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Volatile Constituents of the Green Forage and Hay of Bahiagrass

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Abstract In order to elucidate the qualitative disposition and the quantitative distribution of the aromatic constituents in roughage, the volatile constituents of the green forage and hay of bahiagrass were analyzed by a combined gas chromatograph-mass spectrometer (GC-MS) and a FID-gas chromatograph (FID-GC). The area percentage of each compound in the volatile constituents and the parts per billion (ppb) concentration of each compound in the materials were calculated on a data processor for FID-GC using *n*-pentadecane as the internal standard. As a result, 25 compounds were identified from the volatile constituents of the green forage of bahiagrass; their area percentages and ppm values totaled 78.2 and 22.0, respectively. The volatile constituents of this green forage were quantitatively rich in alcohols, esters, and ketones. On the other hand, 26 compounds were identified from the volatile constituents of the hay of bahiagrass; their area percentages and ppm values totaled 79.4 and 12.0, respectively. The volatile constituents of this hay were also rich in alcohols, esters, and ketones. Both volatile constituents were qualitatively about the same, but were somewhat different quantitatively.

Key words: bahiagrass, GC-MS, green forage, hay, volatile constituents

INTRODUCTION

As a series of our studies (KAMI *et al.*, 1986, 1987, 1988, 1989, 1990) pertaining to the volatile constituents of roughage, in the present work, we have studied the volatile constituents of the green forage and hay of bahiagrass. Bahiagrass is a perennial forage grass that propagates with stolon and seed, and forms a dense pasture. In the warm southwestern district of Japan, bahiagrass is widely cultivated as the major summer crop and is mainly used for grazing of domestic animals because of its strong persistency and regrowth. Therefore, extensive reports exist on yield, chemical composition, morphology, and ecology; however there is little information about the volatile constituents of bahiagrass.

MATERIALS AND METHODS

Materials

Pensacola bahiagrass (*Paspalum notatum* Flügge) was cultivated at the Hiroshima University Experimental Farm ever since sown in May 1982 and was harvested on July 31, 1989 using a mower. The harvest time corresponded to the early-heading stage. The fresh terrestrial parts was chopped with a cutter and 1 kg was used for the experiment (hereafter, the above material will be referred to 'bahia soilage'). A portion of fresh-mown bahiagrass was dried on the ground in the sunlight for 1 week. This hay was chopped with a cutter and 0.5 kg was used for the experiment (the above material will be referred to 'bahia hay'). The moisture percentage and pH value of this hay were measured by the usual methods.

Solvent extraction

One kg of the bahia soilage and 0.5 kg of the bahia hay were immediately immersed in the mixed solvents of distilled *n*-pentane and ethyl ether (1:1), allowed to stand for 2 week, and filtered, as described previously (KAMI *et al.*, 1987). Each residual was again immersed in the mixed solvents for 1 week and subsequently filtered. The combined solvent extract of the bahia soilage yielded a green brown liquid that possessed a strong grassy-smelling odor. On the other hand, the combined solvent extract of the bahia hay yielded a green yellow liquid that possessed a weak grassy-smelling odor. Each solvent extract was then concentrated through a 30 cm long Vigreux-distilling column.

Reduced pressure distillation

Each concentrated solvent extract was distilled several times, by adding distilled water, with a semi-micro distilling apparatus of glass under reduced pressure using handy aspirator. Each distillate was collected into a series of two traps cooled with ice and dry ice-methanol, and was again extracted with the mixed solvents. Then, each solvent solution was concentrated through a 20 cm long Vigreux-distilling column, yielding a colorless liquid that possessed its own characteristic odor.

Analysis of the volatile constituents

Each reduced pressure distillate was first analyzed by GC-MS, then by FID-GC. On the GC-MS, the apparatus and the operating conditions were the same as those described in the previous paper (KAMI *et al.*, 1989). The peaks of GC-MS were assigned through a comparison of the mass spectra with those of authentic specimens and/or with authentic spectra (STENHAGEN *et al.*, 1969). The apparatus and the operating conditions of FID-GC were also the same as those reported previously (KAMI *et al.*, 1989). The FID-GC peaks were assigned by means of the coincidence of retention times with those of authentic samples. The relative peak area of each compound in the volatile constituents and ppb value of each compound in the bahia soilage and bahia hay were determined as previously reported using *n*-pentadecane as the internal standard (KAMI *et al.*, 1987).

RESULTS AND DISCUSSION

The volatile constituents of bahia soilage

Bahia soilage (1.0 kg) was directly extracted with mixed solvents, distilled with water under reduced pressure, and extracted once again with the mixed solvents. The solvent extract was first analyzed by GC-MS using a DB-Wax capillary column, and then by FID-GC using a Thermon 3000 packed column. From the agreement of the results of these two analyses, the volatile constituents of this soilage were identified. The area percentages and the ppb concentrations of identified compounds were calculated on a data processor for FID-

GC using a known weight of *n*-pentadecane as the internal standard. The results are shown in Table 1.

