Comparison of Volatile Compounds in Water- and Oil-Soluble Annatto (*Bixa orellana* L.) Extracts

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Annatto is a natural food colorant extracted from the seeds of the *Bixa orellana* L. plant. Annatto is used in Latin American cuisine to add a deep red color as well as distinctive flavor notes to fish, meat, and rice dishes. In the United States, annatto extracts are primarily used to impart orange/yellow hues to cheese and other dairy foods. The objective of this study was to identify and compare volatile compounds present in water- and oil-soluble annatto extracts. Volatile compounds were recovered using dynamic headspace–solvent desorption sampling and analyzed using GC–MS. Compounds were identified by comparison to a mass spectral database, Kovats indexes, and retention times of known standards. Of the 107 compounds detected, 56 compounds were tentatively identified and 51 were positively identified. Volatile profile differences exist between water- and oil- soluble extracts, and annatto extracts contain odorants with the potential to influence food aroma.

KEYWORDS: Annatto; achiote; *Bixa orellana*; aroma; natural colorant; terpenes; cheese color; humulene

INTRODUCTION

Consumer concerns over the use of synthetic colorants have led to an increase in demand for natural colorants such as annatto. The term "annatto" refers to a series of colorant preparations consisting of carotenoid-type pigments, based on extracts of seeds of the tropical bush *Bixa orellana* L. This 3- to 6-m high bush is native to Central and South America where its seeds are extensively used as a colorant for food and clothes (*1*). The fruit consists of a burr-like pod that contains between 20 and 50 seeds, about the size of grape seeds, which are covered with a thick layer of soft red pulp. Annatto, also known as orlean, achiote, urucum, or rocou, is cultivated in several tropical countries including Jamaica, Mexico, and the Philippines, with Peru, Brazil, and Kenya as the main sources of supply (*2*).

About 80% of the world production, which ranges between 10,000 and 11,000 tons of seeds per year (*3*), is utilized by the U.S. and Western Europe where it is further processed and used to impart the characteristic orange-yellow color to butter, margarine, and cheese (*4*). Annatto extracts may also be used as both a pigment and flavorant in flour and sugar confections, meat products, soft drinks, and snack foods.

The major pigment present in the pulp is *cis*-bixin, the monomethyl ester of the dicarboxylic acid norbixin. Bixin constitutes 80% of the pericarp mass and is responsible for the red coloration obtained from the extracts (*5*). Upon heating, *cis*-bixin is transformed to a more stable trans isomer and, with further heating, degradation compounds are formed. Two methods of pigment extraction are commonly employed, yielding either a water-soluble or an oil-soluble pigment preparation. Although extensive studies have been conducted describing the chemical properties of annatto pigments (*6–9*) little is known about potential aroma-influencing compounds in commercially employed annatto extracts.

Plant leaves, roots, and seeds are natural sources of carotenoids and have long been used to influence flavor character in addition to imparting color to food products. Saffron and paprika are known not only for their coloring properties but also for their flavoring properties and are widely used as spices in specialty foods such as risotto, paella, curry, chili products, soups, stews, and meat products (*10*). For centuries, annatto has been used as a spice for traditional cooking in Mexico and South America where it is mixed with pork, fish, chicken, corn, or rice to enhance the color and to add distinctive flavor notes to the food. Despite being widely used as a spice, little is known of the volatile composition of annatto extracts and powders. Therefore, we conducted an investigation to characterize the volatile compounds present in commercial oil- and water-soluble annatto extracts.

MATERIALS AND METHODS

Sample Preparation. Four annatto (*B. orellana*) extracts were obtained from commercial sources, 2 were water-extracts and 2 were oil-extracts. Volatiles were recovered using dynamic headspace purge and trap sampling conducted in triplicate with 10.0 mL of each of the four annatto samples. Samples were purged in a 100-mL round-bottom flask at 30 °C for 30 min with N₂ gas (purge flow 880 mL/min, purge

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volume 26.4 L) onto Carbotrap adsorbent (Orbo-100, 350/175 mg, Supelco, Bellefonte, PA). Heptanol and ethyl-laurate were added as internal standards at concentrations of 2.0 and 6.0 μg/mL respectively for water extracts, and 100 and 300 μg/mL respectively for oil extracts. Analytes were desorbed with diethyl ether, concentrated to 75 μL, and injected into the GC/MS for analysis.

