Chromatographic Analysis of Vanilla Extracts

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Abstract

Vanilla extracts are made by percolating or macerating chopped vanilla beans in ethanol and water. The Food and Drug Administration (FDA) has specific regulations for manufacturing vanilla extracts. If a company follows these regulations, the product is said to be a "Standard of Identity "product.

The Department of the Treasury's Alcohol and Tobacco Tax and Trade Bureau (TTB) is responsible for regulating the use of ethanol in products manufactured in the United States. One area that falls within TTB's jurisdiction is the regulation of nonbeverage products, which includes vanilla extracts. When a domestic manufacturer produces a vanilla extract according to FDA regulations the distilled spirits used in manufacturing are eligible for drawback of most of the Federal excise tax paid on those spirits.

A series of vanilla extracts were manufactured in the TTB Nonbeverage Products Laboratory according to the FDA regulations. The beans used in the extracts were grown in various countries. Once manufactured, the extracts were analyzed using gas chromatography and mass spectrometry. Different sampling techniques were also investigated: headspace, solid-phase microextraction and liquid injections.

The objectives of this study were to identify predominant components and to determine if chemometric models could be created so that they group the extracts by country of origin. Chemometric models created with headspace sampling showed that extracts cluster according to the year they were prepared. The amount of vanillin present in the extracts is also reported for each extract.

Extract preparation

Standard of identity for vanilla extracts requires one unit of vanilla beans (13.35 oz at 25% moisture or 10.0125 oz dry weight) per gallon. These extracts must have a minimum of 35% alcohol[1]. Twelve vanilla bean types were obtained from industry members and the percentage of moisture was measured by weighing a bean before and after drying overnight at 100 °C (Table 1). The amount needed for a two-fold extract was measured, beans were cut (Figure 1) and were extracted with a solution of 45% ethanol/water for 8 hours at 100 °F using the apparatus shown in Figure 2.

Figure 1 Vanilla beans (whole and cut)

Figure 2 Extraction apparatus

Table 1 Description of vanilla extracts used in this study

Extract #	Country of Origin	% Moisture	Fold	Year prepared
1	Indonesia	12	2	2010
2	Bourbon*	17	2	2010
3	Madagascar	14	2	2010
4	Madagascar	13	2	2010
5	Madagascar	10	2	2010
6	Bourbon*	23	2	2010
7	PNG**	28	2	2010
8	Indonesia	13	1	1998
9	Tahitian	10	1	1998
10	Indonesia	12	1	1998
11	Madagascar	15	1	1998
12	Indonesia	12	2	2010

*Suppliers of beans did not identify country of origin for Bourbon type
**PNG= Papua New Guinea

Results

Once extracts were prepared, they were filtered and stored in amber bottles. Aliquots of these extracts were analyzed according to conditions described in Table 2. Examples of the chromatograms obtained for extract # 5 with each sampling technique are shown in Figure 3. Library search reports were obtained using NIST library (version 2.0, 2005) and summaries for all 12 extracts are shown in Tables 3-5 with the area percentage (Area pct) for compounds detected with match qualities above 80.

Table 2 Experimental conditions

	Headspace (HS)	Liquid (Liq)	Solid Phase Microextraction (SPME)
Gas chromatograph	Agilent 6890	Agilent 6890	Agilent 6890
Autosampler	Gerstel MPS 2	Gerstel MPS 2	Gerstel MPS 2
Sample preparation	1000 µl straight	1:10 with Methanol + Internal Standard	100 uL straight
Autosampler mode	Headspace (HS)	Liquid (Liq)	SPME
Preparation before injection	110 °C for 15 min	none	30 min extraction with PA fiber
Injection volume	1500 uL	1 µL	N/A
Inlet	250 °C, 5:1 split	250 °C, 5:1 split	250 °C, splitless
Oven profile	40 °C, 10 °C/min 220 °C, 10.0'	40 °C, 10 °C/min 250 °C, 10.0'	40 °C, 3 °C/min 250 °C, 10.0'
Column	HP5	Phenomen ZB-WAXplus	Agilent, HP-Innowax
Column dimensions	30 m x 0.25 mm x 0.25 µm	30 m x 0.25 mm x 0.25 µm	30 m x 0.25 mm x 0.25 µm
MSD Detector	Agilent MSD 5975 Inert	Agilent MSD 5975 Inert	Agilent MSD 5973
Solvent delay-(MSD)	4.67 min	4.65 min	4.60 min
Scan-(MSD)	30-300 amu	30-300 amu	40-350 amu

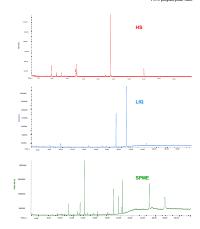* PA = polyacrylate fiber

Figure 3 Total ion chromatograms (TIC) for extract #5 using different sampling techniques
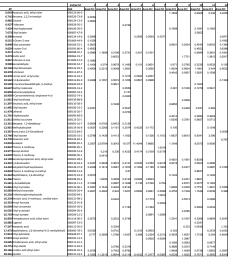
References

1. Code of Federal Regulations (2007). Title 21 Part 169. *U.S. Government Printing Office, Washington, DC 20402-001.*
2. Sostaric, T., Boyce, M. C. and Spickett, E. E. (2000) *J. Agric. Chem.* **48**, 5807.