Of all 62 peaks, 25 compounds consisting of 5 acids, 10 alcohols, 2 aldehydes, 2 esters, 2 ketones, 3 ketols, and 1 lactone were identified. In addition to these compounds, ethyl benzoate, 3-methyl-2-butenoic acid, and benzoic acid were tentatively identified by GC-MS alone. These compounds could not be assigned by FID-GC because of the small size of their peaks. The area percentages and ppb values by functional group of acids, alcohols, aldehydes, esters, ketones, ketols, and lactone were 7.92 and 2228, 32.67 and 9194, 1.69

Table 1. Volatile constituents of bahiagrass soilage

Compound	Peak No.[a]	Retention time, min[b]	Area %[c]	ppb[c]
Acids				
Acetic acid	32	20.7	1.96	552
Isobutyric acid	36	25.5	2.17	610
Isovaleric acid	39	29.2	0.75	210
Isocaproic acid	42	32.7	2.93	826
n-Caproic acid	43	34.0	0.11	30
Alcohols				
Ethanol	7	4.3	2.69	758
2-Butanol	12	6.8	0.05	13
2-Methyl-3-buten-2-ol	13	7.3	11.02	3100
3-Methyl-2-butanol	17	9.1	0.19	54
1-Penten-3-ol	22	11.6	0.15	42
3-Penten-2-ol	23	12.0	9.46	2662
3-Methyl-2-buten-1-ol	29	17.5	5.09	1432
cis-3-Hexen-1-ol	31	19.9	1.15	325
Benzyl alcohol	44	34.6	2.31	650
2-Phenylethyl alcohol	45	36.0	0.56	158
Aldehydes				
1-Hexanal	16	8.9	0.30	84
Phenylacetaldehyde	38	28.2	1.39	390
Esters				
Methyl acetate	3	2.5	11.91	3350
Ethyl acetate	5	3.4	5.44	1531
Ketones				
3-Buten-2-one	8	4.6	6.79	1910
3-Methyl-3-buten-2-one	10	5.7	10.40	2925
Ketols				
3-Hydroxy-3-methyl-2-butanone	27	14.8	0.63	176
1-Hydroxy-2-propanone	28	17.0	0.18	50
4-Hydroxy-3-methyl-2-butanone	37	27.1	0.20	57
Lactones				
γ-Hexalactone	40	29.8	0.33	93

[a] Peak numbers were given in the order of appearance in FID-GC of Thermon 3,000 column.

[b] FID-GC was programmed from 50 to 250 ℃ at 4 ℃/min.

[c] Area percentages and ppb values were calculated on the data processor for FID-GC.

and 474, 17.35 and 4881, 17.19 and 4835, 1.01 and 283, and 0.33 and 93, respectively. The total values were 78.2% in the volatile constituents and 22.0 ppm in the bahia soilage.

The volatile constituents of this bahia soilage contained qualitatively many alcohols and were quantitatively rich in alcohols, esters, and ketones. Of the compounds, methyl acetate, 2-methyl-3-buten-2-ol, and 3-methyl-3-buten-2-one were the major constituents. These three compounds accounted for one-third of total contents. Many compounds consisting of C_4, C_5, and C_6 alcohols, aliphatic carbonys, lower acid esters, and ketols were identified in front of the acetic acid peak (No.32). The total values of these compounds were 65.5% in the volatile constituents and 18.4 ppm in the bahia soilage. On the other hand, the total values of the compounds identified back from the acetic acid peak were 12.7% and 3.6 ppm, respectively. All the identified compounds are considered to contribute to the overall odor of this bahia soilage.

In the identified compounds, *cis*-3-hexen-1-ol seems to give the characteristic green note, but the amount was less than that in soiling dent corn (KAMI and OHSAKI, 1986) and soiling Italian ryegrass (KAMI and OHSAKI, 1988). It may be noteworthy that the small amounts of 3 ketols were detected from this bahia soilage. Among them, 1-hydroxy-2-propanone (acetol) is found in the heated licorice essential oil (FRATTINI *et al.*, 1977) and the volatile compounds in cooked beef (SHIBAMOTO *et al.*, 1981), and also 3-hydroxy-3-methyl-2-butanone in the aroma composition of Swiss Gruyère cheese (BOSSET and LIARDON, 1984). The presence of 4-hydroxy-3-methyl-2-butanone is probably not reported so far. Therefore, it seems that the existence of these ketols is a characteristic found only in bahiagrass. Further investigation of the unknown compounds is necessary.

The volatile constituents of bahia hay

Bahia hay (0.5 kg) was directly extracted with mixed solvents, distilled with water under reduced pressure, and again extracted with the mixed solvents. The bahia hay used in this experiment contained 10.10% moisture and pH was 5.8. The solvent extract was analyzed by GC-MS and FID-GC, and the volatile constituents were identified. The area percentages and ppb concentrations were calculated using *n*-pentadecane as the internal standard. The results are shown in Table 2.

