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Odor-Active Constituents in Fresh Pineapple (*Ananas comosus* [L.] Merr.) by Quantitative and Sensory Evaluation

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By application of aroma extract dilution analysis (AEDA) to an aroma distillate prepared from fresh pineapple using solvent-assisted flavor evaporation (SAFE), 29 odor-active compounds were detected in the flavor dilution (FD) factor range of 2 to 4,096. Quantitative measurements performed by stable isotope dilution assays (SIDA) and a calculation of odor activity values (OAVs) of 12 selected odorants revealed the following compounds as key odorants in fresh pineapple flavor: 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDF; sweet, pineapple-like, caramel-like), ethyl 2-methylpropanoate (fruity), ethyl 2-methylbutanoate (fruity) followed by methyl 2-methylbutanoate (fruity, apple-like) and 1-(*E*,*Z*)-3,5-undecatriene (fresh, pineapple-like). A mixture of these 12 odorants in concentrations equal to those in the fresh pineapple resulted in an odor profile similar to that of the fresh juice. Furthermore, the results of omission tests using the model mixture showed that HDF and ethyl 2-methylbutanoate are character impact odorants in fresh pineapple.

Key words: pineapple; aroma extract dilution analysis; stable isotope dilution assay; sensory evaluation; odor-active compound

Due to its attractive sweet flavor, pineapple is widely consumed as fresh fruit, processed juice, canned fruit, and as an ingredient in exotic foods. The volatile constituents of pineapple have been studied for over 60 years by many researchers, and the results have been published in numerous papers reviewed by Flath,[1)] Berger,[2)] and Nijssen *et al.*[3)] More than 280 compounds have been found among the volatiles of pineapples so far. Only a few of these volatiles have been identified as contributing to pineapple flavor.

Berger *et al.* identified two minor hydrocarbon compounds, 1-(*E*,*Z*)-3,5-undecatriene and 1-(*E*,*Z*,*Z*)-3,5,8-undecatetraene, which were important contributors to fresh-cut pineapple aroma due to their low odor threshold values.[4)] Esters such as 2-methylbutanoates and hexanoates give fruity notes to fresh pineapple as well as other fruits.[2)] 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDF; furaneol®; pineapple furanone), which possesses a pineapple and caramel-like aroma, was first isolated by Rodin *et al.* from pineapple.[5)] Pickenhagen *et al.* reported the amounts of HDF and its methyl ether in pineapple aroma.[6)] Using a stable isotope dilution assay (SIDA), quantitative determination of HDF and its methyl ether was performed by us in fresh and processed pineapples.[7)] The concentration of the former compound in fresh pineapple was 34.9 mg/kg. Takeoka *et al.* identified many sulfur-containing esters among pineapple volatiles, but their concentrations were lower than their odor thresholds.[8)] Umano *et al.* reported many volatile constituents of green and ripened pineapple,[9)] but their contributions to pineapple aroma have not been described. In recent studies, Teai *et al.* have mentioned that esters, lactones, furanoids, and sulfur compounds act as very potent odor components of Polynesian pineapple.[10)]

Though much work has been done, mention of the aroma quality and quantity of each constituent and their contributions to the fresh, sweet aroma of pineapple are still scarce. Using odor unit values calculated from odor threshold and concentration data, Takeoka *et al.* reported that important contributors to fresh pineapple aroma are HDF, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, ethyl acetate, ethyl hexanoate, ethyl butanoate, ethyl 2-methylpropanoate, methyl hexanoate, and methyl butanoate.[11)]

A useful tool to identify the most odor-active compounds among the volatiles of a given food is aroma extract dilution analysis (AEDA),[12)] but this method, aiming at the identification of the key odorants, has not yet been applied to fresh pineapple. Our purpose in the present study was, therefore, to characterize the

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Abbreviations: SIDA, stable isotope dilution assay; AEDA, aroma extract dilution analysis; OAV, odor activity value; SAFE, solvent-assisted flavor evaporation; FD factor, flavor dilution factor; SHA, static headspace aroma extract dilution analysis; HDF, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone

Table 1. Most Odor-Active Volatiles (FD factor ≥ 2) in Distillate Prepared from Fresh Pineapple

