Determination of Synthetic Components in Flavors by Deuterium/Hydrogen Isotopic Ratios

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A study was made relating ^{14}C, $\delta^{13}C$, and δD isotopic abundances for 11 different flavor materials: acetaldehyde, amyl acetate, anethole, benzaldehyde, cinnamic aldehyde, ethyl acetate, ethyl butyrate, ethyl caproate, linalool, methyl salicylate, and vanillin. Deuterium/hydrogen (δD) isotopic ratios, determined in these flavor materials, were found to be sufficiently uniform and different for those of natural sources and fossil fuel derived sources. δD values for the natural or modern derived flavor materials were consistently more depleted in deuterium content than the synthetic material. The mean δD value for the natural and synthetic compounds, respectively, were found to be the following: acetaldehyde, –202 and –76‰; amyl acetate, –292 and –123‰; anethole, –85 and –58‰; benzaldehyde, –111 and –58‰ (synthetic I) or +600‰ (synthetic II); cinnamic aldehyde, –120 and –27‰ (synthetic I) or +515‰ (synthetic II); ethyl acetate, –242 and –98‰; ethyl butyrate, –238 and –57‰; ethyl caproate, –246 and –220‰; linalool, –297 and –196‰; methyl salicylate, –130 and –71‰; and vanillin, –77 and –20‰. Correlation between ^{14}C activities representing the degree of naturalness covaried with δD values, especially with ester compounds. The implication is the deuterium content could be used in determining adulteration by addition of synthetic components or at least suggest further testing for those flavor materials which fall outside the normal range of isotopic values.

INTRODUCTION

The authentication of natural products by isotopic analysis has been well documented and tested for a large array of flavoring materials. The radiocarbon technique has been used in determining the addition of fossil fuel derived products in foods and flavors such as caffein, cinnamic aldehyde, and bitter almond oil (Allen, 1961; Hoffman and Salb, 1980; Krueger, 1987; Butzenlechner et al., 1989; Byrne et al., 1986) as well as the addition of ^{14}C to fossil fuel derived material to simulate a natural activity level (Culp and Noakes, 1990).

Stable carbon and hydrogen isotopes have been used in the deciphering of source materials for flavors such as vanilla, linalool, and citral (Hoffman and Salb, 1979; Bricout, 1982) as well as the characterization of ethanol in wine (Bricout et al., 1975) and in the detection of sugar additives to orange juice and honey (Doner et al., 1987; Lucchesi, 1979).

We previously reported on the advantages and disadvantages of ^{14}C analysis for distinguishing natural materials from those derived from fossil fuels (Culp and Noakes, 1990). We also discussed the theory behind stable isotope analysis ($\delta^{13}C$ and δD) and its applications to the determination of a botanical source for samples of cinnamic aldehyde and benzaldehyde. In this report, we have extended these techniques to nine additional flavor materials: acetaldehyde, amyl acetate, anethole, ethyl acetate, ethyl butyrate, ethyl caproate, linalool, methyl salicylate, and vanillin.

MATERIALS AND METHODS

Samples used in this study were supplied by the Isotopic Studies Committee (ISC) of the Flavor and Extract Manufacturers' Association (FEMA). The samples were prepared and analyzed at the University of Georgia's Center for Applied Isotope Studies (CAIS). Some samples of botanical origin were processed into extracts or oils at CAIS. Samples of previously manufactured or extracted compounds were processed by FEMA and were labeled as natural or synthetic by the supplier.

Anise seeds (anethole samples 41, 42, and 47–52), cassia bark (cinnamic aldehyde samples 25–34), and birch bark (methyl salicylate samples 1 and 3) were processed by first soaking approximately 0.5 kg of material in 0.2 L of water at 50 °C for 24 h. Steam distillation was then carried out on the resulting slurry. The distillate was extracted three times with 50-mL aliquot of methylene chloride which, upon evaporation, yielded a pure enough quantity of isolated oil for isotopic analyses.

Apricot kernels (benzaldehyde samples 36 and 43) were processed as described by Culp and Noakes (1990). Vanilla beans (samples 109 and 112–150), were processed by first soaking the finely cut beans (5-mm slices) in a 35% ethanol aqueous solution for 6 h. Vanillin was extracted using multiple extractions with carbon tetrachloride. After evaporation, the crystalline vanillin was purified using pentane.

