

associated with the synthesis of linolenic acid appeared to be active throughout the growth period of each cultivar. Although we previously showed hydrocarbon profiles of these three orange cultivars to correlate closely with dates of ripening (Nordby and Nagy, 1977), the present study showed no definitive correlation between these cultivars fatty acid patterns and ripening dates.

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Volatiles of Wild Strawberries, *Fragaria vesca* L., Compared to Those of Cultivated Berries, *Fragaria* × *ananassa* cv. Senga Sengana

Tapani Pyysalo,* Erkki Honkanen, and Timo Hirvi

Volatile components of Finnish wild strawberry, *Fragaria vesca* L., and of cultivated strawberry, *Fragaria* × *ananassa* cv. Senga Sengana, were studied by combined gas chromatography-mass spectrometry. In all, 87 compounds were identified in the wild and 58 in the cultivated berries. 2,5-Dimethyl-4-methoxy-3(2*H*)-furanone was identified as the main volatile component in the berries of *F. vesca* L. and as an abundant component also in the cultivated berries. Other compounds reported here for the first time as strawberry volatiles include 3-methyl-2-butenyl acetate, methyl nicotinate, carveyl acetate, methyl anthranilate, methyl *N*-formylanthranilate, verbenone, citronellol, myrtenol, 6-methyl-5-hepten-2-ol, eugenol, vanillin, 2-methoxy-4-vinylphenol, and 4-vinylphenol.

The volatiles of cultivated strawberries have been very extensively studied during the last 25 years. Winter and Willhalm (1964) identified over 60 compounds in *Fragaria* × *ananassa* (var. Surprise des Halles) including *trans*-2-hexen-1-ol, 2-ethylhexanol, 2-hexenal, hexyl acetate, *trans*-2-hexenyl acetate, ethyl butyrate, ethyl acetoacetate, and α -terpineol. Later the same authors (Willhalm et al., 1966) reported that the main volatile acids of strawberries consisted of 2-methylbutanoic, hexanoic, and 2-methylpropanoic acids.

McFadden et al. (1965) reported the identification of *cis*- and *trans*-3-hexenyl hexanoates, *trans*-2-hexenyl hexanoate, linalool, and several other compounds not reported previously as constituents of strawberry aroma. Tressl et al. (1969) identified about 200 compounds in the berries of the cultivar Revata. The main components included such previously unidentified compounds as octyl butanoate, octyl 2-methylbutanoate, octyl hexanoate, and γ -dodecalactone. Stoltz et al. (1970) identified 1,2-dihydro-1,1,6-trimethylnaphthalene in strawberry oil and Mussinan and Walradt (1975) found over 20 volatile acids which had not previously been identified in strawberries.

In a recent study Dirinck et al. (1977) used headspace concentration on Tenax to evaluate the aroma quality of cultivated strawberries. They identified about 30 volatiles eluting before *n*-undecane in a SE-30 capillary column.

The over 60 esters, 30 alcohols, and about 30 carbonyl compounds identified in these studies do of course contribute to the estery and green notes of the odor of strawberries, but they can hardly be considered to contain a "character-impact" compound or compounds. Sundt (1970), however, has reported the identification of 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone in strawberries, and this compound may well be considered the most important aroma constituent of strawberries so far reported.

Drawert et al. (1973) and Staudt et al. (1975) compared the volatiles of the berries of several wild strawberry species with those of the cultivar Revata. The wild berries of species *F. vesca* L. and *F. moschata* L. were shown to contain high amounts of 2-alkanones and 2-alkanols, most of which have not been identified in or are only minor constituents of cultivated berries. On the other hand, fewer esters of aliphatic carboxylic acids and no hydrocarbons were identified among the volatiles of the wild berries.

The odor of Finnish wild strawberry is more herbaceous and markedly stronger than that of cultivated strawberry. The 2-alkanones and 2-alkanols previously identified in

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wild strawberries obviously contribute to the difference between the aromas of wild and cultivated berries. The odor of an imitation reconstituted on the basis of the previously identified components of *F. vesca* L. did not, however, resemble the natural aroma of the wild strawberries growing in Finland. The present study was, therefore, focused on the possible differences between the volatiles of the berries of *F. vesca* L. grown in Finland and elsewhere in Europe.

