



Figure 6. Effect of sludge-amended soil on phenolic content (fresh weight basis) of potatoes.

there was a wide range in phenolic content of sludge-grown potatoes. A high positive correlation between darkening and the phenolic content of tubers has been reported (Mondy et al., 1967). The wide variation of enzymatic discoloration and phenolic content may have been due to the wide variation in trace minerals in the soil and/or to the mineral interactions. Sludge-amended soil had high concentrations of phytotoxic minerals, which may have caused stress to the plants.

CONCLUSION

In both years of the study the cortex tissue of potatoes grown on sludge-amended soil was significantly ($p < 0.05$) higher in total and protein nitrogen than the controls. However, the opposite trend was observed for pith tissue. No significant differences in ascorbic acid content were observed in the first year of the study, but in the second year both cortex and pith from potatoes grown in sludge-amended soil were significantly ($p < 0.05$) lower in ascorbic acid content than the controls. Phenolic content and darkening did not show a significant trend with sludge-amended soil.

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Registry No. N_2 , 7727-37-9; ascorbic acid, 50-81-7.

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Novel Volatiles in Pineapple Fruit and Their Sensory Properties

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Nineteen additional, mainly in trace amounts, occurring volatiles of pineapple fruit—including four nonterpenoid hydrocarbons and a number of carboxylic esters—have been isolated under enzyme inhibition, enriched by liquid-liquid extraction, fractionated on silica gel, and identified by gas chromatography and coupled GC-MS. Among them, 1-(*E,Z*)-3,5-undecatriene and 1-(*E,Z,Z*)-3,5,8-undecatetraene may contribute to the typical flavor of pineapple. They combine a fragrant odor with extremely low odor detection thresholds. The corresponding *E,E* and *E,E,Z* isomers are much less odorous (factors of 10^6 and 10^4 , respectively). Disintegration of the fruit tissue without enzyme inhibition causes a rapid decrease of all undecaenes.

The distinct and pleasant flavor of pineapple fruit has been the subject of a number of studies, resulting in the identification of more than 100 components (van Straten,

1977). More recently, 4-methoxy-2,5-dimethyl-2(*H*)-furan-3-one (Pickenhagen et al., 1981), 2-propenyl hexanoate (Nitz and Drawert, 1982), and a number of sesquiterpene hydrocarbons (Berger et al., 1983) have been found to be genuine components of pineapple flavor.

Nevertheless, the manufacture of commercial pineapple formulations is difficult (Broderick, 1975), and satisfying results are only obtained by addition of various (nonpi-

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Table I. Occurrence and Sensory Properties of Some Pineapple Fruit Volatiles

compound	M_r	av concn, $\mu\text{g}/\text{kg}$	odor detection threshold, ng	odor description sniffing test
1-(<i>E,Z</i>)-3,5-undecatriene ^a (I)	150	1	0.001–0.002	balsamic, spicy, pinewood
1-(<i>E,E</i>)-3,5-undecatriene ^a (II)	150	<0.5	750–1000	musty
1-(<i>E,Z,Z</i>)-3,5,8-undecatetraene ^a (III)	148	1	0.002–0.004	resembling I, more fruity
1-(<i>E,E,Z</i>)-3,5,8-undecatetraene ^a (IV)	148	<0.5	20–30	sweet, fruity
2-propenyl <i>n</i> -hexanoate ethyl	156	<0.5	50–100	fruity, estery, near its detection threshold resembling I
<i>n</i> -hexanoate	144	500	1–2	fruity
(<i>E</i>)-2-hexenoate ^a	142	5	14–20	fruity, slightly pungent
(<i>E</i>)-3-hexenoate	142	15	25–50	pungent, pineapple peel like
(<i>Z</i>)-3-hexenoate	142	<i>b</i>	1–2	fruity, pineapple-like
3-(methylthio)propionate	148	100	1–2	fruity, pineapple-like
2-(methylthio)acetate	134	<0.5	200–300	pungent

^a Identified for the first time in pineapple. ^b Not found in pineapple.

neapple) ingredients or essential oils (Emberger, 1981). It has been the aim of this study to identify new trace compounds with impact character (Jennings, 1969) by correlating the analytical data with sensory judgments by means of a modified capillary gas chromatographic sniffing technique (Drawert and Christoph, 1984; Nitz et al., 1984).

EXPERIMENTAL SECTION

Materials. Pineapple fruits (*Ananas comosus* Merr.) from the Ivory Coast were obtained from a local supplier.