Botanical extracts, once isolated, were stored under nitrogen in Teflon and glass vials until analyzed. To assure purity in both extracts and supplied samples, most samples were submitted to a member company for gas chromatographic/mass spectrometric analysis.

Isotopic analysis were performed for the stable isotopes of carbon $\delta^{13}C$ and hydrogen δD using a Finnigan MAT 251 stable isotope ratioing mass spectrometer. Radiocarbon measurements were performed using a Packard 1050 liquid scintillation counter. Analytical methods are previously described in Culp and Noakes (1990).

Isotopic abundances are tabulated for ^{14}C measurements in units of disintegrations per minute per gram of carbon (dpm/gC). ^{14}C measurements are calibrated relative to the National Institute for Standards and Technology (NIST) oxalic acid. Typical modern ^{14}C activities are approximately 15.5 dpm/gC based on modern atmospheric levels of $^{14}CO_2$. This activity defines the natural or modern end member. Fossil fuel derived material, being depleted in ^{14}C due to their extreme age, have ^{14}C activities of approximately 0 dpm/gC and define the synthetic end member.

The isotope abundances of both carbon and hydrogen isotopes are expressed in δ and written relative to the heavier mass isotope. The δ value is defined as the per mil (‰) or parts per thousand deviation of the sample isotopic ratio relative to that of a standard and expressed by the equation

$$‰ = (R_{sample}/R_{standard} - 1) \times 10^3$$

where $R = {}^{13}C/{}^{12}C$ or D/H.

The standard for reporting carbon isotopes relative to is PDB, a Cretaceous belemnite fossil, *Belemnitella americana*, from the Peedee Formation of South Carolina. Since this standard no longer exists, a working standard is used and calibrated to NIST standards such as NBS-20, NBS-21, and NBS-22 whose isotopic ratios are known relative to PDB (Hoefs, 1980).

Variations in the $\delta^{13}C$ value is a reflection of isotope effects as a result of the different kinetic and equilibration factors for ^{13}C and ^{12}C due to physical and chemical processes such as photosynthesis. Processes common to fossil fuel production such as methanogenesis or petroleum maturation give rise to isotope effects resulting in greater depletion of ^{13}C. Based on these, $\delta^{13}C$ values may be used to verify or suggest botanical source, process, or fossil fuel derivation.

The standard for hydrogen isotopes is V-SMOW: Vienna-standard mean ocean water represents the hydrosphere, the major hydrogen reservoir exhibiting a very constant isotopic ratio for the δD scale. Additional standards exist such as SLAP, standard light Antarctic precipitation, which is used to establish a linear scale and normalize results (Gonfiantini, 1978).

Variations in the δD value is primarily due to the processes of evaporation and condensation of meteoric water. Geographically, higher altitudes and latitudes are associated with more depleted deuterium concentrations as the evaporation/condensation cycle progresses, since water is progressively depleted in deuterium with respect to the water from which it is derived. Other variations, albeit minor, occur in plants during the evapotranspiration cycle and in the CO_2 and H_2O assimilation through the plant stomata (Yapp and Epstein, 1982). Compounds with labile hydroxyl hydrogen atoms such as vanillin and linalool were treated with the standard preparation technique including complete combustion in a sealed Pyrex tube. For the vanillin samples extracted at CAIS, isotopic analysis was performed immediately following extraction to minimize any isotopic fractionation.

RESULTS AND DISCUSSION

Table I lists the isotopic analyses of 11 flavor materials. For each compound, the isotopic values are presented based on whether (1) the sample was derived from botanical material by CAIS or labeled as natural by the submitter and similar in isotopic values to samples prepared by CAIS or (2) the sample was submitted as synthetic and the ^{14}C content was consistent with materials derived from fossil fuels. We have included a separate listing for mixtures for two of the esters, amyl acetate and ethyl butyrate; these mixtures result when the ester is prepared from a combination of botanical and fossil fuel precursors.