Table 3 Library search reports for the extracts using headspace sampling

RT		CAS	1	2	3	4	5	6	7	8	9	10	11	12
3.735	Acetic acid	000064-19-7	2.8811	15.812	24.2253	6.9308	10.488	6.9235	43.6916	7.2985	17.1476	12.1285		9.0583
4.204	1-Butanol, 3-methyl-	000123-51-3	2.1611											6.508
4.400	Hexanal	000066-25-1	12.2766											
5.044	Furfural	000098-01-1	3.2513	3.2513	4.6022	2.1693	0.2145							
6.357	2,3-Butanediol	000513-85-9					2.0972							
7.344	Phenol	000108-95-2	5.0516	5.0516		19.384	0.731							
7.564	Hexanoic acid, ethyl ester	000123-66-0	1.7061							9.2745		15.731	5.1674	4.1018
9.000	Mequinol	000150-76-5									3.0604			
9.009	Phenol, 2-methoxy-	000090-05-1			17.7234	8.3265	3.662							
9.014	Hexane, 1,1-diethoxy-	003658-93-3	2.0454	34.663										
10.524	Octanoic acid, ethyl ester	000106-32-1												
11.426	Benzaldehyde, 4-methoxy-	000123-11-5							1.9867					
11.810	Benzenemethanol, 4-methoxy-	000105-13-5							17.3623					
11.912	Nonanoic acid, ethyl ester	000123-29-5							1.1872	2.0285	6.0428			
12.210	Benzeneacetic acid, 4-methoxy-	000104-01-8							4.2895					
12.217	Furfural	001758-09-0								54.0079				
13.355	Vanillin	000121-33-5	5.5067	15.725	26.3878	22.291	51.098	73.658	28.8712	11.996	3.805	9.9605	21.8258	9.1653
17.733	Benzenesulfonamide, N-butyl-	003622-84-2				6.009								
16.562	2,3-Butanediol	000513-85-9	0.2468	0.505	3.0074	0.4233	0.1172							
19.782	Ethyl tridecanoate	028267-29-0	10.0113		21.093				19.038					
19.782	Decanoic acid, 2,4,6-trimethyl-, methyl ester	000121-33-5	2.4453	2.4453							8.296			
19.786	Undecanoic acid, ethyl ester	000627-90-7			11.3803							8.5269		
21.999	11,14-Eicosadienoic acid, methyl ester	002463-02-7	1.9499											

Table 4 Library search reports for the extracts using solid phase microextraction (PA fiber*)