Aroma Isolation. Whole ripe pineapples were peeled, peel or flesh cut into methanol (Burdick & Jackson, Inc.), and mixed in a Waring Blendor (final methanol concentration 66%; Drawert et al., 1973). The methanol-juice mixture was immediately separated in a Sorvall-type centrifuge (0 °C, 1500g, 10 min) and extracted with pentane-methylene chloride (2:1). The solvent was removed by distillation through a Vigreux column (20 cm, 40 °C; Drawert et al., 1969), and the total concentrate was subjected to descending column chromatography on silica gel 60 (Merck), activity grade II, using a pentane-diethyl ether gradient: 100% pentane yields fraction I, pentane-diethyl ether (9:1 v/v) yields fraction II, and 100% diethyl ether yields fraction III (Schreier and Drawert, 1976). Eluates were concentrated to 0.5 mL for gas chromatographic analysis. Part of the plant material was homogenized in methanol, extracted with pure pentane, concentrated (0.5 mL), and then submitted to further analyses to exclude silica gel catalyzed formation of artifacts in fraction I. All solvents (analytical grade) were redistilled before use.

GLC Conditions. A Carlo Erba Fractovap 2300/AC gas chromatograph with a FID, equipped with an OV-101 glass capillary column (50 m × 0.32 mm i.d., Jaeggi), was used. The carrier gas was H₂ at 1 mL/min, injector and detector temperatures were 225 °C, and the temperature was programmed from 40 to 210 ° at 2 °C/min. The sniffing gas chromatograph was equipped with a wide-bore CW 20 M SCOT glass capillary column (40 m × 0.5 mm i.d., SGE) and is completely described elsewhere (Nitz et al., 1984). Odor detection thresholds were determined by a panel of six experienced persons upon injection of solutions containing various known amounts of reference substances. The values given were found with a gas stream of 100 mL/s and can be empirically compared with olfactometric values (threshold in ng/L of air) by multiplication with a factor of 10.

Combined GLC–Mass Spectrometry. A mass spectrometer, Finnigan 4021 (quadrupole), was directly coupled with a Finnigan 9610 gas chromatograph, equipped with a CW 20 M glass capillary column (25 m × 0.25 mm i.d., Mega). The carrier gas was He at 40 cm/s, injector and transfer line temperatures were 220 °C, and the column

temperature was programmed from 65 (5-min isothermal) to 170 °C at 1.5 °C/min.

A second mass spectrometer, Finnigan 1020 (quadrupole), was directly coupled with a Perkin-Elmer gas chromatograph, equipped with a SE-54 fused silica capillary column (30 m × 0.32 mm i.d., J & W). The carrier gas was He at 2 mL/min, injector and transfer line temperatures were 230 °C, and the temperature was programmed from 50 to 210 °C at 2 °C/min. Both ionization chambers operated at 70 eV. Recording and searching of mass spectra were done by an integrated Nova 4/CDC disk drive system (32 Mbyte, NBS Library).

Qualitative analyses were carried out by means of GLC retention, sniffing, and mass spectral data of authentic substances. Quantitative GLC determinations were performed by internal standard controlled calculations using a Spectra Physics SP4000/4020 laboratory data system without consideration of extraction yields and FID response factors.

RESULTS AND DISCUSSION

The following, genuine volatiles of pineapple fruit have been identified for the first time: four undecatri- and -tetraenes (fraction I; see Table I), methyl and ethyl esters of (*E*)-2-hexenoic, *n*-dodecanoic, *n*-hexadecanoic, and *n*-octadecanoic acid, methyl esters of (*Z*)-9-octadecenoic, (*Z,Z*)-9,12-octadecadienoic, and (*Z,Z,Z*)-octadecatrienoic acid, ethyl 2-(methylthio)acetate, diethyl malonate (fraction II), 1-nonanal, and 1-dodecanol (Fraction 3). Furthermore, mass spectral data suggest the occurrence of ethyl 5-hydroxyoctanoate, a methyl octadienoate, and a yet unknown ethyl octenoate. The concentrations, which vary strongly with the stage of ripeness and purchase data, are in the range of 1 mg/kg for the C₁₆ and C₁₈ fatty acid esters and below 50 μg/kg for all other compounds.

Application of the gas chromatographic sniffing technique has shown that, besides the malonate and the 5-(?)-hydroxyoctanoate [sweet, pineapple-like (Honkanen et al., 1980)], 1-(*E,Z*)-3,5-undecatriene (I) and 1-(*E,Z,Z*)-3,5,8-undecatetraene (III) are of special sensory importance for the pineapple flavor.