No.	Odorant[a]	Odor quality[b]	RI[c] on		FD factor[d]	Earlier identified in pineapple[e]
			FFAP	DB-5		
1	Ethyl acetate	Solvent-like, fruity	898	611	2	26
2	Methyl 2-methylpropanoate	Fruity, sweet	940	684	8	26
3	Ethyl 2-methylpropanoate	Fruity, sweet	973	752	64	26
4	2,3-Butanedione	Buttery	966	—	4	
5	Methyl 2- and 3-methylbutanoates[f]	Fruity, apple-like	1014	773	2048	26
6	Ethyl butanoate	Fruity	1042	804	4	26
7	Ethyl 2-methylbutanoate	Fruity	1048	843	4096	26
8	Ethyl hexanoate	Fruity	1216	1002	4	26
9	Octanal	Citrus, fatty	1268	—	2	
10	(*Z*)-1,5-Octadien-3-one	Geranium-like	1363	982	2	
11	1-(*E*,*Z*)-3,5-Undecatriene	Fresh, pineapple-like	1380	1173	32	4
12	1,3,5,8-Undecatetraene[g]	Fresh, pineapple-like	1431	1173	2	4
13	Unknown	Fruity	1512	—	2	
14	Unknown	Fruity, pineapple-like	1549	—	32	
15	4-Methoxy-2,5-dimethyl-3(2*H*)-furanone	Caramel-like	1578	1055	8	6
16	Butanoic acid	Sour	1610	718	2	27
17	β-Damascenone	Fruity, sweet	1798	1394	8	
18	γ-Octalactone	Fruity, coconut-like	1895	1261	16	28
19	δ-Octalactone	Coconut-like	1941	1288	64	28
20	γ-Nonalactone	Peach-like, fruity	2011	—	4	9
21	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	Sweet, pineapple-like, caramel-like	2022	1063	1024	5
22	γ-Decalactone	Fruity, sweet, peach-like	2131	1475	8	27
23	Unknown	Clove-like, smoky	2146	—	8	
24	δ-Decalactone	Sweet, coconut-like	2165	1500	128	27
25	3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone	Seasoning-like	2181	1105	4	
26	Unknown	Spicy, pepper-like	2237	—	8	
27	γ-Dodecalactone	Fruity, sweet	2333	1678	16	11
28	Phenylacetic acid	Honey-like	2548	—	4	
29	Vanillin	Vanilla-like	2570	1408	128	29

[a]The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on the HRGC stationary phases given in the table, and odor quality as well as odor intensity perceived at the sniffing port.
[b]Odor quality perceived at the sniffing port. Analyses were performed by two assessors.
[c]Retention index.
[d]Flavor dilution (FD) factors were determined on a FFAP capillary.
[e]Reported in the references as a volatile compound in pineapple.[4–29)]
[f]Compounds were not separated on either stationary phase used.
[g]The geometries of the double bonds were not determined.

key aroma compounds in fresh pineapple by application of AEDA, followed by quantitations using SIDA, calculation of their odor activity values (OAVs, ratio of concentration to odor threshold[12)]). To link the analytical results with the original pineapple aroma, sensory studies on model mixture containing the aroma compounds in the same concentrations as determined in fresh pineapple were performed.

Materials and Methods

Pineapples. Fresh, ripe pineapples, "Super Sweet (F-2000)" of the Dole Corporation, were purchased at a local market in Garching, Germany. For sensory experiments, the same variety of pineapples was purchased in Yamanashi, Japan.

Chemicals. The following reference aroma compounds were obtained commercially: nos. 1–4, 5 (methyl 2-methylbutanoate), 6–9, 15, 18, 20–22, 24, 25, 27–29 (Aldrich, Sigma-Aldrich-Chemie, Taufkirchen, Germany); no. 5 (methyl 3-methylbutanoate), no. 19 (Lancaster, Mühlheim am Main, Germany); no. 16 (Fluka, Sigma-Aldrich-Chemie); and no. 17, a gift from Dr. M. Guentert (Symrise, Holzminden, Germany). The following compounds were prepared as described previously: no. 10,[13)] no. 11[14)] (Table 1). The reference compounds for flavor reconstitution were supplied by T. Hasegawa Co. (Tokyo).

Labeled internal standards. The following labeled internal standards were synthesized as reported in the literature (Table 2): 4-hydroxy-2,5-[$^{13}C_2$]-dimethyl-3(2*H*)-furanone (c-1),[7)] [1,1,1-$^2H_3$]-methyl 2-methylpropanoate(d-1) (P. Schieberle, unpublished report), [2,2,2-$^2H_3$]-ethyl 2-methylpropanoate(d-2),[15)] [1,1,1-$^2H_3$]-methyl 2-methylbutanoate(d-3),[16)] [2,2,2-$^2H_3$]-ethyl butanoate(d-4),[17)] [2,2,2-$^2H_3$]-ethyl 2-methylbutanoate(d-5),[15)] [3,3,4,4-$^2H_4$]-octanal(d-6),[18)] β-[$^2H_6$]-damascenone(d-8),[19)] δ-[$^2H_2$]-octalactone(d-9),[20)] δ-[$^2H_2$]-decalactone(d-10),[21)] and [$^2H_3$]-vanillin(d-11).[22)] [10,10,11,11-$^2H_4$]-1-(*E*,*Z*)-3,5-undecatriene(d-7) was synthesized as

Table 2. Selected Ions, Calibration Factors, and Thin-Film Capillaries Used in Stable Isotope Dilution Assays