EXPERIMENTAL SECTION

Isolation and Concentration of the Volatiles. The wild strawberries were collected in northeast Finland in August 1977, deep frozen, and stored at -18°C until used. The berries were allowed to thaw out at 4°C for 15 h and then pressed in a hydraulic press. On average 750 mL of press juice was obtained from 1 kg of berries. The pH of the press juice was 3.5 and the odor characteristic to the fresh berries. In all, about 2 kg of wild strawberries was used in this investigation.

The press juice was neutralized with sodium hydrogen carbonate and extracted with redistilled diethyl ether-pentane (2:1) in a Kutscher-Stuedel extraction apparatus for 20 h. After extraction the aqueous phase was acidified to pH 1.0 with 0.1 M hydrochloric acid and extracted again to obtain the acid fraction. Both extracts were dried over anhydrous magnesium sulfate and concentrated to a small volume (0.1 mL) using a small Vigreux column.

Two kilograms of fresh cultivated berries var. Senga Sengana was extracted, and the extracts were concentrated in a similar manner.

Gas Chromatography and Mass Spectrometry. The gas chromatographic analyses of the concentrates were performed using a 30-m glass capillary column (0.3 mm i.d.) coated with FFAP stationary phase. The temperature was programmed from 60 to 220°C at $4^{\circ}\text{C}/\text{min}$. The gas chromatograph (Carlo Erba, Fractovap 2350) was equipped with a flame ionization detector. The quantitative results were obtained using an Autolab System IV integrator. The combined GC-MS apparatus (JEOL JMS-D100) was equipped with glass capillary columns connected directly to the ion source of the mass spectrometer via a platinum capillary tube (0.1 mm i.d.). The mass spectra were recorded at 70 eV, the electron current was 300 μA , the temperature of the ion source and the interface was 250°C , and the scanning speed was 5 s from m/s 35 to m/s 350. The mass spectral identifications were confirmed by measuring the gas chromatographic retention times of the reference compounds.

Synthesis of the Reference Compounds. 2,5-Dimethyl-4-methoxy-3(2*H*)-furanone was synthesized according to the method of Willhalm and Thomas (1969), and 2-methoxy-4-vinylphenol and 4-vinylphenol according to that of Sovish (1959). Methyl *N*-formylanthranilate was synthesized by formylation of methyl anthranilate with formic acid. Other reference compounds were obtained as commercial chemicals.

Determination of Odor Threshold Values. The odor threshold values of some of the major components were determined as described previously by Pyysalo et al. (1977).

RESULTS AND DISCUSSION

The odors of the neutral fractions of the concentrates of both wild and cultivated berries corresponded to the odors of the respective fresh berries. Therefore, the inhibition of the enzyme activity in the press juice was considered unnecessary. The compounds identified in the press juices of both wild and cultivated berries and the approximate concentrations of the main components are

presented in Table I. The main volatile compound in the press juice of the wild strawberries, *F. vesca* L., was found to be 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (10.7% of the total volatiles). This compound was first reported as a constituent of the mango fruit (*Mangifera indica* L.) by Hunter et al. (1974) and later of arctic bramble (*Rubus arcticus* L.) by Kallio and Honkanen (1976). The same compound was also found in hybrids between raspberry (*R. ideaus* L.) and arctic bramble (Pyysalo, 1976). The cultivated berries of *F. × ananassa* var. Senga Sengana were also shown to contain this compound, although at a considerably lower concentration. 2,5-Dimethyl-4-methoxy-3(2*H*)-furanone has not previously been reported to occur in wild or cultivated strawberries. On the other hand, the corresponding hydroxy compound, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone, which has previously been identified in strawberries (Sundt, 1970), was not found in either the wild or cultivated berries studied in this work.

