

# Vacuum Headspace Method in Aroma Research: Flavor Chemistry of Yellow Passion Fruits

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The volatile flavor components of yellow passion fruits have been isolated using four different isolation techniques. The most representative and typical extract was obtained by vacuum headspace sampling and subsequent liquid–liquid extraction of the aqueous phase. This vacuum headspace concentrate was prefractionated by medium-pressure adsorption chromatography on silica gel. Approximately 180 components were identified in the LC fractions of yellow passion fruit flavor for the first time. Of these compounds, 14 components have not previously been reported as naturally occurring flavor ingredients. Moreover, 47 sulfur-containing volatiles were identified in yellow passion fruits after enrichment by preparative multidimensional capillary gas chromatography; 35 of these components are reported to be present in the tropical fruit flavor for the first time, and 23 of these sulfur-bearing compounds have not been previously reported as constituents of food flavors and are therefore new natural components. In addition, the enantiomeric distributions of several chiral flavor substances were determined by enantioselective multidimensional gas chromatography.

**Keywords:** *Yellow passion fruit flavor; comparison of sampling techniques; vacuum headspace method; sulfur volatiles; enantio-MDGC*

## INTRODUCTION

Yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) is one of the most popular and best known tropical fruits having a floral, estery aroma with an exotic tropical sulfury note. The volatile composition of yellow passion fruit flavor is rather complex and was thoroughly reviewed by Whitfield and Last (1986) and Shibamoto and Tang (1990). To date, >200 components have been identified in yellow passion fruit flavor.

The attractive tropical flavor note of ripe yellow passion fruits has been shown to be associated with trace levels of sulfur volatiles. Volatile sulfur components are important trace constituents of natural products and play an important role in the sensory properties of food flavors. Sulfur-containing components combine high odor intensities and low threshold values and have been identified as character-impact substances in various foods and beverages (Boelens and van Gemert, 1993; Mussinan and Keelan, 1994). To date, 12 volatile sulfur-containing trace constituents have been identified in different varieties of yellow passion fruits as well as in passion fruit juices (Winter et al., 1976; Engel and Tressl, 1991).

In the past decades, flavor volatiles have mainly been isolated by means of liquid–liquid extraction, simultaneous distillation and extraction, or dynamic headspace analysis. It is well-known from the literature that the composition of a flavor extract is dependent on the isolation procedure employed. To obtain aroma concentrates that are truly representative of tropical passion fruit flavor, four different flavor isolation techniques have been applied in our laboratory: vacuum

headspace method (VHS), dynamic headspace method (DHS), simultaneous distillation and extraction at atmospheric pressure (SDE), and simultaneous distillation and extraction under reduced pressure (SDEV).

Flavor extracts were organoleptically evaluated and the aroma composition, of different flavor concentrates was investigated by GC/MS. The VHS concentrate was noted to be of particular sensory interest and was therefore analyzed in more detail after pre-separation of fruit flavor components by medium-pressure liquid chromatography and subsequent enrichment of trace constituents by preparative multidimensional capillary gas chromatography (MDGC). Furthermore, special attention has been paid to the analysis of sulfur-containing components possessing interesting sensory characteristics. In addition, this study reports the evaluation of the enantiomeric composition of some important chiral passion fruit constituents.

## MATERIALS AND METHODS

**Materials.** Fresh ripe yellow passion fruits were obtained by air freight from Colombia via Hamburg and were worked up immediately after arrival.

**Sample Preparation.** 1. *VHS.* This method is basically a form of vacuum steam distillation. A total of 700 g of passion fruit pulp was placed into a 2 L round-bottom flask of the sampling system shown in Figure 1. Subsequently, a vacuum was applied during 6 h ( $\approx 1$ –10 mbar) while the fruit volatiles together with water were condensed in three cooling traps at low temperatures (ice–water/dry ice–acetone/liquid nitrogen).

At the end of the sampling period, the aqueous condensates were combined and extracted with ether/pentane (1:1) by means of a rotational perforator. The extracts obtained from  $\sim 5$  kg of passion fruits were combined and dried over anhydrous sodium sulfate. The organic solvent was then concentrated with a Vigreux column (25 cm) to a final volume of  $\sim 200$   $\mu$ L.