Odorant	Selected ion m/z	Int std[a]	Selected ion m/z	Calibration factor[b]	Capillary
Methyl 2-methylpropanoate	103	d-1	106	0.94	DB-FFAP/DB-1701
Ethyl 2-methylpropanoate	117	d-2	120	0.53	DB-FFAP/DB-5
Methyl 2-methylbutanoate	117	d-3	120	0.96	DB-FFAP/DB-1701
Ethyl butanoate	117	d-4	120	1.00	DB-FFAP/DB-5
Ethyl 2-methylbutanoate	131	d-5	134	0.95	DB-FFAP/DB-5
Octanal	129	d-6	132–134[c]	1.29	DB-FFAP/DB-1701
1-(*E*,*Z*)-3,5-Undecatriene	151	d-7	155	1.05	DB-FFAP/DB-1701
β-Damascenone	191	d-8	195–199[c]	0.80	DB-FFAP/DB-5
δ-Octalactone	143	d-9	145	1.27	DB-FFAP/DB-1701
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	129	c-1	131	1.00	DB-FFAP
δ-Decalactone	171	d-10	173	0.46	DB-FFAP/DB-5
Vanillin	153	d-11	156	1.01	DB-FFAP/DB-5

[a]Isotopic labeling of the internal standard.
[b]Calibration factors were determined in mixtures of equal amounts of the unlabeled odorants and the labeled internal standards by means of mass chromatography, as reported by Sen *et al.*[19)]
[c]The sum of the cluster of ions present in the internal standard was used in the calculation of concentrations.

described for the unlabeled compound[14)] using [5,5,6,6-$^2H_4$]-hexanal instead of hexanal, as detailed previously.[23)]

Isolation of pineapple volatiles. Flesh of ripe pineapples (200 g) was homogenized with a kitchen juicer and stirred with dichloromethane (300 ml) for 1.5 h. The suspension was subsequently extracted with dichloromethane (5 times, total volume 900 ml) using a separatory funnel. The organic layer was separated, dried over sodium sulfate, and concentrated to about 60 ml by distilling off the solvent. The concentrate was subjected to solvent-assisted flavor evaporation (SAFE)[24)] to isolate carefully a distillate containing the pineapple volatiles. For AEDA, this distillate was concentrated at 46 °C to 400 µl.

High resolution gas chromatography (HRGC)/olfactometry (O). HRGC was performed with a type 8000 gas chromatograph (Fisons Instruments, Mainz, Germany) using the following fused silica capillary columns: CP-WAX58 (FFAP)-CB (25 m × 0.32 mm i.d., 0.2 µm; Varian, Darmstadt, Germany) and DB-5 (30 m × 0.32 mm i.d., 0.25 µm; J&W Scientific, Agilent Technologies, Waldbronn, Germany). The samples were applied by the cold-on-column injection technique at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 60 °C, held for 1 min (FFAP) or 2 min (DB-5), then raised at 6 °C/min to 180 °C, and finally raised at 10 °C/min to 240 °C and held for 5 min. The flow rate of the helium carrier gas was 2.5 ml/min. At the end of the capillary, the effluent was split 1:1 (by volume) into a FID and a sniffing port device using deactivated but uncoated fused silica capillaries (50 cm × 0.25 mm). The FID and the sniffing port were held at 260 °C and 230 °C respectively. Linear retention indices (RI) were calculated using *n*-alkanes.[25)]

AEDA. The flavor dilution (FD) factors of the odor-active compounds were determined by HRGC/O of serial dilutions using the AEDA approach.[12)] The following dilution series were evaluated by sniffing: the original distillate (400 µl) was stepwise diluted with dichloromethane (1 + 1) until no odorant was detectable by sniffing of the high dilution. HRGC/O was performed with aliquots (0.5 µl) using capillary FFAP.

Static headspace aroma extract dilution analysis (SHA).[12)] SHA was performed by means of a gas chromatograph CP-9001 with the purge system (PTI-TCT-injection system Model 4001; Chrompack, Mühlheim, Germany) using the experimental procedure described in our previous report.[14)] Homogenized pineapple (20 g) and calcium chloride (20 g) were equilibrated in a septum-sealed vessel (144 ml total volume) for 30 min at room temperature. Decreasing headspace volumes (10, 5, 2.5, 1.25, 0.62, 0.32, 0.16, and 0.08 ml) were taken off by means of gas-tight syringes (SGE, Darmstadt, Germany) and injected into the purge system operating in the desorption mode (10 min). The headspace volatiles were collected at −150 °C in a precooled trap and were flushed with the carrier gas onto the GC-column by heating the trap very rapidly to 250 °C. The fused silica capillaries used were OV-1701 (50 m × 0.53 mm i.d., 1.5 µm, J&W Scientific, Agilent Technologies, Waldbronn, Germany) and RTX5 (equal to an SE-54; 60 m × 0.53 mm i.d., 1.5 µm, Restek, Bad Homburg, Germany). The oven was held at 0 °C for 2 min and then raised by 6 °C/min to 240 °C. The effluent of the column was split 1:1 into a FID and a sniffing port. By odor quality and retention index, the odorants were identified as shown in Table 3. The FD factors given in Table 3 were calculated by dividing the largest volume analyzed (10 ml; FD = 1 by definition) by the lowest volume in which the respective odorants were still detectable.