Gas Chromatography−Mass Spectroscopy GC/MS. Annatto samples were analyzed with a Hewlett-Packard 6890 GC equipped with a Hewlett-Packard 5973 MS detector and Hewlett-Packard B.02.05 ChemStation software. A polar Rtx-wax, 60 m × 0.25 mm i.d., 0.5 μm film thickness poly(ethylene glycol) column (Restek, Bellefonte, PA) was used for the analysis of volatile compounds.

Sample injections of 2 μL were made into the injector port in the splitless mode. Initial column temperature was 40 °C which was held for 5 min, then increased at 5 °C/min to 180 °C and maintained for 20 min. Column flow rate was 1.3 mL/min using helium as the carrier gas. Mass-spectrometry operating conditions were ion source temperature of 230 °C, ionization voltage of 70 eV, and mass scan range of m/z 33−300 at 2.76 scans/s.

Quantification and Identification. Standards were prepared in acetone. Heptanol and ethyl laurate were used as internal standards to quantify the analytes obtained from the annatto extracts. Compounds were identified by comparison to a mass spectral reference collection (NIST 1998 Mass Spectral Library, V1.6d.), published Kovats indexes, and retention times of known standards, when available. Statistical analyses were performed using SAS, version 6.12. Mixed model procedures were used to conduct a one-way analysis of variance (ANOVA). Features available in the mixed procedure were used to examine the ANOVA assumption of homogeneity within treatment variances. When heterogeneous variances were found, the residual variance was partitioned into multiple residuals, using the goodness-of-fit statistic to identify an adequately fitting model. LSD pairwise means comparisons were performed to compare compounds and groups with an α of 0.05.

RESULTS AND DISCUSSION

Results from the GC/MS analysis for the four annatto extracts are summarized in **Table 1**. A total of 107 volatile compounds were detected by GC/MS of which 51 were positively identified. The majority of volatile compounds present in water- and oil-soluble annatto were sesquiterpenes, constituting about 38% of the volatiles in oil extracts and around 89% in water extracts. The compositional difference between oil and water extracts is potentially a consequence of the low solubility of monoterpenes and sesquiterpenes in water. Bixin, the major pigment in annatto extracts, is a compound of less than 40 carbon atoms, but it is classed with the carotenoids because peculiarities in its structure suggest that it has been derived from degradation of higher carotenoids rather than synthesized from smaller units (*11*). The concentrations and types of volatile compounds found in annatto seeds may vary depending on the location, soil, and other factors that were not controlled for this experiment; this variation is reflected on the high SEM of some compounds.

The concentration of alcohols in annatto extracts was low compared to that of other groups of compounds (**Table 1**); most appeared to be derivatives of pentanol and hexanol. As expected, alcohols were present mainly in water extracts. 3-Hexenol, which was detected in one of the water extracts, has been reported as a common degradation compound of linoleic acid (*12*). Other fatty acid degradation products present in annatto extracts are nonanal, hexanal, and 2-heptenal. Nonanal is a degradation compound of oleic acid, whereas hexanal and 2-heptanal come from enzymatic oxidation of linoleic acid. Nonanal and 2,4-heptadienal are major contributors to the aroma of cauliflower and broccoli (*12*). Hexanal was the only aldehyde that appeared in all annatto extracts. This compound has been described as having a green grassy smell (*13*). The concentration

of aldehydes in oil extracts (24.9 and 12.6 μg/mL) was higher than that in water extracts (0.0115 and 0.17 μg/mL). Such differences could be explained on the basis of the extraction method by which the extract was produced, fatty acid degradation, plant variation, and by the fact that aldehydes have more affinity to oil than water. The difference in total aldehyde content between oil extracts is mainly due to the high concentration of 2-methyl-2-butenal in sample A1. It is common to find homologous series in plant material, especially C_6-C_9 series that can include alcohols, aldehydes, and ketones.