Table II lists the mean isotopic values and their 1σ standard deviations for each of the 11 flavor materials and their respective subgroups; a better representation of the range of isotopic values is evident.

Table I indicates natural (modern derived) ^{14}C activities between 13.6 and 17.8 dpm/gC for all 11 flavor materials. This is not unexpected as some deviations occur due to the natural decrease and variation in ^{14}C activity year by year. The corresponding synthetically derived flavors range from −0.17 to 0.41 dpm/gC indicating a purely fossil fuel derivation. The negative values are expressed only as raw data values and indicate ^{14}C measurements statistically less than our background ^{14}C activity. The ^{14}C activities for the amyl acetate mixtures are approximately 75% of the natural activity level (11.48/15.37). Amyl acetate, produced by the condensation of amyl alcohol and acetic acid, has a molecular carbon ratio of 5 to 2 from these respective compounds. When amyl acetate is produced from natural amyl alcohol and fossil fuel derived acetic acid, the ^{14}C activity, representing the total carbon of the compound, should be approximately 5/7 of the natural activity level, 11.00 dpm/gC. This compares statistically with the observed activity of 11.48 dpm/gC.

Similarly, ethyl butyrate, produced by condensation of butyric acid and ethanol, has a molecular carbon ratio from these two compounds of 4 to 2. Therefore ethyl butyrate made from natural ethanol and fossil fuel derived butyric acid should theoretically have a ^{14}C activity of 33% of the natural activity of 15.87 or 5.24 dpm/gC. The observed activity for these mixed ethyl butyrate samples is 5.38 dpm/gC, again indicating good statistical agreement.

The $\delta^{13}C$ values for all naturally derived samples range from −9.67‰ in ethyl butyrate to −33.66‰ in methyl salicylate. This is in close agreement with and indicates both C3 and C4 type plant sources. The production of esters by condensation reactions involves alcohols and acids that often are derived from corn or other C4 type plants displaying an enriched ^{13}C value. Deviations outside the narrower range suggested by Smith and Epstein (1971) for Calvin plants of −24 to −30‰ is to be expected for this variety of plant products.

$\delta^{13}C$ values for the synthetically derived flavors range from −24.57‰ in benzaldehyde to −35.28‰ in ethyl acetate. These values are highly variable and typically cannot be used alone to infer a fossil fuel source. However, it is interesting to note that even though the synthetic and natural methyl salicylate appeared to be of Calvin (C3) biosynthetic origin, this limited sample indicates the ability to differentiate using $\delta^{13}C$ values. More samples should be analyzed to substantiate this conclusion.

As shown in Table II, the $\delta^{13}C$ values are close and occasionally overlap for natural and fossil fuel derived materials. Therefore, this isotopic ratio is not of primary utility other than to signal an anomalous sample for further investigation.

Referring to Table I, the δD values of the botanical extracts and natural flavor materials range from −52‰ for vanillin to −373‰ for amyl acetate. This represents a broad range of abundances but still compares favorably with the hydrogen isotopic distributions indicated by Winkler (1983).

Major variations will occur between the 11 flavor materials studied due to differences in geographic origin, water uptake, physiology, and chemistry of the compound of interest. Minor variations for each flavor material exist due to differences in extraction methodology and geographical origin. Biosynthesis has a variable, but possibly greater, effect on the δD values. Generally, the hydrogen atoms of these extracts are those originally present in the sugar molecules at the time of biosynthesis (Smith and Jacobson, 1976).

The δD of the synthetics, indicated in Table I, show a varied and complex array of values. δD values as depleted as −311‰ exist for synthetic linalool, and enrichments as high as +720‰ are found for synthetic benzaldehyde made from the oxidation of toluene. Such extreme enrichments are atypical for a synthetic process and have only been identified in synthetic benzaldehyde and cinnamic aldehyde. Outside of these the δD values range from −311 to +29‰ for synthetic ethyl acetate. However, when the δD values are averaged for each flavor material and its corresponding subgroups, a more discernable picture is revealed.