Table 3. Results of Static Headspace Aroma Extract Dilution Analysis (SHA) of Fresh Pineapple

Odorant[a]	Odor quality[b]	RI[c] on		Volume (ml)		FD factor	
		RTX-5	OV-1701	RTX-5	OV-1701	RTX-5	OV-1701
Ethyl acetate	Solvent-like, fruity	—	685	—	5.0	—	2
2,3-Butanedione	Buttery	—	696	—	2.5	—	4
Methyl 2-methylpropanoate	Fruity, sweet	694	730	10.0	5.0	1	2
Ethyl 2-methylpropanoate	Fruity, sweet	766	819	1.25	0.32	8	32
Methyl 2- and 3-methylbutanoates[d]	Fruity, apple-like	785	841	0.16	0.08	64	128
Ethyl butanoate	Fruity	806	865	2.5	0.32	4	32
Ethyl 2-methylbutanoate	Fruity	857	910	0.08	0.08	128	128
Ethyl pentanoate	Fruity	905	968	5.0	2.5	2	4
(*Z*)-1,5-Octadien-3-one	Geranium-like	992	—	2.5	—	4	—
Ethyl hexanoate	Fruity	1003	1065	0.62	1.25	16	8
1-(*E*,*Z*)-3,5-Undecatriene	Fresh, pineapple-like	1181	—	0.62	—	16	—

[a,b]Refer to Table 1.
[c]Retention index.
[d]Compounds were not separated on either stationary phase used.

Quantitation by SIDA.

Isolation of flavor compounds and labeled internal standards. Three different extractions using different amounts (10 g, 100 g, or 200 g) of pineapple were performed depending on the concentrations of odorants present in the pineapple. Pineapple was homogenized with a kitchen juicer, and an equal amount of saturated aqueous calcium chloride solution was immediately added in order to inhibit enzymatic reactions. After the addition of known amounts of the labeled internal standards listed in Table 2, the mixture was stirred with dichloromethane or diethyl ether for 1.5 h at room temperature. The subsequent procedure for isolation of the pineapple volatile was the same as that described above.

Isolation of hydrocarbons by column chromatography. To obtain 1-(*E*,*Z*)-3,5-undecatriene for quantitation, the hydrocarbon fraction was isolated using a water-cooled glass column (17 × 1.4 cm) packed with a slurry of silica gel (Kieselgel 60, 230–400 mesh, VWR International, Darmstadt, Germany) in pentane with pentane (100 ml) as eluent. The hydrocarbon fraction was dried over sodium sulfate and concentrated by distilling off the solvent.

HRGC-mass spectrometry (HRGC-MS). Identification and quantitation of the odorants were performed by two-dimensional gas chromatography (TD-HRGC) by means of a Trace 2000 series gas chromatograph (Thermo Finnigan, Dreieich, Germany) as the precolumn system in tandem with a CP-3800 gas chromatograph (Varian, Darmstadt, Germany) as the main column system. MS analyses were performed by means of an ion-trap mass spectrometer Saturn 2000 (Varian) running in the chemical ionization (CI) mode with methanol as the reactant gas. The following fused silica capillaries were used: DB-FFAP (30 m × 0.32 mm i.d., 0.25 µm, J&W Scientific, Agilent Technologies, Waldbronn, Germany) in combination with DB-5 (30 m × 0.32 mm i.d., 0.25 µm, J&W Scientific) or DB-1701 (30 m × 0.32 mm i.d., 0.25 µm, J&W Scientific). The samples were applied by on-column injection at 40 °C. After 2 min, the temperature of the oven was raised at 40 °C/min to 50 °C (DB-5) or 60 °C (DB-FFAP), held for 2 min isothermally, then raised at 6 °C/min to 180 °C, then at 15 °C/min to 230 °C, and finally held for 10 min. In the case of DB-1701, the temperature of the oven was raised at 6 °C/min to 240 °C and finally held for 10 min. The flow of the helium carrier gas was 2.5 ml/min. The cut time intervals were determined by injections of the reference compounds. The selected ions of the labeled standards and the odorants (Table 2) were monitored and their intensities calculated by means of computer programs. The concentrations of the odorants were then calculated from these intensities and the amounts of the internal standards added and subsequently corrected using calibration factors, which had been obtained from a mixture of labeled and unlabeled odorants[19] (Table 2). The concentration of labeled 1-(*E*,*Z*)-3,5-undecatriene was determined by gas chromatography/FID using 1,5,9-decatriene (Aldrich, Sigma-Aldrich-Chemie, Taufkirchen, Germany) as an internal standard.