2. *DHS.* Passion fruit pulp (1.5 kg) was placed in a 4 L glass container, and the system was purged overnight with a

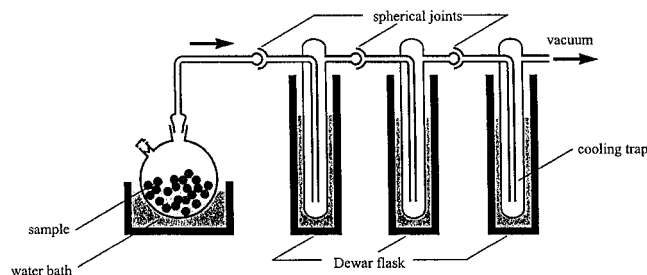
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**Table 1. Organoleptic Evaluation of Different Passion Fruit Flavor Concentrates**

sample	comments
vacuum headspace concentrate (VHS)	tropical, strongly fruity, estery, green, juicy, fresh, sulfury, honey-like, typical passion fruit
dynamic headspace concentrate (DHS) simultaneous distillation-extraction at atmospheric pressure (SDE) simultaneous distillation-extraction at reduced pressure (SDEV)	herbaceous, faintly tropical, very weak aroma, atypical fruity, sulfury, faintly tropical, acidic background, burnt, cooked aroma tropical, sulfury, sweet, fruity, somewhat passion fruit-like

**Figure 1.** Scheme of the vacuum headspace sampling system.

150 mL/min flow of purified helium (passed through a molecular sieve). A Tenax trap was used to collect passion fruit volatiles. The trap consisted of a glass tube packed with 3.1 g of Tenax TA (35–60 mesh, Chrompack). The adsorbed volatiles were eluted from the Tenax trap with 150 mL of pentane/diethyl ether (1:1). The combined extracts obtained from ~5 kg passion fruits were dried over anhydrous sodium sulfate and concentrated with a Vigreux column (25 cm) to a final volume of ~200  $\mu$ L.

**3. SDE and SDEV.** The fruit pulp (1.5 kg) was mixed with 4 L of distilled water and added to a 10 L round-bottom flask. A few drops of antifoam solution were added. The fruit slurry was continuously extracted for 5 h with 200 mL of pentane/diethyl ether (1:1) at atmospheric pressure according to the procedure described by Likens and Nickerson (Nickerson and Likens, 1966). The vacuum steam distillation was also conducted in an SDE apparatus for 5 h using methyl *tert*-butyl ether (80 mbar/50 °C) as an extraction solvent.

The combined organic extracts obtained from ~5 kg of passion fruits were dried over anhydrous sodium sulfate, and the organic solvent was removed on a 25 cm Vigreux distillation column to a final volume of ~200  $\mu$ L.

To study the influence of the four different extraction methods on the chemical composition of passion fruit volatiles, all solvent extracts were subsequently analyzed using completely identical chromatographic and spectroscopic conditions.

**Preparation by Adsorption Chromatography.** A total of 50 kg of yellow passion fruits was used for preparing an extract according to the vacuum headspace method, which was subsequently divided into 20 subfractions. The aroma concentrate was applied to the top of a water-cooled column [480 mm  $\times$  37 mm (i.d.)] of 240 g of silica gel (LiChroprep Si 60/25–40  $\mu$ m; Merck), and the sample was rinsed with a small portion of pentane and subsequently eluted using a pentane/diethyl ether gradient. The flow rate was 10 mL/min at a pressure of 1–2 bar. All eluates were dried over anhydrous sodium sulfate. Fractions were concentrated to a small volume (~100–200  $\mu$ L) using a two-stage procedure: (i) gentle distillation by means of a 25 cm  $\times$  1 cm Vigreux column and (ii) evaporation in a Dünge system (Dünge, 1979).