RT		CAS	1	2	3	4	5	6	7	8	9	10	11	12
6.060	Hexanoic acid, ethyl ester	000124-66-0							1.1939		0.6308	0.336	2.2255
6.716	Benzene, 1,2,3-trimethyl-	000526-73-8	0.0284											
6.860	Octanal	000124-13-0	0.0825											
6.937	Tridecane	000629-50-5				0.0746								
7.430	Ethyl heptanoate	000106-30-9							0.1998		0.1091	0.1565	
7.473	Ethyl lactate	000687-47-8									2.5852		
8.308	Nonanal	000124-19-6	0.2066				0.2956	0.2693	0.5271				0.3971
8.515	4-Octen-2-one	001669-44-9	0.1028									0.1980	
8.648	Ethyl octanoate	000106-32-1	0.3869						0.6901	0.2004	0.4543	0.8003	0.1588
9.029	1-octen-3-ol	003391-86-4	0.4563								0.4552		0.8386
9.217	Furfural	000098-01-1	0.1088	1.1896	0.3108	0.2722	0.451	0.1101		0.3504	0.6600	0.3601	0.1887
9.273	Acetic acid	000064-19-7			0.6053					1.7721		1.3853	0.2977
9.857	4-Nonen-2-one	014309-57-0	0.1980							0.0425		0.1588	
9.998	Benzaldehyde	000100-52-7	0.1458	0.274	0.1483	0.101	0.0601		0.677	0.2765	0.3235	0.0333	0.105
10.156	Ethyl Nonanoate	000123-29-5	0.4939	0.2215	0.3048	1.2603		0.3000	1.3034	0.9824	1.1901	3.444	0.4432
10.370	Octanol	000111-87-5							0.4042	0.0667	0.5831	0.1588	
10.378	Formic acid, octyl ester	000112-32-3			0.1215	0.2508	0.2087						
10.562	2,3-Butanediol	000513-85-9	0.2468	0.505	3.0074	0.4233	0.1172						
10.599	Furancarboxaldehyde, 5-methyl-	000620-02-0									0.1482		0.1284
10.698	Dimethyl malonate	000108-59-8							0.381	0.1039	0.1279	0.5947	
10.890	beta-Caryophyllene	000087-44-5	0.141										
10.958	Carvomenthenol, terpinene-4-ol	000562-74-3				0.0648							
11.061	Ethyl levulinate	000539-88-8				0.0109							
11.387	Decanoic acid, ethyl ester	000110-38-3							0.6850		0.4406	0.531	0.960
11.465	Ethyl laurate	000106-33-2	0.2331		0.1608	0.5527							
11.479	Carveol	000116-26-7				0.0649							
11.701	Ethylbenzoate	000093-89-0							0.4012		0.680		
11.831	Diethyl succinate	000123-25-1		0.0809	0.0766	0.0452	0.236						
12.352	Benzene, 1,2-dimethoxy-	000091-16-7	0.0800	0.0702	0.1988	0.1872	0.109	0.1172	0.195				
12.367	Methylsalicylate	000119-36-8	0.029	0.068	0.0951	0.3070							
12.363	trans,trans-2,4-Decadienal	025152-84-5				0.0746							0.2059
12.748	Ethyl laurate	000106-33-2	0.3298	0.1436	0.4113	1.1933	0.1186	0.4231	1.6837	0.8005	0.9347	3.396	
12.789	Ethyl levulilinate	000539-88-8					1.0674						8.767
13.818	Guaiacol	000090-05-1											
13.833	Phenol, 2-methoxy-	000090-05-1	3.2007	23.9789	9.3045	19.377	11.4044	7.8665	1.5019	1.1548	0.5073	0.9306	
14.039	Phenol, 2-methoxy-	000090-05-1			0.216	0.230	0.4232	0.4179	0.5064				
14.128	Benzenepropanoic acid, ethyl ester	002021-28-5			0.0491							
14.391	1,4-Butanediol	000110-63-4	0.4347	0.3608	0.3675	0.4121	0.5038	0.4762	0.6218	0.5019	0.5504	0.4580	
14.442	butylated hydroxytoluene	000128-37-0	0.2409	0.1612	0.1988	0.0872	0.109	0.1185	0.1824		0.1500	0.1220	
14.834	Phenol, 2-methoxy-4-methyl-	000093-51-6			0.61					0.9627			
15.058	Benzaldehyde, 4-methoxy-	000123-11-5	0.0573								0.1498		
15.464	Phenol	000108-95-2		1.4030	0.944	0.4600	0.8852		0.3494	0.2431	1.8601		
15.581	4-Anisaldehyde	000123-11-5	0.1269	0.0987	0.0948	0.130	0.3062	0.3586		4.8701	0.2577	0.1302	
15.865	Ethyl myristate	000124-06-1	0.3491	0.1544	0.4986	0.987	0.4684	0.3021	0.0506	0.4055	0.7751	1.4603	0.1805
15.883	Methyl cinnamate	000103-26-4	0.2205	0.4462	0.941	0.5602	1.5820	0.5005	0.7054	1.5548	0.2559	0.2286	
16.229	4-Methoxybenzyldehyde	000123-11-5	0.777	0.3594	0.1587	1.542	0.448	0.4234	1.6557	1.7158	0.5004	0.0904	
16.228	benzoic acid, 4-methoxy-, methyl ester	000121-98-2		0.0442			1.1773		0.503		0.2386		
16.352	hexyl formate	000112-31-8					0.0988						
16.604	Ethyl cinnamate	000103-36-6			0.1182		1.1382						
16.636	Ethyl-p-anisate	000094-30-4			0.0491					0.9783			
16.758	Butyl acetate	000104-21-2						0.0881	1.3055				
16.959	Pentadecanoic acid, ethyl ester	041114-00-5	0.2520	0.2523	0.3748				0.5450	0.9806	0.8894	0.2047	
16.885	Eugenol	000097-53-0		0.0491						0.1302			
17.247	Nonanoic acid	000112-05-0									0.032	5.0936	1.320
17.473	naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	000483-78-3	0.0426	0.0714	0.1144	0.0903	0.183						
17.453	Ethyl palmitate	000628-97-7	0.777	0.3594	1.2794	1.542	0.448	4.9334	1.6557	1.7158	5.0094	0.0904	
17.672	hexyl alcohol	000111-27-3					0.085		1.387				
17.513	Octadecanoic acid, ethyl ester	000111-61-5								0.0083		0.007	
19.775	Ethyl oleate	000111-62-6	0.378		0.2788	0.2218						0.7148	
20.166	Linoleic acid ethyl ester	000544-35-4			0.7453	0.9782			1.8468	1.4069	0.7488	0.3947	
20.355	Linolelic	000121-33-5	1.1581	11.3215	5.6464	6.2118	8.4332	17.2447	6.6897	1.0025	9.7071	6.3695	6.6374