Sensory evaluation.

Panelists. The test panel consisted of 15 female students of the Home Economics Education Course at the University of Yamanashi. Before the sensory evaluation, they were trained by evaluating suprathreshold aroma solutions of ethyl 2-methylbutanoate (fruity), 1-(*E*,*Z*)-3,5-undecatriene (fresh, pineapple-like, green), β-damascenone (fruity, woody), and HDF (sweet, pineapple-like).

Flavor reconstitution. The following 12 reference compounds, dissolved in 0.5 ml of ethanol, were added to 1-liter of distilled water at concentration levels equal to those determined in fresh pineapple: methyl 2-methylpropanoate (150 µg); ethyl 2-methylpropanoate (48 µg); methyl 2-methylbutanoate (1,200 µg); ethyl butanoate (75 µg); ethyl 2-methylbutanoate (160 µg); octanal (19 µg); 1-(*E*,*Z*)-3,5-undecatriene (9 µg); β-damascenone (0.08 µg); δ-octalactone (78 µg); HDF

(26.8 mg); δ-decalactone (33 µg); and vanillin (6 µg). The mixture was stirred for 30 min (pineapple juice aroma model mixture).

Fresh pineapple juice. Fresh pineapple juice was obtained by hand-squeezing of fruit with a kitchen juicer immediately before the sensory evaluation. The sensory profile of the flavor was evaluated by eight well-trained students. The pineapple juice sample was tested orthonasally and seven odor qualities (sweet, citrus-like, fresh, fruity, green and grassy, woody, and pineapple-like) were selected for evaluation of the odor profiles of fresh pineapple juice as well as for the corresponding model mixture.

The fresh pineapple juice and the model mixture (25 ml each) were presented in covered glass beakers (capacity, 55 ml; internal diameter 40 mm) immediately after preparation. For evaluation, the glass cover was removed and the sample was sniffed by the panelists. The intensities of the seven odor qualities were scored on a seven-point scale from 0 to 6 (0, absent; 6, strong). The values given by the panelists were averaged and analyzed by *t*-test.

Omission tests. Six model solutions were prepared by omitting only one compound from the complete aroma model mixture. Each of the six solutions was presented to 15 panelists for comparison with the complete model in a triangle test.

Results and Discussion

AEDA of fresh pineapple distillate

The volatiles from 200 g of fresh pineapples were isolated by solvent extraction and SAFE.[24] An overall sensory evaluation of the flavor distillate revealed the typical fruity and sweet aroma impression of fresh pineapple. By application of AEDA on this pineapple distillate, a total of 29 odor-active regions was detected in the FD factor range of 2–4,096 (Table 1). The results of AEDA together with the identification experiments subsequently performed showed ethyl 2-methylbutanoate (no. 7, fruity) followed by methyl 2- and 3-methylbutanoates (no. 5, fruity, apple-like), HDF (no. 21, sweet, pineapple-like, caramel-like), δ-decalactone (no. 24, sweet, coconut-like), vanillin (no. 29, vanilla-like), ethyl 2-methylpropanoate (no. 3, fruity, sweet), δ-octalactone (no. 19, coconut-like), 1-(*E*,*Z*)-3,5-undecatriene (no. 11, fresh, pineapple-like), and an unknown compound (no. 14, fruity, pineapple-like) as the most odor-active compounds.

SHA

The volatiles isolated by SAFE have to be prepared by distillation and concentration steps. These operations might cause losses of highly volatile compounds, and consequently an underestimation of their flavor contributions when AEDA is applied. By combining the AEDA principle with static headspace samples, called SHA, this difference in the evaluation of the contributions of volatiles can be overcome.

By application of SHA on a pineapple homogenate, 11 odor-active compounds were detectable (Table 3). The identification experiments for the odorants were performed on the basis of odor quality, odor intensity, and retention indices on two capillary columns (RTX5 and OV-1701). All odorants detected by SHA had been found before during AEDA of the pineapple distillate (Table 1). The fruity-smelling esters ethyl 2-methylbutanoate and methyl 2- and 3-methylbutanoates were detectable in the very small volume of 0.08 ml. Ethyl 2-methylpropanoate and ethyl butanoate were detected when a headspace volume of 0.32 ml was used. These results indicate that these ester compounds contribute to the top note flavor of pineapple. 1-(*E*,*Z*)-3,5-Undecatriene, which was previously reported by Berger *et al.*[4] as an important pineapple odorant, was detected in a volume of 0.62 ml. This hydrocarbon exhibits a fresh, green, pineapple-like smell and a low threshold value and, therefore, is held responsible for the fresh top note of pineapple.