The undecatriene isomers I and II have been isolated from the essential oil of members of the family of Umbelliferae [*Ferula galbaniflua*; *Ferula rubicaulis* (Chrétien-Bessière et al., 1967; Teisseire et al., 1967; Naves, 1967)]. Both also occur, together with the 1-(*E,Z,Z*)-3,5,8-(III) and 1-(*E,E,Z*)-3,5,8-undecatetraenes (IV), in Phaeophyceae of the order Dictyotales [*Dictyopteris plagiogramma*; *Dictyopteris australis* (Pettus and Moore, 1970, 1971; Moore et al., 1972, 1974)].

Up to now, only 1-(*E,Z,Z*)-3,5,8-undecatetraene (III), which has also been detected in *Spermatochneus paradoxus* (Müller et al., 1981) and in *Ascophyllum nodosum*, where

it acts as a sperm attractant ["finavarrene" (Müller et al., 1982)], has been found in fruits of higher plants [*Mangifera indica* (Idstein and Schreier, 1983)].

According to Jaenicke et al. (1974) and Moore (1976), the biosynthesis starts from linoleic or linolenic acid by concerted lipoxygenase action, β -oxidation, and heterolytic fragmentation and runs via 1-(*Z*)-5-undecadien-3-ol or 1-(*Z,Z*)-5,8-undecatrien-3-ol to 1-(*E,Z*)-3,5- or 1-(*E,Z,Z*)-3,5,8-undecaene. The corresponding *E,E* or *E,E,Z* isomers result from photosensitized isomerization in the algal cells (Moore, 1976). These pathways may also function in cells of higher plants.

During recent work on chemosynthesis of undecaenes, a number of authors observed a peculiar odor of some of the isomer forms (Näf et al., 1975; Yamada et al., 1980; Boland et al., 1981; Marner et al., 1982; Näf et al., 1982; Schneider and Bruch, 1982; Giraudi and Teisseire, 1983; Hayashi et al., 1983). Evaluation of subsequent sniffing runs of solutions containing descending substance concentrations proved the isomers I and III to possess unique odor properties and the lowest odor detection thresholds in air reported for hydrocarbons (van Gemert and Nettenbreijer, 1977; Table I). Despite their low concentrations, they determine the odor of the nonpolar fraction and may therefore contribute to the overall impression of pineapple flavor. The corresponding *E* isomers II and IV are both much less odorous (factors of 10^6 and 10^4 , respectively).

The odor detection thresholds of some pineapple volatiles and an additional ethyl hexenoate have been comparatively investigated (Table I). Forming—analogue to the calculation of odor units (Guadagni et al., 1966)—the quotients of average substance concentrations and odor detection thresholds, one obtains higher values for the isomer I than for ethyl hexanoate, although the latter exhibits good sensory activity and belongs to the main volatiles in pineapple.

The well-known contribution of ethyl 3-(methylthio)propionate to pineapple flavor is confirmed by our data. A comparison with the sensory properties of ethyl (*Z*)-3-hexenoate, which has not been detected in pineapple, shows, in agreement with observations of Boelens and Heydel (1973), that neither the odor character nor the odor detection threshold is markedly affected by replacement of the C=C double bond with a sulfur atom.

Both the triene isomer I and the tetraene isomer III occur in similar amounts in peel and flesh of pineapple fruit. Their concentrations decrease in mechanically prepared, non-enzyme-inhibited homogenates below the sensory detection thresholds within several hours. As a consequence, pronounced losses have to be expected during conventional processing of pineapple fruits. It is worth mentioning in this context that 2-propenyl hexanoate, a compound widely used to improve commercial pineapple formulations (Merory, 1968; Broderick, 1975), smells at concentrations near its odor detection threshold like the triene I, and both compounds were sometimes confused with each other in sniffing runs. In fact, a contribution of the ester to natural pineapple flavor can be excluded due to its low actual concentration and its rather high odor detection threshold (Table I). Further, considering the known toxicity of allylic esters, a quality improvement of pineapple flavor extracts and other processed products from pineapple should be achieved by preventing the enzymic and/or oxidative degradation of 1-(*E,Z*)-3,5-undecatriene and 1-(*E,Z,Z*)-3,5,8-undecatetraene.

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Registry No. I, 19883-27-3; II, 19883-29-5; III, 29837-19-2; IV, 50277-31-1; methyl (*E*)-2-hexenoate, 13894-63-8; methyl *n*-dodecanoate, 111-82-0; methyl *n*-hexadecanoate, 112-39-0; methyl *n*-octadecanoate, 112-61-8; ethyl (*E*)-2-hexenoate, 27829-72-7; ethyl *n*-dodecanoate, 106-33-2; ethyl *n*-hexadecanoate, 628-97-7; ethyl *n*-octadecanoate, 111-61-5; methyl (*Z*)-9-octadecenoate, 112-62-9; methyl (*Z,Z*)-9,12-octadecadienoate, 112-63-0; methyl (*Z,Z,Z*)-9,12,15-octadecatrienoate, 301-00-8; ethyl 2-(methylthio)acetate, 4455-13-4; diethyl malonate, 105-53-3; 1-nonanal, 124-19-6; 1-dodecanol, 112-53-8; ethyl 5-hydroxyoctanoate, 75587-05-2; ethyl 3-(methylthio)propionate, 13327-56-5.