Table 5 Library search reports for the extracts using liquid injections

RT		CAS	1	2	3	4	5	6	7	8	9	10	11	12
7.832	2-Propanone, 1-hydroxy-	000116-09-6	1.1241	1.1851	1.5217		1.1466	1.9384	1.7518	1.7344	1.6203	2.2247	2.3809	1.9342
8.077	1-Propanol, 3-ethoxy-	000111-35-3											0.2494	
8.790	2-Chloroethanol	000107-07-3	1.8792	1.450										
9.708	Acetic acid	000064-19-7		1.8947	3.8513	3.5898	2.547	2.7675	3.0337	1.224	1.3688	1.6983	1.5919	4.3466
11.267	2,3-Butanediol	000513-85-9	1.3843	0.7124	0.4683	0.5586	0.4613	2.5006	0.1237	0.5678	0.4198	0.2425	0.3469	
11.866	1,2-Ethanediol	000107-21-1	1.3686	1.0226					0.2909	0.3098	0.0356	0.1035		
12.781	2-Furanmethanol	000098-00-0		0.67	0.4843	0.2622	0.4297		0.4868	0.1538	0.4868	0.6374		
13.320	L-2-Cyclopentanedione	000698-10-2	0.844	0.4824	1.463	1.053	0.511	0.1011	0.5339	0.394	0.4495	0.4848	0.7981	
14.474	Phenol, 2-methoxy-	000090-05-1	0.7523	1.7163	1.8511	2.6340	1.9088	1.507	1.159	1.6559	1.8262	2.9644	1.9573	2.3004
14.966	Phenol	000108-95-2		0.642	0.3882	0.4374	0.3152	1.5491	1.1315	0.687	0.809	0.5988	0.5594	
16.775	2-Propanone, 1,3-dihydroxy-	000096-26-4	1.3784	2.2004	1.7753	1.3071	0.2601	2.0163	1.7569	1.4441	1.4345	2.7987		
16.560	benzenemethanol, 4-methoxy-	000105-13-5											0.468	0.9347
19.168	2,4'-(Methylenedioxy)acetophenone*	003162-29-4	27.7500	22.4898	31.7805	28.7097	28.164	28.8939	27.5134	29.4035	24.7573	27.7846	24.3922	1.3422
20.413	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	000067-47-0	0.6697	1.19	1.380	0.9176	0.6558	0.6355	0.450	0.017	0.1972	1.7169	1.0968	0.4879
20.704	Phenol, 4-(ethoxymethyl)-	057726-26-8								0.9372	0.5081	27.923	30.883	0.3047
23.885	Benzoic acid, 4-methoxy-	000100-09-4						0.3960						
24.303	Benzaldehyde, 4-hydroxy-	000123-08-0	0.7818	0.75	1.3223	2.6464	2.3953	2.711	2.3089	2.7469				
28.961	Octaethylene glycol monododecyl ether	000123-33-5			1.5511	0.4042								

* Internal Standard used for quantitation of vanillin

Chemometric Analysis

Principal component analysis of data collected with headspace sampling indicated that the total variance captured with the first three principal components was 97.91%. However, this high percentage of variance captured did not appear to be related to the country of origin of the extract. Instead Figure 4 indicates that extracts clustered according to the year they were prepared.
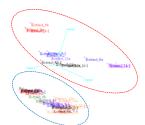

Figure 4 Principal component analysis of HS data (4 replicas per extract). Ellipsoids do not represent statistical information, they are drawn for visual identification of clusters.

Vanillin content

A three point calibration curve was created using liquid injections. The vanillin content of the 12 extracts was obtained using 3',4'-(Methylenedioxy)acetophenone as internal standard. Results are shown in Table 6.

Table 6 Vanillin content obtained using liquid injection

Extract #	Vanillin (ppm)
1	724
2	1412
3	1624
4	1611
5	1964
6	2678
7	1071
8	996
9	643
10	735
11	1810
12	1091

Conclusions and future work

➢ Solid Phase Microextraction provided a simple and fast sampling technique that identified 62 compounds.
➢ Liquid injections required an extra step in the sample preparation (dilution with methanol) in order not to overload the chromatographic column.
➢ Headspace sampling was the least sensitive. In some extracts only 4 compounds were identified with a good quality match.
➢ The potential of discriminating extracts according to country of origin will be investigated using data collected with SPME sampling
➢ Future work includes addition of more extracts to this database plus analysis using liquid chromatography.

* PA = polyacrylate fiber. No other fibers were studied since Sostaric[2] et al. found the PA fiber to be the most efficient at extracting vanillin.