Quantitation by SIDA

Among pineapple volatiles, HDF is known to be relatively labile, and the esters (*e.g.*, methyl 2-methylpropanoate) are very volatile. To compensate for losses of these compounds during the experiment procedure, SIDA was used for quantitation.

According to the results of AEDA and SHA, 12 aroma compounds were quantified in pineapple by SIDA using the 12 labeled internal standards shown in Table 2. The results of the quantitation experiments are summarized in Table 4. HDF was present in a very high concentration, 26.8 mg/kg (ppm), in fresh pineapple. Using an internal standard on GC analysis, the concentrations of HDF in fresh pineapple juice were determined to be 7.4 mg/l and 0.7 mg/kg by Pickenhagen *et al.*[6] and Wu *et al.*[29] respectively. Takeoka *et al.*[11] have reported that HDF in pineapple volatile was not recovered by either steam distillation-extraction or dynamic headspace sampling. In a previous study, Sen *et al.* determined a concentration of HDF of 34.9 mg/kg using SIDA.[7] This value and our result indicate that SIDA combined with solvent extraction and the SAFE method is useful for the quantitation of labile and highly polar compounds like HDF.

The concentration of fruity-smelling ester methyl 2-methylbutanoate was 1.19 mg/kg. Methyl 2- and 3-methylbutanoates were not completely separable on the different stationary phases used (Tables 1 and 3). Using characteristic fragments in the MS/EI (m/z88, methyl 2-methylbutanoate; m/z74, methyl 3-methylbutanoate), an 86:1 ratio in each isomeric compound was estimated. Hence, the concentration only of methyl 2-methylbutanoate was determined by SIDA. Slightly lower concentrations were found for ethyl 2-methylbutanoate and methyl 2-methylpropanoate, with values in the range of 150 µg/kg (ppb).

Table 4. Odor Thresholds, Concentrations, and Odor Activity Values (OAVs) of Potent Odorants in Fresh Pineapple

Odorant	Odor quality	Threshold (μg/l in water)[a]	Concn. μg/kg	OAV[f]
Methyl 2-methylpropanoate	Fruity, sweet	6.3	154[d]	24.4
Ethyl 2-methylpropanoate	Fruity, sweet	0.02	48.0[e]	2400
Methyl 2-methylbutanoate	Fruity, apple-like	2	1190[e]	595
Ethyl butanoate	Fruity	1	75.2[e]	75.2
Ethyl 2-methylbutanoate	Fruity	0.15	157[e]	1050
Octanal	Citrus, fatty	8	19.1[e]	2.40
1-(*E*,*Z*)-3,5-Undecatriene	Fresh, pineapple-like	0.02[b]	8.89[d]	445
β-Damascenone	Fruity, sweet	0.00075	0.083[e]	111
δ-Octalactone	Coconut-like	400	78.2[e]	0.20
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	Sweet, pineapple-like	10[c]	26800[d]	2680
δ-Decalactone	Sweet, coconut-like	160	32.7[e]	0.20
Vanillin	Vanilla-like	25	5.99[d]	0.24

[a]Odor threshold values according to Ref. 30.
[b]Steinhaus, M., and Schieberle, P., unpublished report.
[c]Ref. 17.
[d]The data are mean values of duplicates.
[e]The data are mean values of triplicates.
[f]The odor activity values were calculated by dividing the concentrations of the odorants by their orthonasal odor thresholds.

The fresh and pineapple-like smelling 1-(*E*,*Z*)-3,5-undecatriene was present in a concentration of 8.89 μg/kg. On the other hand, β-damascenone was present in an extremely low concentration at the ppt level. In the quantitation of trace volatiles such as β-damascenone and 1-(*E*,*Z*)-3,5-undecatriene, SIDA is an effective method.

OAVs

To estimate precisely the sensory contribution of the 12 odorants to the flavor of fresh pineapple, their OAVs[12)] were calculated on the basis of nasal odor thresholds in water and concentrations quantified by SIDA (Table 4). This approach revealed sweet and pineapple-like smelling HDF, fruity smelling ethyl 2-methylpropanoate, and ethyl 2-methylbutanoate as the three most odor-active compounds, followed by methyl 2-methylbutanoate, 1-(*E*,*Z*)-3,5-undecatriene, and β-damascenone.

The odor unit values of several esters in fresh pineapple were calculated from their odor thresholds and concentrations determined using two internal standards on the headspace GC analysis by Takeoka *et al.*[11)] They estimated the odor unit value of HDF using an odor threshold value (0.03 ppb) and a concentration (7.4 ppm) published in the literature. On the basis of high odor unit values, they concluded that HDF (odor unit, 250,000), methyl 2-methylbutanoate (odor unit, 8,316), and ethyl 2-methylbutanoate (odor unit, 220) were the most important contributors to fresh pineapple aroma. Comparing their odor unit values with our own OAVs, the contributions of HDF and fruity-smelling esters to fresh pineapple aroma are evident.