Other major neutral volatiles identified in the wild berries included *trans*-2-hexen-1-ol (4.1% of total volatiles), methyl butanoate (3.1%), hexanol (3.0%), 2-pentadecanone (2.8%), benzyl alcohol (2.7%), ethyl butanoate (1.8%), 2-heptanone (1.8%), 2-pentadecanol (1.7%), and eugenol (1.6%). The main volatile acids in the press juice of the wild berries were acetic (8.9%), hexanoic (5.1%), and butanoic (3.9%) acids. Alcohols formed the largest separate group of compounds in the berries of *F. vesca* L., whereas according to previous studies esters dominate both qualitatively and quantitatively in cultivated strawberries. The odd-carbon-numbered 2-alkanols and 2-alkanones from C_7 to C_{15} have previously been identified in *F. vesca* L. and *F. moschata* L. by Drawert et al. (1973). The same compounds were also found in the wild berries in this investigation, but the relative amounts of the compounds differed from those reported by Drawert et al. (1973), according to whom 2-heptanol, 2-nonanol, and the corresponding ketones are the most abundant of these compounds in *F. vesca* L. In the berries studied in the present work, however, 2-pentadecanol and 2-pentadecanone dominated. No traces of C_{11} , C_{13} , or C_{15} 2-alkanones or 2-alkanols could be found in the berries of the strawberry cultivar Senga Sengana. Other alcohols and carbonyl compounds identified only in the berries of *F. vesca* L. include citronellol, myrtenol, 6-methyl-5-hepten-2-ol, 3-phenylpropanol, *trans*-cinnamyl alcohol, verbenone, and vanillin. These compounds have not previously been reported to occur in strawberries (Table I).

The esters identified were largely the same in both strawberry species studied. The most abundant esters included methyl and ethyl butanoate, ethyl hexanoate, hexyl acetate, and *trans*-2-hexenyl acetate. Five of the esters identified in the wild berries have not previously been reported to occur in strawberries: 3-methyl-2-butenyl acetate, carveyl acetate, methyl nicotinate, methyl anthranilate, and methyl *N*-formylanthranilate. Methyl *N*-formylanthranilate has not previously been found in any fruits or berries. Mass spectrum of the synthesized reference compound was: M^+ 179 (30%); m/e 119 (100), 46 (59), 151 (48), 45 (44), 44 (42), 92 (33), 120 (18), 65 (16). Other compounds identified in the press juice of the berries of *F. vesca* L., but not in the cultivated berries studied, included γ -hexalactone, γ -heptalactone, and γ -octalactone. Eugenol, 2-methoxy-4-vinylphenol, and 4-vinylphenol were identified in the berries of both strawberry species, whereas linalool oxides and some of the identified hydrocarbons were found only in the cultivated strawberries.

Table I. Compounds Identified in the Berries of *Fragaria vesca* L. and *Fragaria* × *ananassa* cv. Senga Sengana and the Concentrations of the Major Components in the Press Juices of the Berries