A distinct difference can be observed between the natural flavors of aromatic structure and those of aliphatic structure. The latter group, acetaldehyde, linalool, and the esters, have δD values more depleted than −200‰. This is in contrast to the enriched δD values of the aromatic flavor materials which range on average from −77 to −120‰. This appears to be in agreement with earlier investigations by Bricout and Koziet (1978), where a

Table I.   Carbon and Hydrogen Isotopic Values for Acetaldehyde, Amyl Acetate, Anethole, Benzaldehyde, Cinnamic Aldehdye, Ethyl Acetate, Ethyl Butyrate, Ethyl Caproate, Linalool, Methyl Salicylate, and Vanillin

sample	^{14}C, dpm/gC	$\delta^{13}C$, ‰	δD, ‰
Acetaldehyde, Natural			
65	16.41	−12.15	−201
117	16.43	−19.07	−138
132	16.30	−21.76	−266
mean	16.38	−17.66	−202
SD	0.07	4.96	64
Acetaldehyde, Synthetic			
77	0.26	−30.73	−68
185	0.06	−29.05	−80
189	0.02	−27.74	−72
202	0.05	−26.29	−75
359	−0.17	−28.89	−83
mean	0.04	−28.54	−76
SD	0.15	1.65	6
Amyl Acetate, Natural			
105	15.60	−28.06	−282
236	16.70	−24.36	−373
241	16.00	−11.68	−305
250	15.15	−11.32	−284
266	15.75	−18.28	−300
306	14.29	−15.61	−282
328	13.60	−17.55	−246
3	15.88	−12.91	−266
mean	15.37	−17.47	−292
SD	1.00	6.05	38
Amyl Acetate, Synthetic			
176	0.082	−28.19	−145
186	−0.02	−26.69	−133
188	−0.09	−28.58	−117
194	−0.02	−29.63	−128
334	0.23	−26.49	−89
342	0.04	−31.48	−123
mean	0.04	−28.51	−123
SD	0.11	1.88	19
Amyl Acetate, Mixtures			
121	11.49	−28.46	−271
213	11.26	−15.23	−271
219	11.52	−23.53	−243
230	11.25	−18.64	−250
356	11.80	−23.59	−258
367	11.53	−23.64	−261
mean	11.48	−22.18	−252
SD	0.20	4.61	15
Anethole, Natural			
41	16.36	−25.86	−68
42	15.97	−29.35	−96
47	15.95	−29.47	−67
48	15.36	−26.27	−69
49	16.24	−26.93	−80
50	15.75	−29.40	−84
51	15.29	−29.14	−92
52	15.62	−29.20	−89
228	16.66	−33.02	−94
249	14.98	−26.95	−72
322	15.73	−28.99	−102
5	15.84	−27.68	−91
6	15.46	−28.93	−93
7	15.43	−27.48	−90
mean	15.76	−28.48	−85
SD	0.46	1.81	12
Anethole, Synthetic			
170	17.12	−32.08	−58
171	18.50	−30.72	−39
254	16.00	−31.83	−61
310	13.63	−30.73	−110
335	17.34	−31.95	−55
352	13.64	−31.05	−82
361	13.56	−30.93	−103
mean	15.87	−31.30	−58
SD	1.91	0.78	28

