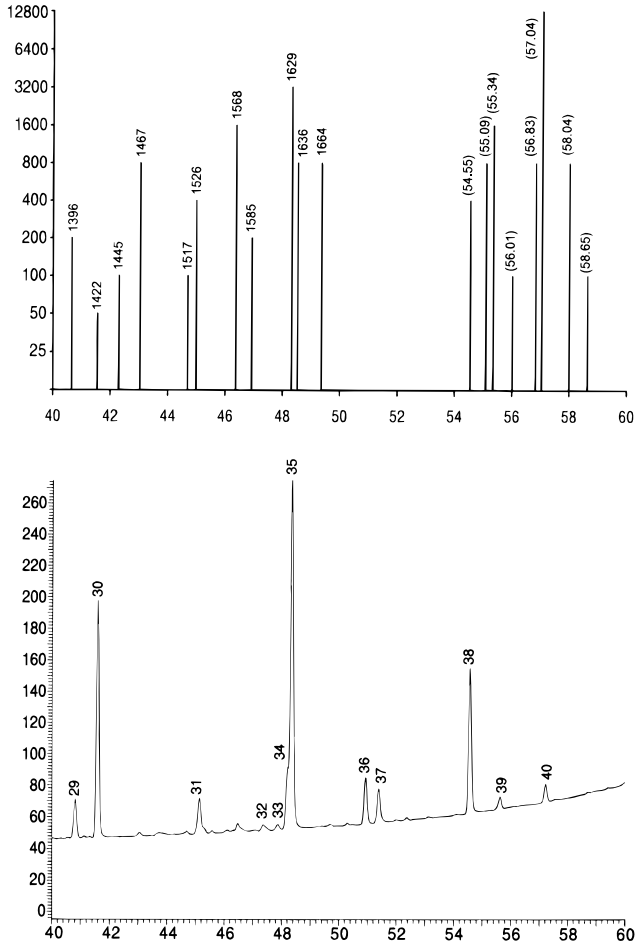

Figure 4. Analysis of tequila extract by (a) GC−FID (lower trace) and (b) GC−AED (upper trace), retention time 40−60 min. *x*-axis is time (minutes); *y*-axis is (a) FID response or (b) aroma extract dilution. Numbers on peaks in AED trace are GC retention indices; those in parentheses refer to retention time in minutes. For identities of peaks in FID trace (where given) see Table 1.

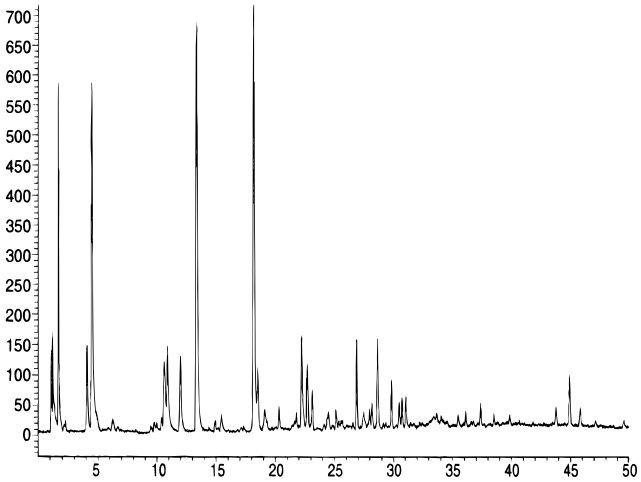

Figure 5. Analysis of tequila extract by GC−SCD. *x*-axis is time (minutes); *y*-axis is SCD response.

cooking and/or distillation steps of tequila production. For example, β-cyclocitral and β-damascenone are most likely degradation products of carotenoids (Mordi, 1993; Näf *et al.*, 1990), while 4-methyl-5-vinylthiazole can be formed by dehydration of 4-methyl-5-(2-hydroxyethyl)-thiazole, a known breakdown product of thiamine (Güntert *et al.*, 1992). The aldehydes, ketones, furans, and pyrazines are probably derived in most cases through nonenzymic browning of sugars and/or Maillard reactions between sugars and amino-containing substances. It is speculated that the phenols may form by breakdown of phenolic acids, originally present in the agave cactus; alternatively, they may arise in tequila by extraction from the oak vats during aging. In fact, when another lot of the same gold tequila was compared with a sample of white tequila (presumably not aged in oak), the phenols vanillin and syringic aldehyde could be detected only in the former. Other differences in chemical composition noted were higher levels of linalool, *cis*- and *trans*-linalool oxides, and various other terpenoid constituents in the gold tequila but generally lower levels of several acetaldehyde acetals than were detected in the white tequila.