**Capillary Gas Chromatography (HRGC).** Analytical separations were performed on a Carlo Erba Type 5360 Mega series gas chromatograph equipped with a split/splitless injector (220 °C, split ratio 1:20), a flame ionization detector operating at 250 °C; a nitrogen-phosphorus detector (NPD), and a flame photometric detector (FPD) operating in the sulfur mode at 394 nm. Chromatographic data were processed with a HP Chem Server Model 4930 (Hewlett-Packard). The column used was a 60 m  $\times$  0.32 mm (i.d.) fused silica capillary column coated with DB-Wax (0.25  $\mu$ m film thickness) (J&W Scientific, Folsom, CA). The oven temperature was pro-

grammed from 60 °C at 3 °C/min to 220 °C. Hydrogen was used as a carrier gas (inlet pressure = 1.0 bar).

**Preparative Capillary Gas Chromatography.** A mass flow controlled multidimensional switching system MCS (Gerstel GmbH, Mülheim/Ruhr, Germany) was employed for the preparative isolation of unknown flavor components as well as for the preparative enrichment of sulfur-containing trace constituents. A combination of a 5 m thick film DB-1 [0.53 mm (i.d.)/ $d_f$  = 3  $\mu$ m] and a 30 m thick film DB-1 column [0.53 mm (i.d.)/ $d_f$  = 3  $\mu$ m] was used: flow rate, 5 mL/min helium; temperature program, 60 °C, raised at 3 °C/min to 250 °C; temperature programmed cold injection system, 60 °C  $\rightarrow$  12 °C/s  $\rightarrow$  350 °C; automated fraction collection system, –40 °C.

**Gas Chromatography/Mass Spectrometry (HRGC/MS).** For GC/MS analysis a Carlo Erba Mega 5300 gas chromatograph equipped with a split/splitless injector was directly coupled to a Finnigan MAT 8200 double-focusing high-resolution mass spectrometer. The operating conditions were as follows: ion source, 220 °C; EI, 70 eV; cathodic current, 1 mA; accelerating voltage, 3 kV; and resolution, 900. A J&W DB-Wax column (60 m  $\times$  0.32 mm i.d.,  $d_f$  = 0.25  $\mu$ m) was used with the following temperature program: 60 °C, raised at 3 °C/min to 240 °C (15 min).

The temperature of the injector and of the transfer line was 240 °C. The mass spectrometer was operated in scan mode over a mass range from 25 to 370 amu (1 s/decade).

**Infrared (IR) and Nuclear Magnetic Resonance (NMR) Analyses.** For GC-FTIR analyses a Bio-Rad Digilab FTS-45A spectrometer connected to a Bio-Rad Tracer (Bio-Rad, Krefeld, Germany) equipped with a liquid nitrogen cooled narrow-band MCT detector and coupled to a HP 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) was applied. The samples were separated on a J&W DB-1 column [30 m  $\times$  0.25 mm (i.d.)/0.25  $\mu$ m film thickness] with helium as carrier gas (split injection mode). Deposition tip and transfer line were held above 200 °C. Absorbance spectra were recorded from 4000 to 700  $\text{cm}^{-1}$  at a spectral resolution of 1  $\text{cm}^{-1}$ .

<sup>1</sup>H NMR spectra of collected and synthesized samples were obtained at 400 MHz in CDCl<sub>3</sub>, C<sub>6</sub>D<sub>6</sub>, or C<sub>6</sub>D<sub>12</sub> on a Varian VXR-400 instrument with Si(CH<sub>3</sub>)<sub>4</sub> as internal standard.

**Component Identification.** Sample components were identified by comparison of their mass spectra and linear retention indices with those of reference standards. Reference compounds were synthesized in our laboratory. The respective structures were confirmed by NMR, MS, and IR spectra.

**Sensory Evaluation.** The sensorial evaluation of selected components was performed by an expert panel of flavorists. The synthesized compounds were evaluated in water at certain concentrations (1–10 ppm) or were dissolved in ethanol and tested on a smelling blotter. Taste threshold determinations were performed analogous to DIN 10959.