sample	^{14}C, dpm/gC	$\delta^{13}C$, ‰	δD, ‰
Benzaldehyde, Natural			
4	16.05	−28.44	−114
7	16.18	−29.15	−122
11	15.61	−27.06	−93
14	15.73	−29.84	−105
16	16.15	−28.37	−119
18	15.20	−29.81	−109
20	16.07	−29.95	−117
36	15.38	−27.95	−84
43	15.74	−27.50	−86
68	16.66	−28.54	−121
71	16.55	−27.96	−114
72	16.74	−28.55	−92
73	16.62	−29.15	−91
84	16.71	−29.58	−88
87	16.00	−28.18	−103
101	16.47	−30.35	−101
103	16.85	−29.31	−109
104	16.52	−28.20	−145
108	16.50	−28.55	−118
125	17.78	−28.65	−75
197	15.55	−28.36	−132
237	16.25	−28.16	−116
265	15.44	−27.80	−121
305	15.70	−27.85	−131
329	15.26	−27.75	−106
368	15.89	−28.10	−134
369	15.09	−29.09	−136
mean	16.09	−28.51	−111
SD	0.61	1.03	18
Benzaldehyde, Synthetic (I)			
53	−0.01	−29.06	−47
54	−0.06	−29.99	−48
82	0.11	−28.52	−67
178	−0.01	−28.79	−45
−200	0.02	−28.57	−84
336	−0.01	−29.47	−57
mean	0.01	−29.07	−58
SD	0.06	0.57	15
Benzaldehyde, Synthetic (II)			
58	−0.07	−26.50	+641
63	0.03	−25.51	+623
114	−0.04	−26.55	+603
151	0.30	−25.84	+669
179	0.09	−24.95	−514
229	0.03	−28.57	+633
247	−0.11	−26.00	+720
311	0.06	−24.70	+608
343	−0.05	−25.36	+588
350	0.18	−24.57	+584
360	−0.08	−25.16	+526
mean	0.03	−25.99	+600
SD	0.61	1.29	67
Cinnamic Aldehyde, Natural			
25	16.38	−27.50	−109
26	16.86	−27.88	−124
27	16.30	−26.81	−142
28	17.89	−27.75	−124
29	15.83	−27.36	−124
30	15.46	−27.76	−132
31	15.83	−28.16	−127
32	18.25	−27.24	−129
33	16.75	−27.73	−120
34	15.86	−27.49	−134
55	16.03	−27.50	−118
56	16.73	−27.65	−115
57	13.67	−27.18	−111
102	16.41	−27.36	−125
136	16.16	−27.12	−117
153	15.44	−26.78	−113

sample	^{14}C, dpm/gC	$\delta^{13}C$, ‰	δD, ‰
Cinnamic Aldehyde, Natural			
156	16.37	−30.07	−75
157		−26.77	−110
158	16.75	−27.73	−103
159	15.89	−27.11	−125
160	16.18	−27.31	−125
161	16.61	−25.97	−148
162	16.25	−27.60	−141
163		−26.11	−114
164		−27.68	−133
165	16.86	−27.07	−103
166	15.97	−26.98	−145
167	13.78	−27.25	−140
168		−26.70	−98
169	14.73	−26.59	−107
239	16.07	−26.88	−116
324	15.59	−26.92	−122
mean	16.11	−27.30	−120
SD	0.94	0.69	15
Cinnamic Aldehyde, Synthetic (I)			
180	−0.02	−28.81	−11
337	0.00	−29.18	−43
mean	−0.01	−29.00	−27
SD	0.01	0.26	23
Cinnamic Aldehyde, Synthetic (II)			
62	0.13	−25.02	+464
152	0.15	−25.70	+569
177	0.03	−25.65	+499
223	−0.01	−25.65	+482
231	0.04	−25.03	+549
319	0.07	−26.69	+508
344	−0.08	−26.68	+569
351	−0.04	−24.61	+478
mean	0.04	−25.63	+515
SD	0.08	0.76	42
Ethyl Acetate, Natural			
74	15.63	−13.80	−219
76	16.29	−16.91	−203
80	16.43	−19.62	−235
123	16.37	−28.40	−201
205	15.81	−11.48	−235
217	14.65	−15.90	−240
248	14.60	−17.16	−268
251	15.46	−29.26	−259
258	14.74	−17.30	−280
259	15.05	−16.48	−250
261	15.35	−29.28	−295
270	15.81	−19.92	−256
307	16.60	−13.45	−208
mean	15.60	−19.19	−242
SD	0.70	6.05	30
Ethyl Acetate, Synthetic			
78	0.38	−29.65	+29
90	0.26	−35.28	−96
119	0.01	−32.64	−118
191	0.01	−27.09	−79
220	0.01	−25.31	−133
260	0.15	−28.10	−140
312	−0.08	−28.25	−131
317	0.00	−26.95	−119
mean	0.09	−29.16	−98
SD	0.16	3.29	55
Ethyl Butyrate, Natural			
64	15.84	−9.67	−172
67	16.58	−10.65	−182
107	16.74	−16.74	−256
203	15.95	−13.77	−251
212	15.43	−18.27	−251
226	15.84	−17.98	−270