Only 3 of the alkane compounds listed in **Table 1** were positively identified. Small alkanes are typically found in quantities well below their detection threshold and hence have little contribution to flavor. The majority of alkanes were obtained from oil extracts. Sample A2 presented a wide variety of alkanes, although the overall concentration for oil extracts is not significantly different ($p > 0.05$). *cis-* and *trans*-1,3-Dimethylcyclohexane were consistently found in all annatto extracts; they were especially concentrated in sample A1. 2,5-Dimethylhexane and 2-methylheptane had similar concentrations in all samples except for sample A1, where they appeared at higher concentrations.

δ-Elemene was the major alkene found in annatto extracts, with concentrations that varied from 1.14 to 15.0 μg/mL. The difference in δ-elemene across samples could be due to normal plant variations. In this case we cannot explain the differences based on the extraction method as, according to our analysis, similar concentrations can be found in water and oil extracts.

Of the seven ketones listed in **Table 1**, six were positively identified. Two of them, 3-penten-2-one and 3-octanone, were present in only the water annatto extracts. 4-Methyl-3-penten-2-one, a fatty acid derivative that has a sweet flowery aroma (*14*), was found in similar amounts in all four samples ($p > 0.05$). 6-Methyl-5-hepten-2-one was found across all extracts; it is usually a product of oxidative cleavage of lycopene, phytofluene, or phytoene and has a sweet, fruity-green aroma (*12*). The major ketone in both water and oil extracts was 4-hydroxy-4-methyl-2-pentanone, and its concentration was 4 times higher in oil extracts than in water extracts. As expected, overall ketone concentration was higher in oil extracts as compared with water extracts and this can be explained by the greater affinity of ketones to oil.

Esters and acids are usually regarded as great contributors to flavor and they are widely distributed in essential oils of fruits and spices. They contribute with an overall "fruity" character. Acetic acid was the only acid found in the headspace and was detected only in oil extracts. This phenomenon can be explained by the solubility of acetic acid, which is miscible with water and in low concentrations will tend to stay in solution rather than moving to the headspace. Ethyl butyrate was the only ester found in all extracts, and its concentration was similar in 3 of the samples. The concentration of esters and acids in annatto shows again the wide variation of the extracts. The high concentration of 1,2-propanediol-2-acetate in sample A2 accounts for the differences in the total values for esters and acids. This difference may well be due to plant variations or to degradation of triglycerides from the oil used to extract the pigment. The concentration of 1,2-propanediol-2-acetate varied widely in all replicates and when added to the total, it inflated the SEM to 15 μg/mL.

Small concentrations of heterocyclic compounds were found in annatto extracts, mainly pyridine and furan derivatives. In small concentrations, pyridines, especially 3-methylpyridine, contribute to the green aroma of plants. Pyridines are readily

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Table 1. Volatile Compounds Present in Oil- and Water-Soluble Annatto Extracts