Of all 60 peaks, 26 compounds consisting of 7 acids, 10 alcohols, 2 aldehydes, 2 esters, 2 ketones, 2 ketols, and 1 lactone were identified. In addition, γ-nonalactone and *n*-caprylic acid were tentatively identified by GC-MS alone. The area percentages and ppb values by functional group of acids, alcohols, aldehydes, esters, ketones, ketols, and lactone were 8.62 and 1305, 37.06 and 5622, 3.86 and 586, 15.07 and 2285, 13.70 and 2079, 0.60 and 90, and 0.44 and 67, respectively. The total values were 79.4% in the volatile constituents and 12.0 ppm in the bahia hay.

The volatile constituents of this bahia hay contained qualitatively many alcohols and acids, and were quantitatively rich in alcohols, esters, and ketones. Of the compounds, 3-pentan-2-ol, 3-methyl-3-buten-2-one, and 2-methyl-3-buten-2-ol were major constituents. These three compounds accounted for about one-fourth of total contents. The total values of the compounds identified in front of the acetic acid peak (No. 24) were 55.5% in the volatile constituents and 8.4 ppm in the bahia hay. On the other hand, those of the compounds identified back from the acetic acid peak were 23.9% and 3.6 ppm, respectively.

From the above results, it became clear that the volatile constituents of bahia hay were qualitatively about the same as those of bahia soilage. However, some quantitative differences were found between the two. Namely, the total ppm concentrations of the volatile constituents in bahia hay and in bahia soilage were 12.0 and 22.0, respectively. Each item was also as follows; ppm values of the compounds detected in front of the acetic acid peak were 8.4 in bahia hay and 18.4 in bahia soilage, and on the other hand, those of the compounds detected back from the acetic acid peak were the same 3.6. The decrease of the volatile constituents of bahia hay was principally the decrease of the compounds detected in

Table 2. Volatile constituents of bahiagrass hay

Compound	Peak No.[a]	Retention time, min[b]	Area %[c]	ppb[c]
Acids				
Acetic acid	24	20.7	0.30	45
Isobutyric acid	28	25.5	1.03	156
Isovaleric acid	32	29.1	4.64	703
n-Valeric acid	34	30.8	0.30	45
Isocaproic acid	36	32.8	0.11	17
n-Caproic acid	38	34.1	1.85	280
n-Capric acid	47	44.7	0.39	59
Alcohols				
Ethanol	6	4.3	1.78	270
2-Butanol	9	6.5	0.04	7
2-Methyl-3-buten-2-ol	10	7.2	8.31	1260
3-Methyl-2-butanol	13	9.1	1.18	179
1-Penten-3-ol	15	11.6	0.25	38
3-Penten-2-ol	16	11.9	9.76	1480
3-Methyl-2-buten-1-ol	22	17.5	2.17	330
cis-3-Hexen-1-ol	23	19.8	2.92	443
Benzyl alcohol	39	34.7	3.66	555
2-Phenylethyl alcohol	40	35.7	6.99	1060
Aldehydes				
1-Hexanal	12	8.9	0.07	11
Phenylacetaldehyde	31	28.2	3.79	575
Esters				
Methyl acetate	2	2.5	7.31	1108
Ethyl acetate	4	3.3	7.76	1177
Ketones				
3-Buten-2-one	7	4.5	4.64	704
3-Methyl-3-buten-2-one	8	5.6	9.06	1375
Ketols				
3-Hydroxy-3-methyl-2-butanone	20	15.0	0.24	36
4-Hydroxy-3-methyl-2-butanone	30	27.1	0.36	54
Lactones				
γ-Hexalactone	33	30.0	0.44	67

[a], [b] and [c] see Table 1.

front of the acetic acid peak. Therefore, it may be inferred that the low-boiling components of bahia hay vaporized with moisture during natural drying of its soilage. As the result, an odor of the concentrated solvent extract of bahia hay was weakly felt as compared with that of bahia soilage. Further investigation is necessary to verify this inference.

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バヒアグラスの生草および乾草の揮発性成分

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粗飼料中の香気成分の種類とその量的分布を知るために，バヒアグラスの生草と乾草の揮発性成分を，ガスクロマトグラフ・マスペクトロメーター (GC-MS) および水素炎イオン化検出器のガスクロマトグラフ (FID-GC) によって分析した。揮発性成分中の各化合物の面積%および飼料中の各化合物の ppb 濃度は，正ペンタデカンを内部標準に用いてクロマトグラフ用データ処理装置によって算出した。それによって，バヒアグラス生草の揮発性成分から25化合物を同定し，それらの面積%および ppm 値は，それぞれ総計78.2および22.0であった。この生草の揮発性成分は，量的にアルコール類，エステル類，ケトン類が多かった。一方，バヒアグラス乾草の揮発性成分から26化合物を同定し，それらの面積%および ppm 値は，それぞれ総計79.4および12.0であった。この乾草の揮発性成分もまた，アルコール類，エステル類，ケトン類が多かった。両者の揮発性成分は，質的にほとんど同じであったが量的に若干の差があった。

キーワード：乾草，ガスクロマトグラフ質量分析計，揮発性成分，生草，バヒアグラス