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Table I (Continued)

sample	^{14}C, dpm/gC	$\delta^{13}C$, ‰	δD, ‰	sample	^{14}C, dpm/gC	$\delta^{13}C$, ‰	δD, ‰	sample	^{14}C, dpm/gC	$\delta^{13}C$, ‰	δD, ‰
	Ethyl Butyrate, Natural				Linalool, Natural				Vanillin, Beans		
240	15.48	-21.21	-243	106	15.39	-27.09	-303	109		-19.74	-115
281	15.13	-13.89	-264	137	15.32	-27.14	-281	110		-20.65	-84
304	15.69	-13.57	-235	139	17.45	-28.93	-259	112		-20.08	-76
308	15.67	-13.08	-248	141	17.05	-25.16	-295	113		-20.05	-74
325	16.23	-12.00	-243	142	16.33	-25.78	-310	116		-19.44	-80
330	16.43	-23.26	-232	184	18.34	-28.48	-297	118		-19.55	-59
372	16.60	-16.73	-266	196	15.60	-27.99	-321	120		-19.23	-85
1	14.59	-14.76	-220	238	16.62	-26.58	-267	128		-19.70	-64
mean	15.87	-15.33	-238	320	15.96	-25.66	-359	130		-19.57	-81
SD	0.61	3.86	29	327		-26.88	-226	131		-21.49	-79
				331	15.80	-27.90	-286	133		-18.48	-73
	Ethyl Butyrate, Synthetic			340	12.65	-30.24	-244	134		-21.18	-91
70	0.41	-15.73	-9	355	14.54	-28.01	-294	135		-21.02	-80
187	0.02	-25.30	-12	362	17.27	-25.52	-289	138		-20.87	-66
192	0.20	-25.90	-107	mean	16.31	-27.02	-297	150		-19.76	-52
222	0.06	-22.95	-102	SD	1.08	1.27	26	mean		-20.05	-77
253	-0.07	-31.89	-86					SD		0.83	15
313	-0.02	-27.26	-15		Linalool, Synthetic						
318	-0.06	-25.60	-9	140	0.12	-26.53	-156		Vanillin, Lignin		
338	-0.07	-29.30	-84	181	0.03	-27.77	-203	174	14.97	-26.79	-170
345	0.07	-29.43	-90	315	-0.01	-26.20	-168	175	14.76	-26.54	-182
mean	0.06	-27.04	-57	321	0.04	-27.82	-311	199	17.06	-26.85	-195
SD	0.16	2.72	44	347	-0.06	-32.00	-176	210	14.91	-26.57	-172
				353	-0.01	-25.83	-160	221	15.70	-27.54	-178
	Ethyl Butyrate, Mixtures			mean	0.02	-27.69	-196	227	14.44	-26.88	-177
66	5.34	-21.91	-145	SD	0.06	2.26	59	234	14.99	-27.19	-200
233	5.31	-22.05	-140					256	15.48	-27.27	-180
349	5.49	-24.57	-32		Methyl Salicylate, Natural			264	14.67	-26.64	-194
mean	5.38	-22.84	-106	1	17.35	-33.15		274	15.55	-26.61	-204
SD	0.10	1.50	64	3	16.09	-33.66	-56	mean	15.25	-26.89	-185
				323	15.95	-32.65	-143	SD	0.75	0.34	12
	Ethyl Caproate, Natural			332	15.74	-32.85	-156				
129	15.59	-29.35	-221	363	17.66	-33.23	-163		Vanillin, Guaiacol		
208	14.43	-29.21	-251	mean	15.65	-33.11	-130	111		-26.13	-17
214	15.22	-27.65	-239	SD	0.88	0.39	50	124		-24.86	-23
245	15.21	-28.28	-259					mean		-25.50	-20
252	15.60	-27.28	-232		Methyl Salicylate, Synthetic			SD		0.90	4
275	15.79	-30.14	-267	61	0.00	-29.85	-51				
309	16.69	-27.88	-266	183	0.05	-28.85	-95				
339	15.62	-31.37	-262	190	0.54	-29.77	-75				
365	17.23	-27.51	-251	209	-0.04	-28.32	-62				
370	15.83	-32.20	-194	246	-0.01	-30.84	-75				
371	15.22	-28.38	-263	314	1.41	-28.91	-58				
mean	15.68	-29.02	-246	316	0.73	-29.12	-78				
SD	0.75	1.63	23	341	0.81	-28.83	-97				
				348	0.33	-30.08	-73				
	Ethyl Caproate, Mixtures			354	0.80	-28.75	-50				
172	12.54	-31.47	-199	mean	0.46	-29.33	-71				
173	12.20	-33.08	-217	SD	0.48	0.77	16				
201	11.81	-32.88	-209								
242	12.42	-30.36	-234								
243	11.44	-31.93	-203								
262	12.64	-30.43	-235								
282	12.91	-32.48	-242								
358	12.69	-31.58	-224								
mean	12.33	-31.78	-220								
SD	0.49	1.03	16								