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Carotene-Xanthophyll in Field-Wilted and Dehydrated Alfalfa and Coastal Bermuda Grass

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Dehydrated coastal Bermuda grass (CBG) [*Cynodon dactylon* (L.) Pers.] and alfalfa (ALF) (*Medicago sativa* L.) are important sources of carotene and xanthophylls for pigmenting broilers and eggs. Recently, commercial dehydrators have resorted to preliminary field wilting to partially dry these crops. Although this practice saves fuel, some carotene and xanthophyll are lost. This study was conducted to compare changes in the carotene-xanthophyll content of fresh cut vs. field-wilted and then mechanically dehydrated CBG and ALF and also to determine if major changes occur in the relative amounts of monohydroxy-, dihydroxy-, and polyoxyxanthophylls due to processing. Field wilting for 6 h resulted in losses of up to 25% carotene and 20% xanthophyll. Field wilting overnight did not decrease total carotene or xanthophyll in CBG; however, ALF lost about 25% carotene and 14% xanthophyll. Although carotene was relatively stable during dehydration, up to 45% xanthophyll was lost. No appreciable changes were found in the relative amounts of the monohydroxy-, dihydroxy-, and polyoxyxanthophylls in the fresh or dehydrated forages.

Dehydrated Coastal Bermuda grass (CBG) [*Cynodon dactylon* (L.) Pers.] and alfalfa (ALF) (*Medicago sativa* L.) are used in poultry feeds as sources of carotenoids for pigmenting egg yolks and broilers. In the past, both forages were dehydrated immediately following harvest. However, because of the current high cost of fossil fuel, forages processors now resort to field wilting to partially dry the crop. The cut forage is usually field dried for about 6 h during the day; however, when cut in the late afternoon or evening, it may remain in the field overnight and be dehydrated the next morning. Although partial field wilting appreciably reduces the fuel required for mechanical dehydration (Butler et al., 1969), some carotene and xanthophyll are lost. When exposed to heat, light, and air, these labile compounds undergo oxidation and isomerization (Kohler et al., 1967; Livingston et al., 1970; Marusich and Bauernfeind, 1981). As a result, forages that have been field wilted and mechanically dehydrated may vary widely in the relative proportions of monohydroxy-, dihydroxy-, and polyoxyxanthophylls, which differ in pigmentation potency (Marusich and Bauernfeind, 1981; Kuzmicky et al., 1969). Middendorff et al. (1980) reported that the nonepoxide (dihydroxy) xanthophyll content in sun-cured and dehydrated ALF meals varied considerably as did the levels of these substances in the blood serum of poultry.

In fresh forage, there is a positive relationship between carotene and xanthophyll content (Livingston et al., 1968a,b). Because of this, feed ingredient buyers assume that if carotene is high, xanthophyll also will be high. However, this may not be true for dehydrated forage products as field wilting and dehydration may result in differential losses in carotene and xanthophyll. Livingston et al. (1977) reported large losses in carotene and xanthophyll when ALF was field wilted for 4 h and mechanically dehydrated. Compared to ALF, few data have been published on factors that influence carotene-xanthophyll content in sun-cured and dehydrated CBG (Middendorff et al., 1980). In certain studies where CBG and ALF were compared, either the forages differed widely in quality or information was not available on origin, processing conditions, etc. (Dua and Day, 1964; Barnett and Morgan, 1959; Middendorff, 1980). In assessing potential pigmentation potency of different forage species, materials produced under similar conditions should be compared.

The objective of this study was to compare changes in the carotene-xanthophyll content of fresh cut vs. field-wilted and then mechanically dehydrated CBG and ALF were produced and processed under commercial conditions and also to determine if changes occur in the relative proportions of carotene and the monohydroxy-, dihydroxy-, and polyoxyxanthophylls in these forages that may be related to processing conditions.

MATERIALS AND METHODS

CBG and ALF were harvested from fields managed by a commercial forage processor (dehydrator). Both forages were grown on a Wagram sand (Arenic Paleudult loamy siliceous thermic family) under optimum conditions of pH and fertility. In 1981, the CBG and ALF fields were

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