Separation of the gold tequila extract on silica gel yielded seven fractions of widely differing organoleptic characteristics. Fractions 2−6 appeared to be of most interest, on the basis of comparison with the original product, but each of these fractions remained highly complex, judging by the results of GC analysis. It was decided to submit the original tequila extract to organoleptic analysis employing the technique of GC with odor port evaluation/aroma extract dilution (AED) analysis (Grosch, 1994; Boelens *et al.*, 1995). In GC−AED analysis the extract is diluted in a serial manner, and each dilution is analyzed by GC with odor port evaluation until no further odors can be detected. This enables one to describe the organoleptic characteristics of individual components present in the extract and to determine which have the most potent odors by establishing their relative importance to the aroma of the whole, as well as to that of the original product. The tequila extract was diluted as follows: 25, 50, 100, 200, 400, 800, 1600, 3200, 6400, and 12 800 times, and each dilution was analyzed by GC on DB-Wax stationary phase, employing simultaneous flame ionization detection and odor port evaluation. Fraction 2 was also analyzed by GC−AED at 200× dilution; in addition, the whole extract was analyzed by GC−AED on a DB-1 capillary column.

Table 2 summarizes the large amount of organoleptic information obtained during the various GC−AED experiments undertaken in the present study. However, the results of carrying out AED analysis can best be visualized graphically by means of so-called "aromagrams"—plots of maximum dilution (the highest dilution at which an odor can still be detected) vs retention time or retention index. The use of aromagrams allows one to determine where in a gas chromatographic run the most potent odorants appear. Figures 2b, 3b, and 4b show aromagrams corresponding to the GC−FID traces presented, respectively, in Figures 2a, 3a, and 4a; odor description and chemical identity (where determined) are given in Table 2. It should be noted that retention indices correspond to the point in time at which odors were first perceived. It is apparent from a comparison of Figures 2b, 3b, and 4b with Figures 2a, 3a, and 4a, respectively, that many of the most potent odorants of tequila elute from the capillary column at retention times at which there is little or no response from the FID; *i.e.*, the nose for these components is a more sensitive detector than is the FID (*e.g.*, *trans*-2-nonenal—see Table 2).

Of the more than 60 odorants detected, 32 could be correlated with compounds identified in the extract. Further, it was possible to identify the compounds