compound[a]	RT min	concentration μg/mL (SEM)				RI$_k$
		oil extract samples		water extract samples		
		A1	A2	B1	B2	
alcohols						
4-methyl-2-pentanol[1,2,3]	21.62	nd	nd	0.111^a (0.003)	0.09^a (0.038)	1170
1-pentanol[1,2,3]	24.69	nd	2.6 (0.33)	nd	nd	1258
2-pentenol[1,2]	26.84	nd	0.5 (0.20)	nd	nd	1318
3-hexenol[1,2]	29.26	nd	nd	0.04 (0.023)	nd	1394
4-methyl-1-hexanol[1]	30.57	nd	0.5 (0.33)	nd	nd	1445
6-methyl-5-hepten-2-ol[1,2]	31.47	nd	nd	0.33^a (0.021)	0.29^a (0.02)	1466
2-ethyl-1-hexanol[1,2,3]	32.31	nd	nd	2.4^a (0.17)	1.23^b (0.019)	1493
propanediol[1]	35.30	nd	nd	0.97 (0.016)	nd	1585
sum		nd	3.6^{ab} (0.78)	3.8^a (0.16)	1.61^b (0.059)	
aldehydes						
hexanal[1,2,3]	18.87	3.42^a (0.02)	5.5^b (0.24)	0.115^c (0.008)	0.10^c (0.04)	1095
2-methyl-2-butenal[1,2,3]	19.44	18.6^a (0.72)	0.3^b (0.11)	nd	nd	1108
heptanal[1,2,3]	22.79	nd	0.6 (0.26)	nd	nd	1201
3-methyl-2-butenal[1]	23.34	2.84 (0.026)	nd	nd	nd	1233
2-heptenal[1,2]	27.80	nd	1.0 (0.18)	nd	nd	1349
nonanal[1,2,3]	29.88	nd	1.3^a (0.42)	nd	0.07^a (0.044)	1437
2,4-heptadienal[1]	33.10	nd	3.8 (0.62)	nd	nd	1520
sum		24.9^a (0.73)	12.6^b (0.93)	0.115^c (0.008)	0.17^c (0.071)	
alkanes						
heptane[1]	6.52	nd	0.8 (0.24)	nd	nd	738
2,5-dimethylhexane[1]	6.68	0.92^a (0.06)	0.3^b (0.14)	0.081^b (0.009)	0.086^b (0.034)	742
2-methylheptane[1]	7.42	0.74^a (0.091)	0.3^b (0.12)	0.066^b (0.003)	0.064^b (0.024)	764
octane[1,2]	8.38	nd	19.2 (4.8)	nd	nd	792
2,4-dimethylheptane[1]	8.52	nd	3.9^a (0.89)	0.76^a (0.023)	0.88^a (0.063)	797
cis-1,3-dimethylcyclohexane[1]	8.96	17.9^a (0.43)	5.5^b (1.46)	1.22^{bc} (0.087)	1.7^c (0.12)	810
2,4-dimethylhexane[1]	9.79	1.36^a (0.041)	nd	0.04^b (0.014)	0.067^b (0.024)	834
trans-1,3-dimethylcyclohexane[1]	10.10	4.25^a (0.068)	0.18^{bc} (0.043)	0.05^b (0.023)	0.173^c (0.003)	843
nonane[1,2]	11.38	nd	0.25 (0.093)	nd	nd	880
butylcyclopentane[1]	14.71	nd	0.8 (0.18)	nd	nd	979
propylcyclohexane[1]	14.82	nd	0.27 (0.095)	nd	nd	982
decane[1,2]	15.14	nd	0.22^a (0.068)	nd	0.022^a (0.009)	1000
sum		26.5^a (0.63)	31.7^a (7.11)	2.2^b (0.14)	3.0^b (0.25)	
alkenes						