compound	concentration, mg/kg		References	compound	concentration, mg/kg		References
	<i>F.</i> <i>vesca</i>	<i>F.</i> × <i>anana-</i> <i>ssa</i>			<i>F.</i> <i>vesca</i>	<i>F.</i> × <i>anana-</i> <i>ssa</i>	
acids				esters			
acetic acid	1.5	0.45	<i>a-g</i>	butyl formate	0.10	—	<i>g</i>
propanoic acid	+	+	<i>b, e-h</i>	hexyl formate	0.05	+	
2-methylpropanoic acid	+	0.20	<i>b, c, e-h</i>	ethyl acetate	0.05	0.10	<i>a, e, f, g</i>
butanoic acid	0.65	0.45	<i>b-h</i>	butyl acetate	+	+	<i>d, f, g, k, l</i>
2-methylbutanoic acid	+	0.80	<i>g, h</i>	3-methyl-2-butenyl acetate	0.05	+	
hexanoic acid	0.85	1.2	<i>a-c, f-i</i>	hexyl acetate	0.10	+	<i>d, f, g, k, l</i>
octanoic acid	+	+	<i>g, h</i>	<i>trans</i> -2-hexenyl acetate	+	0.05	<i>d, f, g, l</i>
decanoic acid	+	+	<i>g, h</i>	<i>cis</i> -3-hexenyl acetate	+	+	<i>g, l</i>
dodecanoic acid	+	+	<i>g, h</i>	octyl acetate	0.05	—	<i>g, l</i>
tetradecanoic acid	+	0.40	<i>g</i>	decyl acetate	0.05	—	<i>g, l</i>
hexadecanoic acid	+	2.1	<i>g, h</i>	ethylacetoacetate	+	—	<i>i, f</i>
<i>trans</i> -cinnamic acid	—	0.50	<i>a, f-j</i>	benzyl acetate	+	—	<i>g, k, l</i>
alcohols				carveyl acetate			
ethanol	+	+	<i>b-d, f-h</i>	methyl butanoate	0.50	0.20	<i>e-g</i>
3-methyl-2-butanol	0.05	—	<i>g, k</i>	ethyl butanoate	0.30	0.15	<i>d, f, g, k, l</i>
3-methyl-1-butanol	+	+	<i>g</i>	hexyl butanoate	+	+	<i>g, l</i>
1-pentanol	0.05	+	<i>d, f, g</i>	decyl butanoate	+	—	
3-penten-1-ol	0.05	—		ethyl crotonate	+	—	<i>f</i>
1-hexanol	0.50	0.20	<i>a, d, f, g, k, l</i>	methyl hexanoate	+	+	<i>g, k, l</i>
<i>trans</i> -2-hexen-1-ol	0.65	0.10	<i>d, f, g, k</i>	ethyl hexanoate	+	0.15	<i>d, f, g, k, l</i>
<i>cis</i> -3-hexen-1-ol	0.05	+	<i>g, k</i>	methyl decanoate	—	+	<i>g, k, l</i>
2-ethyl-1-hexanol	+	—	<i>b, f</i>	methyl dodecanoate	+	+	<i>g, l</i>
1-heptanol	0.05	—	<i>g</i>	methyl salicylate	—	+	
2-heptanol	0.10	0.05	<i>g, l</i>	methyl nicotinate	+	—	
6-methyl-5-hepten-2-ol	+	—		methyl cinnamate	+	+	<i>g, k, l</i>
1-octanol	0.05	—	<i>g, l</i>	methyl anthranilate	+	—	
2-nonanol	0.15	+	<i>g, l</i>	methyl <i>N</i> -formylanthranilate	+	—	
1-decanol	+	—	<i>g</i>	micellaneous			
2-undecanol	+	—	<i>g, l</i>	diethyl ether ^m	+	+	
2-tridecanol	+	—	<i>l</i>	pentane ^m	+	+	
2-pentadecanol	0.30	—	<i>l</i>	dichloromethane ^m	+	+	
linalool	+	0.15	<i>g, k, l</i>	chloroform ^m	+	+	
α-terpineol	+	+	<i>a, f, g, k</i>	dodecane	—	+	<i>g</i>
citronellol	+	—		tetradecane	—	+	
myrtenol	+	—		pentadecane	—	+	
benzyl alcohol	0.45	+	<i>g</i>	styrene	+	+	
2-phenylethanol	+	—	<i>f, g, j</i>	phenylacetylene ^m	+	+	
3-phenylpropanol	+	—		linalool oxide } five-membered	—	+	
<i>trans</i> -cinnamyl alcohol	0.05	+		linalool oxide } six-membered	—	+	
carbonyl compounds				linalool oxide }	—	+	
hexanal	+	+	<i>b, f, g</i>	linalool oxide }	—	+	
2-hexenal	0.05	0.05	<i>b, c, f, g, k, l</i>	γ-hexalactone	+	—	<i>g</i>
2-heptanone	0.30	0.15	<i>g, l</i>	γ-heptalactone	+	—	<i>g</i>
2-nonanone	0.15	+	<i>g, l</i>	δ-hexalactone	+	+	
2-undecanone	+	—	<i>g, l</i>	γ-octalactone	+	—	<i>g</i>
2-tridecanone	+	—	<i>l</i>	δ-octalactone	+	+	<i>g</i>
2-pentadecanone	0.45	—	<i>l</i>	2,5-dimethyl-4-methoxy			
acetoin	0.05	—	<i>a, f</i>	3(2 <i>H</i>)-furanone	1.7	0.20	
benzaldehyde	+	+	<i>g, k</i>	2-methoxy-4-vinylphenol	+	+	
verbenone	+	—		4-vinylphenol	+	0.05	
vanillin	+	—		eugenol	0.25	+	

^a Compound previously identified in strawberries by Coppens and Hoejenbos (1939). ^b Winter et al. (1958). ^c Dimick and Makower (1956). ^d Dimick and Corse (1958). ^e Teranishi et al. (1963). ^f Winter and Willhalm (1964). ^g Tressl et al. (1969). ^h Mussinan and Walradt (1975). ⁱ McGlumphy (1951). ^j Seidel et al. (1958). ^k McFadden et al. (1965). ^l Drawert et al. (1973). ^m Solvent or compounds originating from the solvents. ⁿ Compound identified as a minor constituent (+); not identified (—).