Table 2. Aroma Extract Dilution Analysis of Tequila Extract

T_R	I_{EE}	max dilution	odor descriptors	flavor component(s)
1.36	<100	800	chemical, sharp	acetaldehyde
1.74	105	800	sweet, caramel	isobutyraldehyde
2.44	249	6400	sweet, cocoa, chocolate	isovaleraldehyde
2.94	300	800	sweet, butterscotch, fruity	ethyl propionate
3.11	325	800	butter	diacetyl
3.58	364	400	chemical, ether	?
4.06	400	50	fruity, banana	ethyl butyrate
4.40	416	50	sweet	?
4.60	425	200	butter	acetylpropionyl
4.73	430	50	green, fruity?	?
5.43	460	800	sweet, chemical	isobutyl alcohol
6.19	492	200	green, chemical	?
7.01	519	50	sweet, fusel	butyl alcohol
8.37	561	100	sharp, chemical	?
9.00	581	6400	sweet, fruity, fusel	isoamyl alcohol
9.62	600	100	fruity, apple	ethyl hexanoate
11.70	654	200	sharp, chemical	furfuryl ethyl ether?
11.95	661	400	butter	acetoin
12.30	670	200	sweet, fruity	?
14.99	737	100	sulfury, pungent	dimethyl trisulfide
16.95	785	100	dry, leafy, brown	?
17.60	801	400	earthy, woody, leafy	?
18.60	824	200	mushroom, earthy	oct-1-en-3-ol
19.11	836	200	stale, papery, bready	?
20.04	859	100	sharp, papery, green	?
20.30	865	100	bready, sweet, chocolate	?
21.82	901	100	dry, leafy, green	*trans*-2-nonenal? (tentative identification based on odor and I_{EE})
22.57	920	800	floral, sweet	linalool
23.54	943	50	sweet, berry	?
23.73	948	50	fruity, sweaty	isobutyric acid
25.88	1000	50	sweet, dairy	ethyl decanoate
26.37	1013	200	floral, sharp	phenylacetaldehyde
26.91	1026	200	sulfury, bready	?
27.47	1041	800	fruity, sweaty	2-methylbutyric acid
30.40	1115	50	sweet, phenolic	?
30.59	1120	50	sharp, fruity	?
31.07	1133	50	sharp, floral, fruity	acetaldehyde ethyl phenylethyl acetal?
31.18	1136	25	sweet, floral	citronellol?
32.83	1179	12800	fruity, woody, winey, berry	β-damascenone (some contribution to odor by coeluting 2-phenylethyl acetate)
34.19	1215	200	woody	?
34.55	1225	3200	smoky, phenolic	guaiacol
35.12	1241	200	woody	?
35.49	1252	50	fatty, dairy	?
36.52	1280	6400	floral	2-phenylethyl alcohol
37.37	1304	100	woody, berry, ionone-like	?
37.53	1309	200	smoky, phenolic	?
38.02	1322	200	sweet	creosol
40.65	1396	200	smoky, phenolic	4-ethylguaiacol
41.54	1422	50	fatty acid, dry, dairy	octanoic acid
42.30	1445	100	plastic	?
43.03	1467	800	woody, burnt, fatty, phenolic, medicinal	?
44.69	1517	100	fruity, woody, sweet	6(*E*)-dihydrofarnesyl acetate?
44.99	1526	400	spicy, clove	eugenol
46.37	1568	1600	warm, spicy, curry powder	thymol + unknown?
46.93	1585	200	smoky, phenolic	?
48.32	1629	3200	fatty acid, dry, woody	decanoic acid + ethyl hexadec-9-enoate
48.53	1636	800	spicy, curry powder	?
49.36	1664	800	powdery, fatty, sharp	unidentified C_{10} acid?
54.55	>1800	400	fatty acid	dodecanoic acid
55.09	>1800	800	fatty, animal	?
55.34	>1800	1600	woody	?
56.01	>1800	100	woody	?
56.83	>1800	800	pungent, floral, honey	phenylacetic acid
57.04	>1800	12800	sweet, creamy	vanillin
58.00	>1800	800	woody, phenolic	?
58.65	>1800	100	fatty, cheesy	?

representing the five most potent odorants (see Table 2). The two odors detected with the highest dilution factor of 12800× did not correspond to large peaks in the gas chromatogram. The first of these, at I_{EE} 1179 (Figure 3b), was identified as β-damascenone. Its odor is described as woody, sweet, fruity, and floral (Mosciano *et al.*, 1991). Under the chromatographic conditions used in this study, β-damascenone coeluted with 2-phenylethyl acetate (Figure 3a, peak 25), which was present at a much higher concentration. It is possible that the latter compound made a contribution to the odor perceived in this region. However, the similarity of the odor of β-damascenone to that perceived during odor port evaluation, as well as the extremely low odor

threshold of β-damascenone [0.002 ppb, in water, according to Leffingwell (1994)], makes it likely that by itself it is one of the most potent odorants of tequila. The other odor detected at maximum dilution, at retention time 57.04 min (Figure 4b), was attributed to vanillin. Vanillin has a characteristic sweet, creamy, vanilla-like odor (Leffingwell, 1994), and its importance as a character impact compound of tequila is supported by the fact that "vanilla" was identified as a significant attribute of tequila flavor during sensory evaluation of tequila by a panel of experienced tasters. Vanillin also has a low odor threshold [20−200 ppb, in water, according to Leffingwell (1994)] and, at the level present in the extract (0.04%), contributes significantly to tequila aroma and flavor.