2,4-dimethyl-1-pentene[1]	6.39	1.3 (0.11)	nd	nd	nd	734
4-methyl-1,3-pentadiene[1]	8.49	12.4 (0.52)	nd	nd	nd	796
2,4-dimethyl-2-hexene[1]	22.90	nd	nd	0.121 (0.008)	nd	1220
δ-elemene[1,2]	32.54	2.5^a (0.58)	15.0^b (1.4)	10.7^b (1.19)	1.14^a (0.012)	1501
elixene[1]	32.90	nd	nd	0.121 (0.058)	nd	1514
1,3-dimethyl-1-cyclohexene[1]	32.96	1.22 (0.067)	nd	nd	nd	1516
4-ethenyl-4-methyl-3-cyclohexene[1]	34.55	nd	nd	0.27 (0.012)	nd	1563
sum		16.1^a (0.262)	15.0^a (1.4)	11.3^a (1.2)	1.14^b (0.012)	
ketones						
3-penten-2-one[1,2]	20.57	nd	nd	0.136^a (0.004)	0.09^a (0.036)	1142
4-methyl-3-penten-2-one(mesityl oxide)[1,3]	20.92	3.54^a (0.068)	2.4^a (0.21)	5.6^a (0.98)	3.2^a (0.90)	1152
2-heptanone[1,2,3]	22.66	nd	0.34 (0.12)	nd	nd	1198
3-hexen-2-one[1]	23.92	nd	2.0 (0.17)	nd	nd	1250
3-octanone[1,2]	25.24	nd	nd	0.06^a (0.028)	0.08^a (0.03)	1273
6-methyl-5-hepten-2-one[1,2,3]	28.05	13.8^a (0.63)	6.7^b (0.37)	3.86^c (0.075)	5.3^b (0.15)	1358
4-hydroxy-4-methyl-2-pentanone[1,3]	28.99	24.2^a (0.27)	28.5^a (1.95)	7.1^b (2.8)	0.56^b (0.077)	1385
sum		41.6^a (0.96)	40.0^a (2.2)	16.8^b (3.6)	9.3^b (0.84)	
esters and acids						
ethyl-butyrate[1,2,3]	17.01	1.93^a (0.034)	2.2^a (0.31)	1.8^a (0.36)	0.07^b (0.03)	1050
methylhexanoate[1,2,3]	22.79	1.08 (0.074)	nd	nd	nd	1202
heptylformate[1]	27.55	nd	0.32 (0.11)	nd	nd	1357
methyloctanoate[1,2]	29.63	1.4 (0.14)	nd	nd	nd	1404
acetic acid[1,2]	31.04	4.9^a (0.20)	3.2^a (0.42)	nd	nd	1451
1,2-propanediol-2-acetate[1,2]	36.28	nd	30.0 (16.0)	nd	nd	1621
sum		9.3^a (0.31)	36.0^{abc} (15.0)	1.8^b (0.36)	0.07^c (0.03)	
heterocycles						
γ-caprolactone[1]	13.37	nd	nd	0.12 (0.024)	nd	939
2,5-dimethylfuran[1,2]	13.81	0.82 (0.039)	nd	nd	nd	952
2,4-dimethylfuran[1]	14.70	0.91 (0.008)	nd	nd	nd	978
2-methylpyridine[1,2]	24.11	nd	0.32 (0.11)	nd	nd	1241
2-pentylfuran[1]	24.44	nd	2.4 (0.37)	nd	nd	1265
3-methylpyridine[1]	26.94	nd	0.21^a (0.12)	0.06^a (0.025)	0.11^a (0.047)	1338
4-methylpyridine[1]	27.16	nd	nd	0.16^a (0.02)	0.30^a (0.051)	1345
5-methyl-2-furancarboxaldehyde[1]	33.06	1.49 (0.03)	nd	nd	nd	1518
sum		3.22^a (0.05)	2.93^a (0.50)	0.34^b (0.057)	0.41^b (0.04)	