1-(*E*,*Z*)-3,5-Undecatriene, which exhibits a low odor threshold value, had been suggested to be the main contributor to fresh-cut fruit flavor.[4)] The high OAV determined in the present study clearly corroborates this thesis and indicates that this compound was responsible for the fresh pineapple-like odor note.

On the other hand, in spite of high FD factors determined by AEDA, due to their high threshold values, δ-octalactone, δ-decalactone, and vanillin showed OAVs lower than 1. This result indicates that their contribution to the overall aroma of pineapple was lower.

Sensory evaluations

To corroborate the analytical data, sensory evaluations were performed. In a first step, a trained sensory panel determined the odor profile of fresh pineapple juice. They described the odor as sweet, citrus-like, fresh, fruity, green and grassy, woody, and pineapple-like (Fig. 1).

In a following step, the 12 odorants were dissolved in water in concentrations equal to those determined in pineapple (Table 4). The model mixture showed the typical fresh and fruity aroma associated with pineapple flavor. Then the odor profile of the model mixture was evaluated by the sensory panel in comparison to fresh pineapple juice. The results of the sensory evaluations as shown in Fig. 1 revealed a high similarity of the model

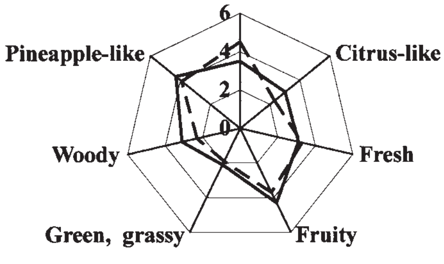

Fig. 1. Odor Profiles of Freshly Prepared Pineapple Juice (straight line) and the Pineapple Aroma Model Mixture (dotted line).

Table 5. The Model Mixture Effected by the Omission of One Compound

Odorant omitted from the model mixture	Number of panelists (out of 15) detecting an odor difference
Ethyl 2-methylpropanoate	8
Methyl 2-methylbutanoate	4
Ethyl 2-methylbutanoate	9*
1-(*E*,*Z*)-3,5-Undecatriene	7
β-Damascenone	5
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	11**

*, $p < 0.05$; **, $p < 0.01$.

mixture with fresh pineapple juice. There were no significant differences between the model and the fresh juice as to seven odor qualities. Fresh, fruity, and pineapple-like odor notes scored almost the same intensities in the model as in the juice. Only the sweet aroma note was more intense in the model than in the juice.

In further experiments, the contributions of six highly odor-active odorants (ethyl 2-methylpropanoate, methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 1-(*E*,*Z*)-3,5-undecatriene, *β*-damascenone, and HDF) were evaluated by means of omission tests. Each of the six odorants was singly omitted from the model mixture, and this omission mixture was orthonasally evaluated by 15 sensory panelists in comparison to the full model mixture by means of a triangle test. When HDF and ethyl 2-methylbutanoate were omitted, respectively, those incomplete model mixtures were clearly detectable by the panel (Table 5). The model without HDF lacked a sweet, pineapple-like aroma, whereas the model without ethyl 2-methylbutanoate lacked a fresh, fruity odor note. Hence, these results show that both compounds are character-impact odorants in fresh pineapple flavor.