Two of the three odors detected with an extract dilution factor of 6400× corresponded to major peaks in the gas chromatogram. One of these, at I_{EE} 581 (Figure 2a), was identified as isoamyl alcohol. Under the chromatographic conditions used, the two isomers 2- and 3-methylbutan-1-ol were not separated and for odor port evaluation were considered as one material, isoamyl alcohol. The odor of isoamyl alcohol from fusel oil has been described as breathtaking, alcoholic, and (when suitably diluted) wine/brandylike (Leffingwell, 1994), as well as penetrating, woody, sweet, fruity, and whiskey-like (G. Mosciano, Bush Boake Allen, Inc., personal communication, 1995). The attributes "fusel" and "whiskey", which were identified by panelists during sensory evaluation of tequila, reflect the presence of the large amount, over 77%, of isoamyl alcohol in the tequila extract. The floral odor at I_{EE} 1280 was attributed to 2-phenylethyl alcohol which, at 0.60% of the extract, was one of the main peaks in the chromatogram (Figure 3a). The third odor detected with a dilution factor of 6400×, at I_{EE} 249, was described as sweet, cocoa, and chocolate. It was attributed to isovaleraldehyde. Again, the 2- and 3-methyl isomers were not separated under the chromatographic conditions employed. The odors of both 2- and 3-methylbutanal in solution have been described as malty (Badings, 1991; Amoore *et al*., 1976).

Two odorants were found having a maximum dilution factor of 3200×. Guaiacol, a trace component of the extract, is responsible for the smoky, phenolic odor detected at I_{EE} 1225. The odor of this compound is described as powerful, smoke-like, medicinal, and sweet (Arctander, 1969). Several other trace components also gave rise to similar odor descriptors, such as woody, smoky, burnt, and phenolic, at lower dilution factors. Three of these, at I_{EE} 1467 and retention times 55.34 and 58.00 min, respectively, had maximum dilution factors of at least 800×. These compounds all remained unidentified, due to their low abundances in the extract. The odor at I_{EE} 1629 appears to be due mainly to decanoic acid, with some contribution from other compounds. Decanoic acid has a fatty/waxy and rancid odor (Leffingwell, 1994), while the odor perceived during odor port evaluation in this region of the chromatogram was also dry and woody. This may have been due to the presence of ethyl hexadec-9-enoate, but this remains unconfirmed in the absence of odor data for this compound.

Other identified compounds which were detected at a maximum dilution of 800×, and which probably contribute significantly to the aroma of tequila, were acetaldehyde (T_R 1.36 min; chemical, sharp); isobutyraldehyde (I_{EE} 105; sweet, caramel); ethyl propionate (I_{EE} 300; sweet, butterscotch, fruity); diacetyl (I_{EE} 325; butter); isobutyl alcohol (I_{EE} 460; sweet, chemical); linalool (I_{EE} 920; floral, sweet); 2-methylbutyric acid (I_{EE} 1041; fruity, sweaty); and phenylacetic acid (T_R 56.83 min; pungent, floral, honey).

In addition to the aforementioned unidentified compounds having woody and phenolic odors, two further unidentified compounds, having odors described as warm, spicy, and reminiscent of curry powder, were detected. The first of these, at I_{EE} 1568, detected at a maximum dilution factor of 1600×, coeluted with thymol, which may have contributed to the odor. However, thymol, which has been described as medicinal, herbal, and spicy/phenolic (Leffingwell, 1994), has an odor different from that perceived during odor port evaluation in the present studies. It is more likely that an unidentified compound was responsible for this odor. The compound responsible for the similar odor perceived at I_{EE} 1636 also remains unidentified.

An attempt was made to reconstitute the flavor of tequila from its identified component parts. An ethanolic solution was made of 47 chemicals found in the tequila extract, including all 32 character impact compounds identified during odor port evaluation. The amount of each compound used in the reconstitution was based on the level detected in the extract. The flavor of the reconstituted "tequila" (at the appropriate dilution in water) was found to be more sweet, chocolate, fruity, fusel, and estery than that of the genuine product. Further, it lacked bready, woody, and spicy character. While several odorants with these descriptors were identified during odor port evaluation, few of them could be attributed to compounds identified in the extract, indicating that other significant contributors to tequila flavor remain as yet unidentified.