Table 1. (Continued)

compound[a]	RT min	concentration µg/mL (SEM)				RI$_k$
		oil extract samples		water extract samples		
		A1	A2	B1	B2	
arenes						
toluene[1,2,3]	17.40	91.2a (9.4)	8.5b (1.1)	0.071c (0.037)	0.103c (0.042)	1059
p-xylene[1,2,3]	21.38	269.0a (24.0)	208.0ab (51.0)	3.3b (0.36)	3.7b (0.12)	1164
allyl-p-methylbenzyl ether[1]	38.76	nd	5.4 (2.8)	nd	nd	1686
1-methyl-3-hexylbenzene[1]	38.89	1.6a (1.5)	2.0a (1.9)	7.5a (1.3)	2.2a (2.1)	1690
3-methylacetophenone[1]	42.76	nd	nd	0.63a (0.30)	2.0b (0.20)	1804
sum		362a (32.0)	224a (50.0)	11.5b (1.9)	8.1b (2.4)	
monoterpenes						
α-pinene[1,2,3]	16.61	220a (11.2)	40.4b (5.9)	4.6c (0.80)	2.8c (0.14)	1039
camphene[1,2,3]	18.48	1.64a (0.08)	0.26b (0.08)	nd	nd	1085
β-pinene[1,2,3]	20.14	93.4a (1.2)	28.5b (3.5)	3.1c (0.53)	1.43c (0.058)	1130
β-phellandrene[1]	20.55	1.54a (0.077)	0.9b (0.16)	nd	nd	1151
β-myrcene[1,2,3]	21.88	5.1a (0.24)	2.9a (0.52)	0.06c (0.029)	nd	1178
limonene[1,2,3]	23.54	1.53a (0.074)	2.5a (0.44)	0.03b (0.022)	0.04b (0.024)	1224
eucalyptol[1,2,3]	23.98	nd	nd	nd	2.51 (0.072)	1237
trans-ocimene[1,2]	24.44	1.33a (0.067)	0.5b (0.19)	nd	nd	1251
α-terpineol[1]	31.94	nd	nd	0.114a (0.007)	0.11a (0.045)	1486
2-carene[1]	33.90	nd	nd	0.4 (0.14)	nd	1543
3,7-dimethyl-1,6-octadien-3-ol[1]	33.96	nd	nd	nd	0.67 (0.055)	1545
terpinen-4-ol[1,2]	36.53	nd	nd	nd	0.19 (0.085)	1631
sum		325a (11.5)	75.9b (10.6)	8.3c (1.3)	7.7c (0.31)	
sesquiterpenes						
β-thujene[1]	23.96	0.86 (0.082)	nd	nd	nd	1251
α-cubebene[1,2,3]	32.24	3.4a (0.54)	2.5a (0.42)	nd	nd	1483
ylangene[1,2]	33.23	nd	nd	0.28a (0.037)	0.20a (0.088)	1518
cycloisosativene[1,3]	33.42	6.6a (0.68)	2.9b (0.40)	0.8c (0.14)	0.59c (0.012)	1530
copaene[1,2,3]	33.56	15.7a (1.7)	9.3b (1.4)	3.5c (0.57)	1.67c (0.03)	1531
isoledene[1]	34.44	nd	nd	0.16 (0.074)	nd	1559
germacrene-D[1]	34.82	nd	nd	1.2a (0.16)	0.3b (0.13)	1570
α-bergamotene[1]	35.58	3.8a (0.59)	nd	0.94b (0.067)	0.35c (0.014)	1592
santalene[1]	35.84	8.2a (1.0)	nd	1.6b (0.21)	0.88b (0.046)	1601
β-elemene[1]	36.39	1.9ab (1.0)	nd	4.0a (0.40)	1.34b (0.058)	1617
epi-bicyclosesquiphellandrene[1]	36.93	0.2a (0.19)	nd	nd	0.41a (0.056)	1633
caryophyllene[1,2]	37.17	5.49a (1.028)	nd	2.7a (0.34)	1.68a (0.055)	1644
allo-aromadendrene[1,2]	37.55	2.3ab (0.57)	nd	1.02a (0.082)	0.49b (0.06)	1662
eudesma-4(14),11-diene[1,2]	37.86	nd	nd	3.8 (0.45)	nd	1670
isosativene[1]	38.40	nd	1.4a (1.2)	nd	3.9a (0.51)	1676
β-panasinsen[1,3]	38.51	nd	nd	6.0a (1.03)	1.6a (1.6)	1689
β-humulene[1,3]	39.56	187a (22.0)	193a (35.5)	220a (36.3)	129a (64.0)	1710
δ-aristolene[1]	39.81	7.4a (0.73)	8.6a (2.5)	8.6a (1.1)	9.0a (0.68)	1717
γ-muurolene[1,2]	40.21	2.6 (0.26)	nd	nd	nd	1729
β-maaliene[1]	40.31	nd	14.4a (3.4)	19.1ab (3.3)	29.8b (2.1)	1732
β-selinene[1,2]	40.53	nd	nd	2.2a (0.34)	1.2a (0.54)	1739
α-gurjunene[1]	40.70	3.9a (0.33)	nd	1.6ab (0.86)	0.5b (0.46)	1773
γ-neoclovene[1]	41.06	nd	nd	9.1a (1.6)	8.4a (0.61)	1754
chamigren[1]	41.07	6.8a (0.68)	6.6a (3.2)	nd	nd	1755
germacrene-B[1,2]	41.30	15.3a (1.5)	44.5a (11.2)	56.6a (9.5)	17.0a (0.98)	1761
valencene[1,3]	41.68	10.0a (0.95)	11.6a (2.7)	11.0a (1.9)	15.6a (0.96)	1772
γ-selinene[1]	41.90	nd	15.8a (1.4)	17.7a (2.9)	nd	1779
γ-elemene[1]	42.36	24.5a (2.5)	34.8ab (7.8)	48.0a (7.6)	7.8b (3.9)	1792
patchoulene[1]	42.40	nd	nd	nd	21.9 (1.3)	1793
δ-cadinene[1]	42.91	8.7a (0.63)	7.4a (1.8)	7.4a (0.97)	2.7a (2.6)	1809
β-sesquiphellandrene[1]	43.13	nd	0.6a (0.39)	0.8a (0.10)	nd	1815
γ-cadinene[1]	43.36	nd	nd	1.2a (0.67)	1.5a (0.72)	1821
α-panasinsen[1]	43.91	4.8a (3.1)	4.3a (2.05)	11.5a (1.6)	8.6a (0.46)	1840
cyperene[1]	46.70	nd	nd	3.7a (0.46)	2.0a (1.97)	1920
spathulenol[1]	47.72	21.0a (10.4)	4.8a (4.6)	0.8a (0.74)	4.2a (4.2)	1950
sum		372a (63.7)	362a (62.1)	445a (70.3)	272a (69.8)	