**Enantioselective Separation.** A Siemens Sichromat double-oven gas chromatograph equipped with a temperature-programmed cold injection system and two flame ionization detectors was used for direct enantiodifferentiation of chiral flavor components. Preseparation was achieved in oven 1 on a 30 m  $\times$  0.25 mm (i.d.) DB-Wax fused silica capillary column ( $d_f$  = 0.25  $\mu$ m). The temperature was programmed from 60 to 240 °C at 3 °C/min (0.7 bar of helium). The second oven was equipped with appropriate chiral capillary columns including different O-alkylated and -acylated  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins from various suppliers. Several temperature programs were

**Table 2. Flavor Constituents of Yellow Passion Fruits/Comparison of Sample Preparation Techniques**

compound <sup>a</sup>	area %				compound <sup>a</sup>	area %			
	VHS	DHS	SDE	SDEV		VHS	DHS	SDE	SDEV
ethyl acetate	0.7	3.0	1.4	2.0	(E)-2-hexenyl acetate	<0.1			
2-butanone	0.3	1.3	0.3		1-hexanol	8.0	3.3	2.5	5.4
2- and 3-methylbutanal			0.4		2-cyclopenten-1-one	<0.1	<0.1	0.3	<0.1
ethyl propanoate	<0.1	<0.1	0.1	<0.1	3-nonanone	<0.1	<0.1		<0.1
2-pentanethiol			<0.1		(E)-3-hexen-1-ol	0.6	0.1	0.2	0.3
2-ethylfuran			<0.1		(Z)-3-hexen-1-ol	2.5	0.6	1.0	1.7
2,3-butanedione			<0.1		2-methyl-3(2H)-furanone			<0.1	
propyl acetate	<0.1	<0.1		<0.1	butyl hexanoate	<0.1	<0.1	0.1	<0.1
2- and 3-pentanone	<0.1	0.1	0.1	0.1	hexyl butanoate	8.9	15.5	2.9	8.7
pentanal			<0.1		α-angelica lactone			<0.1	
methyl butanoate	<0.1	<0.1	<0.1	<0.1	hexyl 2-methylbutanoate	0.1	0.1	<0.1	0.1
2-methyl-2-butanol			<0.1		(E)-2-octenal				<0.1
isobutyl acetate	<0.1	0.1	<0.1	<0.1	1-isopropenyl-4-methylbenzene	<0.1		<0.1	<0.1
2-butanol	<0.1	0.1	<0.1	<0.1	ethyl octanoate	0.3	0.1	0.1	0.3
α-pinene	<0.1	0.3	<0.1	<0.1	trans-linalool oxide (f)	0.2		1.2	0.4
ethyl butanoate	4.7	23.0	7.9	9.1	acetic acid	<0.1	<0.1	<0.1	<0.1
1-propanol	<0.1	<0.1	<0.1	<0.1	methional			<0.1	
(E)-2-butenal			<0.1	<0.1	ethyl (methylthio)acetate	<0.1			
2,3-pentanedione	<0.1	<0.1	<0.1	<0.1	hexyl 3-methylbutanoate				<0.1
ethyl 2-methylbutanoate	<0.1	<0.1	<0.1	<0.1	(E)-3-hexenyl butanoate	0.3	0.3	0.1	0.2
S-methyl acetothioate			<0.1		furfural	<0.1	<0.1	2.3	<0.1
butyl acetate	0.1	0.6	<0.1	0.1	1-heptanol	<0.1			<0.1
hexanal	0.1	0.1	0.1	0.2	(Z)-3-hexenyl butanoate	2.1	2.2	0.5	1.3
2-methyl-1-propanol	0.1	0.2	<0.1	<0.1	nerol oxide			<0.1	
1-hexen-3-one			<0.1	<0.1	cis-linalool oxide (f)	<0.1		0.6	0.1
2-methyl-(E)-2-butenal			<0.1		2-acetyl-furan and			0.4	
2,6,6-trimethyl-2-vinyltetrahydropyran			0.4		2,5-dimethyl-3(2H)-furanone				