CONCLUSIONS

More than 175 constituents were identified in a dichloromethane extract of tequila; these accounted for more than 99% of the total peak area measured by GC−FID analysis. The importance of individual chemical constituents in the extract was determined by sensory analysis employing the technique of GC with odor port evaluation/aroma extract dilution. More than 60 odorants were detected, at least 30 of which could be correlated with specific GC peaks arising from components found in the extract. On the basis of their detection in the most highly diluted extracts analyzed, five constituents were determined to be the most powerful odorants of tequila; these were isovaleraldehyde, isoamyl alcohol, β-damascenone, 2-phenylethanol, and vanillin. However, efforts at reconstituting tequila flavor by mixing together the various components identified in the present studies were largely unsuccessful, indicating that at least some of the key contributors to tequila flavor have yet to be chemically characterized.

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LITERATURE CITED

Amoore, J. E.; Forrester, L. J.; Pelosi, P. Specific anosmia to isobutyraldehyde: the malty primary odor. *Chem. Senses Flavour* **1976**, *2*, 17−25.

Anon. Liquor giants increase clout: as market posts another down year. *Advertising Age* **1993**, *64 (7)*, 51.

Arctander, S. *Perfume and Flavor Chemicals* (*Aroma Chemicals*); Arctander: Elizabeth, NJ, 1969.

Badings, H. T. Milk. In *Volatile Compounds in Food and Beverages*; Maarse, H., Ed.; Dekker: New York, 1991; pp 91−106.

Bluhm, L. Distilled beverages. In *Biotechnology*, *Volume 5—Food and Feed Production with Microorganisms*; Reed, G., Ed.; Verlag Chemie: Weinheim, Germany, 1983; Chapter 10.

Boelens, M. H.; Boelens, H.; van Gemert, L. J. Trends in aroma research and gas chromatography-olfactometry. *Perfum. Flavor.* **1995**, *20*, 1.

Federal Register. Title 27, 5.22(g), **1973**.

Gentry, H. S. *The Agaves of Continental North America*; University of Arizona Press: Tucson, AZ, 1982; 670 pp.

Grosch, W. Determination of potent odourants in foods by aroma extract dilution analysis (AEDA) and calculation of odour activity values (OAVs). *Flavour Fragrance J.* **1994**, *9*, 147−158.

Güntert, M.; Brüning, J.; Emberger, R.; Hopp, R.; Köpsel, M.; Surburg, H.; Werkhoff, P. Thermally degraded thiamin. A potent source of interesting flavor compounds. In *Flavor Precursors—Thermal and Enzymatic Conversions*; Teranishi, R., Takeoka, G., Güntert, M., Eds.; American Chemical Society: Washington, DC, 1992; Vol. 490, Chapter 11.

Hollingsworth, P. It ain't hip to sip. *Food Technol.* **1994**, *48 (11)*, 26.

Hortus Third, A Concise Dictionary of Plants Cultivated in The United States and Canada; The Liberty Hyde Bailey Hortorium; MacMillan: New York, 1976; 739 pp.

Incitti, S.; Tommasini, A.; Pascucci, E. Determination of minor volatile constituents of spirits. I. Tequila. *Riv. Soc. Ital. Sci. Aliment.* **1980**, *9 (1)*, 43−50.

Leffingwell, J. C. FLAVOR-BASE, version 2.5; Leffingwell & Associates: Canton, GA, 1989−1994.

Manjarrez, A.; Llama, M. Quantification of the volatile components of tequilas and mescals by gas chromatography. *Rev. Soc. Quim. Mex.* **1969**, *13 (1)*, 1−5.

Mordi, R. C. Carotenoids: functions and degradation. *Chem. Ind.* **1993**, 79−83.

Mosciano, G.; Fasano, M.; Michalski, J.; Sadural, S. Organoleptic characteristics of flavor materials. *Perfum. Flavor.* **1991**, *16 (1)*, 31−33.

Näf, R.; Velluz, A.; Thommen, W. Isolation of a glucosidic precursor of damascenone from *Lycium halimifolium* Mil. *Tetrahedron Lett.* **1990**, *31 (45)*, 6521−6522.

Peppard, T. L. Volatile flavor constituents of *Monstera deliciosa*. *J. Agric. Food Chem.* **1992**, *40 (2)*, 257−262.

Valenzuela-Zapata, A. G. The tequila industry in Jalisco, Mexico. *Desert Plants* **1985**, *7 (2)*, 65−70.

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