[a] ID match: 1 MS, 2 Kovats, and 3 Standard. Means with same superscript (a, b or c) are not significantly different (LSD, $p > 0.05$). nd = not detected.

obtained from the reaction of acetaldehydes with ammonia, a product of amino acid degradation (*15*).

Arenes were the second largest group of volatiles present in oil extracts. They constituted around 29% of all volatiles present in oil extracts. In contrast, water extracts had small concentrations of arenes, accounting for only 2.5% of all volatiles. m-Xylene and toluene were previously reported in commercial annatto formulations in the range of 30−200 mg/kg and ≤12

mg/kg, respectively (*16*). We found considerably higher amounts of p-xylene and toluene in oil-soluble annatto extracts. Our results show p-xylene levels of 208 and 269 µg/mL for oil extracts, and about 3.3−3.7 µg/mL for water extracts. As predicted, the concentration of p-xylene is higher in oil extracts because of its low solubility in water. There is a difference between oil and water extracts with respect to p-xylene concentration. However, p-xylene concentrations are not statisti-

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cally different for samples A2, B1, and B2 due to the high standard error among different annatto samples. A 10-fold increase in toluene was detected in extract A1 (91.2 μg/mL) as compared to that detected in sample A2. The difference could result from plant cultivar or manufacturer extraction method variations. Water extracts contained similar concentrations of toluene ($p > 0.05$). Some extraction procedures used to obtain oil-soluble annatto involve a heat treatment, which can initiate carotenoid degradation. A characteristic fragmentation pattern of conjugated polyene carotenoids observed with mass spectroscopy analysis is the loss of toluene and xylene, which might explain the high concentrations found in oil annatto extracts (*17*). Toluene and xylene are also commonly found as native constituents in plant materials (*12*) and as extraction solvent residual or environment contaminants. Their presence in annatto extracts may be explained based on the extraction procedure, or, as stated previously, from breakdown of higher carotenoids. It has been suggested that repeated exposure to concentrations of 100 ppm and higher of xylene can cause gastrointestinal disturbances, and workers exposed to 200−300 ppm toluene have been observed to have an impaired speed of perception (*18*). Chronic exposure to toluene produces an irreversible progressive high-frequency hearing loss in animals that is dose and time dependent (*18*). Weak or absent genotoxic tests suggest no carcinogenic activity although it has not been proved systematically (*18*).

Simple terpenes have gained attention as agents of communication and defense among insects and can act as attractants or repellants in food products (*19*). Monoterpenes and sesquiterpenes are major components of many spices, fruit, and flower essential oils and as such have a great economic importance (*11*). The variety of monoterpenes and sesquiterpenes seen in the extracts is a result of the readiness with which they undergo rearrangement (*11*). In nature, terpenoids are synthesized from two isoprene equivalents: isopentyl-pyrophosphate and dimethylallyl-pyrophosphate (*20*). The proportions of oxidized and reduced monoterpenes vary according to the metabolic status of the plant; monoterpenes may act as an important store of physiological reducing power (*19*). Many monoterpenes inhibit the growth of competing plants and can be insecticidal or toxic to higher animals.

Several monoterpenes were found in annatto extracts as shown in **Table 1**. Interestingly, α-pinene appeared to be a major component of one of the oil samples (220 μg/mL), although its concentration in water-extracts is very small (4.61 and 2.75 μg/mL). α-Pinene has one of the lowest boiling points of all monoterpenes (157 °C). It has a resinous pine-like smell and is commonly found in essential oils of spices such as black pepper (*21*). It is normally extracted from turpentine and is often used in artificial pine fragrances and as a minor component in the production of artificial lemon, bergamot, nutmeg, geranium, and lavandin aromas (*22*). β-pinene was also found in higher amounts in the oil-soluble extracts than in water-soluble extracts. This compound has been described as dry-woody resinous piney odor of poor tenacity (*22*). Other monoterpenes found in trace amounts in annatto, that are worth mentioning owing to their distinctive aroma attributes, include limonene, β-myrcene, eucalyptol (found only in sample B2), β-phellandrene, and terpinen-4-ol. These compounds are widely used in the flavor industry and are considered isomerization products of other major monoterpenes found in annatto. In fact, β-myrcene, a compound also found in hops, bay leaf oil, and laurel, is widely used as a precursor to synthesize fragrance and flavor chemicals such as citral, citronellal, geraniol, linalool, and ionones (*22*).