dependency on volatility of plant substances was observed with δD values.

The highly depleted values indicated for the esters of natural origin agree well with the δD values for biogenic ethanol, one of the precursors of the ethyl esters. The more enriched values for the synthetically derived esters indicate that δD values may be useful in differentiating synthetic from natural compounds. Even though the isotopic range for each compound and its subgroup is broad, there is little if any overlap of the ranges for natural and synthetic flavor materials.

The utility of the δD values in differentiating synthetic materials from botanically derived materials is demonstrated by the amyl acetate samples of mixed origin. With respect to amyl acetate, the mixture was shown to be approximately 75% natural based on the ^{14}C activity and the chemistry of the precursors. In terms of the δD value, this 75% natural material would be -250‰. This agrees well with the observed mean value for the six samples of -252‰. For the samples of ethyl butyrate, the ^{14}C and corresponding δD values can be used to determine the percentage of synthetic precursors used to prepare the flavor compound. The mixtures of ethyl butyrate whose mean ^{14}C activity is 5.38 dpm/gC (34% of natural) indicate the reaction of natural ethanol with synthetic butyric acid. This should be equivalent to a δD value of -119‰ which is between the mean δD value of the synthetic -57‰ and the natural -238‰. Again, the agreement with the observed δD value of -106‰ is well within the range of one standard deviation for this data and can be used to differentiate materials.

Table II. Carbon and Hydrogen Isotope Summary for Acetaldehyde, Amyl Acetate, Anethole, Benzaldehyde, Cinnamic Aldehyde, Ethyl Acetate, Ethyl Butyrate, Ethyl Caproate, Linalool, Methyl Salicylate, and Vanillin

	stable isotope summary		
	^{14}C, dpm/gC	δ^{13}C, ‰	δD, ‰
Acetaldehyde			
natural (3)	16.38 ± 0.07	−17.66 ± 4.96[a]	−202 ± 64
synthetic (5)	0.04 ± 0.15	−28.54 ± 1.65	−76 ± 6
Amyl Acetate			
natural (8)	15.37 ± 1.00	−17.47 ± 6.05[a]	−292 ± 38
synthetic (6)	0.04 ± 0.11	−28.51 ± 1.88	−123 ± 19
mixtures (6)	11.48 ± 0.20	−22.18 ± 4.61[a]	−252 ± 15
Anethole			
natural (14)	15.76 ± 0.46	−28.48 ± 1.81	−85 ± 12
synthetic (8)	15.87 ± 1.91	−31.30 ± 0.78	−58 ± 28
Benzaldehyde			
natural (28)	16.09 ± 0.61	−28.51 ± 1.03	−111 ± 18
synthetic (12)	0.03 ± 0.12	−25.99 ± 1.29	+600 ± 67
synthetic (6)	0.01 ± 0.06	−29.07 ± 0.57	−58 ± 15
Cinnamic Aldehyde			
natural (30)	16.11 ± 0.94	−27.30 ± 0.69	−120 ± 15
synthetic (8)	0.04 ± 0.08	−25.63 ± 0.76	+515 ± 42
synthetic (2)	−0.01 ± 0.01	−29.00 ± 0.26	−27 ± 23
Ethyl Acetate			
natural (13)	15.60 ± 0.70	−19.19 ± 6.05[a]	−242 ± 30
synthetic (8)	0.09 ± 0.16	−29.16 ± 3.29	−98 ± 55
Ethyl Butyrate			
natural (14)	15.87 ± 0.61	−15.33 ± 3.86[a]	−238 ± 29
synthetic (9)	0.06 ± 0.16	−27.04 ± 2.72	−57 ± 44
mixtures (3)	5.38 ± 0.10	−22.84 ± 1.50[a]	−106 ± 64
Ethyl Caproate			
natural (11)	15.68 ± 0.75	−29.02 ± 1.63	−246 ± 23
mixtures (8)	12.33 ± 0.49	−31.78 ± 1.03	−220 ± 16
Linalool			
natural (13)	16.31 ± 1.08	−27.02 ± 1.27	−297 ± 26
synthetic (6)	0.02 ± 0.06	−27.69 ± 2.26	−196 ± 59
Methyl Salicylate			
natural (5)	16.56 ± 0.88	−33.11 ± 0.39	−130 ± 50
synthetic (10)	0.46 ± 0.48	−29.33 ± 0.77	−71 ± 16
Vanillin			
beans (15)		−20.05 ± 0.83	−77 ± 15
lignin (10)	15.25 ± 0.75	−26.89 ± 0.34	−185 ± 12
quaiacol (2)		−25.50 ± 0.90	−20 ± 4