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References

1) Flath, R. A., Pineapple. In "Tropical and Subtropical Fruits. Composition, Properties and Uses", eds. Nagy, S., and Shaw, P. E., AVI, Westport, pp. 157–183 (1980).
2) Berger, R., Pineapple. In "Volatile Compounds in Foods and Beverages", ed. Maarse, H., Marcel Dekker, New York, p. 296 (1991).
3) Nijssen, L. M., Visscher, C. A., Maarse, H., Willemsens, L., and Boelens, M. H., Volatile Compounds in Foods. Qualitative and Quantitative Data, 7th ed., TNO Nutrition and Food Research Institute, Zeist, The Netherlands, pp. 14.1–14.8 (1996).
4) Berger, R. G., Drawert, F., Kollmannsberger, H., Nitz, S., and Schraufstetter, B., Novel volatiles in pineapple fruit and their sensory properties. *J. Agric. Food Chem.*, **33**, 232–235 (1985).
5) Rodin, J. O., Himel, C. M., Silverstein, R. M., Leeper, R. W., and Gortner, W. A., Volatile flavor and aroma components of pineapple. I. Isolation and tentative identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone. *J. Food Sci.*, **30**, 280–285 (1965).
6) Pickenhagen, W., Velluz, A., Passerat, J.-P., and Ohloff, G., Estimation of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (FURANEOL) in cultivated and wild strawberries, pineapples and mangoes. *J. Sci. Food Agric.*, **32**, 1132–1134 (1981).
7) Sen, A., Schieberle, P., and Grosch, W., Quantitative determination of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone and its methyl ether using a stable isotope dilution assay. *Lebensm.-Wiss. Technol.*, **24**, 364–369 (1991).
8) Takeoka, G. R., Buttery, R. G., Teranishi, R., Flath, R. A., and Guentert, M., Identification of additional pineapple volatiles. *J. Agric. Food Chem.*, **39**, 1848–1851 (1991).
9) Umano, K., Hagi, Y., Nakahara, K., Shoji, A., and Shibamoto, T., Volatile constituents of green and ripened pineapple (*Ananas comosus* [L.] Merr.). *J. Agric. Food Chem.*, **40**, 599–603 (1992).
10) Teai, T., Claude-Lafontaine, A., Schippa, C., and Cozzolino, F., Volatile compounds in fresh pulp of pineapple (*Ananas comosus* [L.] Merr.) from French Polynesia. *J. Essent. Oil Res.*, **13**, 314–318 (2001).
11) Takeoka, G., Buttery, R. G., Flath, R. A., Teranishi, R., Wheeler, E. L., Wieczorek, R. L., and Guentert, M., Volatile constituents of pineapple (*Ananas comosus* [L.] Merr.). *ACS Symp. Ser.*, **388**, 223–237 (1989).
12) Schieberle, P., New developments in methods for analysis of volatile flavor compounds and their precursors. In "Characterization of Food: Emerging Methods", ed. Gaonkar, A. G., Elsevier Science B. V., Amsterdam, pp. 403–431 (1995).
13) Ullrich, F., and Grosch, W., Identification of the most intense odour compounds formed during autoxidation of methyl linolenate at room temperature. *J. Am. Oil Chem. Soc.*, **65**, 1313–1317 (1988).
14) Steinhaus, M., and Schieberle, P., Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety Spalter Select) based on GC-olfactometry and odor dilution techniques. *J. Agric. Food Chem.*, **48**, 1776–1783 (2000).
15) Guth, H., and Grosch, W., Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc.*, **70**, 513–518 (1993).
16) Fuhrmann, E., Studies on the flavor of apples of different varieties and correlations between the structure and odor of "apples" esters (in German). Ph. D. Thesis, Technical University of Munich (1998).
17) Schieberle, P., and Hofmann, T., Evaluation of the character impact odorants in fresh strawberry juice by quantitative measurements and sensory studies on model mixtures. *J. Agric. Food Chem.*, **45**, 227–232 (1997).
18) Blekas, G., and Guth, H., Evaluation and quantification of potent odorants of Greek virgin olive oils. *Dev. Food Sci.*, **37A**, 419–427 (1995).
19) Sen, A., Laskawy, G., Schieberle, P., and Grosch, W., Quantitative determination of *β*-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.*, **39**, 757–759 (1991).

20) Pfnuer, P., Investigations on the aroma of chocolate (in German). Ph. D. Thesis, Technical University of Munich (1998).
21) Schieberle, P., Gassenmeier, K., Guth, H., Sen, A., and Grosch, W., Character impact odour compounds of different kinds of butter. *Lebensm.-Wiss. Technol.*, **26**, 347–356 (1993).
22) Guth, H., Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.*, **45**, 3027–3032 (1997).
23) Steinhaus, M., Important aroma substances in hops (*Humulus lupulus* L.) (in German). Ph. D. Thesis, Technical University of Munich (2001).
24) Engel, W., Bahr, W., and Schieberle, P., Solvent assisted flavour evaporation—a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.*, **209**, 237–241 (1999).
25) Halang, W. A., Langlais, R., and Kugler, E., Cubic spline interpolation for the calculation of retention indices in temperature-programmed gas-liquid chromatography. *Anal. Chem.*, **50**, 1809–1832 (1978).
26) Flath, R. A., and Forrey, R. R., Volatile components of Smooth Cayenne pineapple. *J. Agric. Food Chem.*, **18**, 306–309 (1970).
27) Ohta, H., Kinjo, S., and Osajima, Y., Glass capillary gas chromatographic analysis of volatile components of canned Philippine pineapple juice. *J. Chromatogr.*, **409**, 409–412 (1987).
28) Creveling, R. K., Silverstein, R. M., and Jennings, W. G., Volatile components of pineapple. *J. Food Sci.*, **33**, 284–287 (1968).
29) Wu, P., Kuo, M.-C., Hartman, T. G., Rosen, R. T., and Ho, C.-T., Free and glycosidically bound aroma compounds in pineapple (*Ananas comosus* L. Merr.). *J. Agric. Food Chem.*, **39**, 170–172 (1991).
30) Rychlik, M., Schieberle, P., and Grosch, W., Compilation of Odor Thresholds, Odor Qualities and Retention Indices of Key Food Odorants. Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany (1998).