Limonene is the major component of citrus fruits such as orange, but it also occurs in many essential oils. It has a fresh, light, and sweet-balsamic gum-like odor and in small concentrations has a sweet-herbaceous taste, but at higher concentrations it tends to give pungency and bitterness (*22*). Apart from contributing to the aroma of fruits, limonene has been cited as having anticarcinogenic and antimicrobial properties (*23, 24*). β-Phellandrene (bp 171 °C), which was detected only in oil extracts, has a peppery-minty and slightly citrusy odor with poor tenacity. It is usually isolated from waterfennel oil and eucalyptus. Terpinen-4-ol, which was found only in sample B2 (**Table 1**), has been described as having a floral, mildy earthy and sweet lilac-like aroma (*22*). It is commonly used in small amounts in the production of berry, lime, lemon, orange, ginger, anise, and spice flavors.

Sesquiterpenes constituted the major group of volatile compounds found in annatto extracts, showing similar average concentrations in all four samples. β-Humulene is by far the major volatile compound found in annatto extracts and did not vary between either source or type of extract. β-Humulene has been reported to be a major aromatic component of hops, and a major contributor to the flavor of beer (*25*). It has been described as having a woody-spicy, dry, clove-like tenacious odor, and somewhat bitter taste. It is usually accompanied by its isomer caryophyllene (*22*), a compound which was also found in annatto extracts but in smaller amounts. Also of importance are γ-elemene, germacrene-B, spathulenol, and copaene, which were present in high concentrations in water-soluble as well as in oil-soluble annatto extracts (**Table 1**). Copaene has been described as having a fresh, earthy, mango-like smell. During sesquiterpene biosynthesis several isomers are formed that can readily undergo isomerization depending on environmental conditions. Ylangene and copaene are examples of such isomers. Ylangene, a compound found sometimes in raw corn (*17*), was found in small concentrations in water annatto extracts. Copaene and ylangene differ only by the configuration on the carbon substituted with an isopropyl group (*19*). Presence of ylangene could be due to either rearrangement of copaene during the extraction procedure or as a byproduct of the biosynthesis of the latter.

Minor sesquiterpenes found in annatto extracts that have distinctive aromas include α-cubebene, δ- and γ-cadinene, santalene, and β-selinene. α-Cubebene has been described as having a fruity, sweet, citrus-like smell (*26*). δ- and γ-Cadinene, are compounds occasionally used as fixatives in candy flavors and have a dry-woody, slightly medicinal-tarry odor with some similarity to spices in the cumin-thyme family (*22*). Santalene, which was found in oil and water annatto extracts, possesses a mild woody odor with high tenacity and is usually isolated from sandalwood oil (*22*). β-Selinene is a compound with warm, herbaceous-peppery odor found in small amounts in water annatto extracts.

Some of the monoterpenes and sesquiterpenes found in annatto extracts have been previously described as having antimicrobial properties (*27*). Among those compounds are β-humulene, α- and β-pinene, β-phellandrene, α-terpineol, and caryophyllene, suggesting that annatto extracts may exhibit antimicrobial activities.

Volatile compounds detected in annatto extracts consisted mainly of sesquiterpenes, monoterpenes, and arenes. Most monoterpenes and sesquiterpenes have been previously detected in other spices and plants. β-Humulene is the major volatile present in annatto extracts followed by p-xylene, toluene, α- and β-pinene, γ-elemene, and spathulenol. Differences between

oil and water extracts were potentially due to differences in the extraction method of the manufacturer. This study demonstrates that annatto extracts contain numerous odorants with the potential to influence food aroma. The profile of compounds identified may also impart other properties to annatto extracts, such as antimicrobial, anticarcinogenic, or antioxidant activities.

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