[a] Extreme σ values due to both C3 and C4 source materials.

CONCLUSION

This study has demonstrated the effectiveness of isotopic analysis for the authentification of flavor materials. As with previous CAIS work with FEMA, the utility of this technique for identifying isotopic ranges for both natural and synthetic sources has now been expanded to 11 compounds.

As the number of samples increases within each compound class, the range of acceptable values for each of the isotopic abundances will narrow, and the ability to distinguish natural derivation from that of synthetic will become simpler and more credulous. For now the ^{14}C method is superior for defining a synthetic source of origin. However, due to the potential for ^{14}C addition to synthetics to simulate natural derivation, the method is limited. The stable isotopes of carbon, δ^{13}C, are quite useful in defining or suggesting isotopic manipulation or adulteration of flavors by virtue of their distinct ranges for the C3 and C4 type plant groups and even narrower ranges for those compounds within each group. The data presented here indicate a covariance of δ^{13}C values with ^{14}C activities for nearly all 11 compounds. However, for those compounds with values too similar between synthetic and natural

derivations the δ^{13}C values are of little utility when used alone. For the ester compounds the disadvantage is more severe in that the precursor acid and alcohol may be from two different plant types, creating difficulty in source interpretation.

The deuterium/hydrogen, δD, isotopic ranges for these 11 compounds have been shown to be much more effective in detecting adulteration and isotopic manipulation. The greater isotope effect and more sensitive measurement, due to the mass difference, imparts a more specific range of values for these 11 compounds. Even though some overlap of values may exist, a minimum reasonable value can be suggested for each compound which one could use to suggest a synthetic source of origin. The δD values covary with ^{14}C activities and indicate that depleted deuterium concentrations are associated with natural products and the more enriched or less negative δD values are associated with the synthetic precursors. Where compounds have been produced by a mixture of synthetic and natural materials such as some of the esters described here, the δD values have duplicated the molecular proportions of synthetic and natural material quite accurately. By using the mean δD values for each of the known endmembers, for example ethyl butyrate, −238‰ for natural and −57‰ for synthetic, a mixture of synthetic butyric acid and natural ethanol to produce ethyl butyrate should exhibit a ^{14}C activity of 5.24 dpm/gC based on a 4 to 2 molecular carbon ratio and a δD value of −133‰ based on a 7 to 5 molecular hydrogen ratio. What is found is a ^{14}C of 5.38 dpm/gC and −106‰ for the δD. The nearness of results to those predicted for at least these esters studied here indicate the potential for classifying these flavors based solely on their δD value.

The δD measurement is more complex and suffers from competing reactions that are less than predictable; however, it can be concluded that for most compounds listed here δD values have found utility on their own to classify and authenticate flavor materials. Where the δD values may not solely define the source material, they may be used in combination with other isotopic abundances to enhance interpretation and define a flavor materials origin.

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