The main qualitative difference between the results of this investigation and those obtained by Drawert et al. (1973) is the identification of 2,5-dimethyl-4-methoxy-3(2*H*)-furanone (odor threshold value 0.01 mg/L, this work) as the main volatile component of Finnish wild strawberries. According to our preliminary sensory experiments with reconstituted wild strawberry flavor preparations this compound makes a great contribution to the characteristic odor of the berries. Some of the fairly abundant alcohols and carbonyl compounds are obviously also important constituents of the wild strawberry aroma.

At least 2-heptanol (odor threshold in water 0.07 mg/L, this work), 2-heptanone (0.37 mg/L, Pyysalo et al., 1977), linalool (0.006 mg/L, Buttery et al., 1971), benzyl alcohol (0.62 mg/L, Pyysalo et al., 1977), acetoin (0.055 mg/L, Pyysalo et al., 1977), and 1-hexanol (0.5 mg/L, Flath et al., 1967) are present in the berries in concentrations close to or above their respective threshold concentrations in water. 2-Pentadecanol and 2-pentadecanone are obviously not volatile enough to effect the odor of the berries, but they may have some influence on the taste. The contribution of other components identified only in the wild

berries remains to be determined. Vanillin, eugenol, and carveyl acetate are, however, considered to be important for the odor of Finnish wild strawberries.

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Kudzu (*Pueraria lobata*) Root Starch as a Substrate for the Lysine-Enriched Baker's Yeast and Ethanol Fermentation Process

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At best, kudzu is considered to be an escaped plant in the southern United States. It was introduced to the South during the 1930's to help restore the soil and reduce erosion since it is a legume with an extensive root structure. This vine has adapted so successfully that it is presently overgrowing roads and trees. It is proposed here to use the starchy root of this plant as a fermentation medium in order to develop a commercial outlet for kudzu and add a starch to supplement our renewable food and fuel supplies. Purified kudzu starch as well as the kudzu root extract is compared with the other starches, manioc and corn dextrin, to determine the relative rate at which α -amylase degrades the polymer for fermentation purposes. Fermentations of the purified kudzu root starch are conducted to determine the fermentation vitamin and mineral requirements. Intracellular lysine and cell yields from the fermentation process for the raw and purified kudzu starch are compared with corn dextrin and manioc starch substrates.

In a previous paper (Tanner et al., 1977), a fermentation process was proposed for producing lysine-enriched baker's yeast to help alleviate human protein malnutrition, and ethanol, for use as a liquid fuel such as a gasoline extender. In that earlier paper, glucose was used as a model substrate. Typical substrates are sugar cane, sugar beet, and sorghum, or saccharified corn, wheat, and manioc starches. In light of impending world shortages of all of these conventional sugar sources, a new substrate source is examined in this work: kudzu, a plant which can be grown on marginal farming lands.

Since kudzu (*Pueraria lobata* or *Pueraria thunbergiana*) is a legume, it has minimal nitrogen fertilizer requirements and its leaves and vines can be used as protein-rich forage (Hill, 1937; Schery, 1952). Having an extensive root structure, in addition to its primary starch tap root, kudzu

has excellent soil erosion control properties (Bailey and Mayton, 1931; Pieters, 1932; and McKee and Stephens, 1943) and may have an important role in meeting the global problem of soil erosion (Carter, 1977).

Kudzu is not without disadvantages, however. To one who lives in the southeastern United States, kudzu is considered to be, at best, an escaped plant. It was originally planted in large amounts in the 1930's to help restore the soil, being a legume, and keep down erosion because of its extensive root structure. But it has gotten out of control in the warmer areas of the South because the winters do not kill back the vines to the roots, as they do in the North and, presumably, as in its native Japan and China. The plant is tenacious, primarily because of the reservoir of starch in its thick root, whose use for fermentation is the subject of this paper.

MATERIALS AND METHODS

The yeast culture, lysine assay culture, and cell optical density measurement procedure are described in a previous

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