3-pentanol	<0.1	<0.1		<0.1	theaspirane A	<0.1		<0.1	<0.1
2-pentanol	<0.1			<0.1	benzaldehyde	1.9	<0.1	1.9	2.9
β-pinene		<0.1	<0.1	<0.1	ethyl 3-hydroxybutanoate	1.6	<0.1	0.2	<0.1
2- and 3-methylbutyl acetate	<0.1	0.3	<0.1	<0.1	cis-2-methyl-4-propyl-1,3-oxathiane	<0.1		<0.1	<0.1
propyl butanoate	<0.1	<0.1	<0.1	<0.1	theaspirane B	<0.1			<0.1
3-penten-2-one			<0.1	<0.1	linalool	0.5	0.3	4.6	4.9
ethyl pentanoate	<0.1	<0.1	<0.1	<0.1	hexyl 2-butenate	<0.1	<0.1		<0.1
4-methyl-3-penten-2-one	<0.1		<0.1	<0.1	trans-2-methyl-4-propyl-1,3-oxathiane	<0.1	<0.1	<0.1	<0.1
(Z)-3-hexenal			<0.1	<0.1	1-octanol	2.3	0.4	0.3	0.8
1-butanol	0.2	0.1	0.2	0.3	ethyl 3-(methylthio)propanoate	<0.1	<0.1	<0.1	<0.1
(E)-3-hexenal	<0.1		<0.1	<0.1	5-methylfurfural			0.1	
3-heptanone	<0.1	<0.1	<0.1	<0.1	4-terpineol	0.3		0.1	0.2
Δ-3-carene	<0.1	<0.1	<0.1	<0.1	hotrienol	<0.1		<0.1	<0.1
myrcene	0.3	4.4	0.7	1.2	p-1-menthenal-9 P1			<0.1	<0.1
ethyl 2-butenate	<0.1	<0.1	<0.1	<0.1	p-1-menthenal-9 P2			<0.1	<0.1
isobutyl butanoate	<0.1	<0.1		<0.1	hexyl hexanoate	26.7	7.6	10.3	24.4
α-phellandrene		<0.1	<0.1	0.1	octyl butanoate	<0.1			0.2
α-terpinene		0.1	<0.1	0.2	phenylacetaldehyde			0.8	<0.1
2-heptanone	<0.1	0.1	<0.1	<0.1	butanoic acid			<0.1	<0.1
cyclopentanone	0.2	0.1	<0.1	0.1	(E)-3-hexenyl hexanoate			0.2	
methyl hexanoate	<0.1	<0.1	<0.1		Riesling acetal			<0.1	
limonene	0.2	1.8	0.4	0.7	furfuryl alcohol			0.1	
3-methyl-2-butenal	<0.1			<0.1	(Z)-ocimene			0.1	
2- and 3-methyl-1-butanol	0.2	<0.1	0.1	0.2	(Z)-3-hexenyl hexanoate	4.7	1.2	1.6	3.7
trans-anhydrolinalool oxide			0.3		diethyl succinate	0.3		<0.1	0.2
β-phellandrene		<0.1		0.2	(E)-ocimene			0.4	
1,8-cineole	<0.1	<0.1	<0.1	<0.1	2- and 3-methylbutanoic acid			<0.1	
(E)-2-hexenal	0.1		0.4	0.4	ethyl 3-hydroxyhexanoate	0.2		0.1	0.1
butyl butanoate	<0.1	<0.1		<0.1	α-terpineol	0.5	<0.1	2.0	1.7
ethyl hexanoate	5.2	7.2	2.0	5.8	γ-terpineol			<0.1	
cis-anhydrolinalool oxide			0.2		3-mercaptohexyl acetate			<0.1	
cis-β-ocimene		<0.1	<0.1		benzyl acetate	1.0	0.1	0.2	0.3
γ-terpinene	0.1	0.3	0.1	0.2	(Z)-3-, (E)-6-farnesene	<0.1			0.1
trans-β-ocimene	0.3	4.1	0.7	1.0	ethyl 3-(methylthio)-(E)-2-propenoate	<0.1		<0.1	0.1
1-pentanol			<0.1		(E)-3-, (E)-6-farnesene	0.1		<0.1	0.5
2- and 3-methylbutyl butanoate	<0.1	<0.1			1-decanol	0.1		<0.1	0.1
hexyl acetate	2.0	7.9	0.7	1.6	β-citronellol	0.1			0.1
3-hydroxy-2-butanone and terpinolene	0.4	1.3	0.4	0.7	methyl 2-hydroxybenzoate	0.2	<0.1	<0.1	0.1
octanal	<0.1		<0.1	<0.1	neryl 2-methylpropanoate	<0.1			<0.1
ethyl (E)-3-hexenoate	<0.1	<0.1	<0.1	0.1	nerol	0.1		0.3	0.2
cyclopentanol	<0.1	<0.1	<0.1	<0.1	ethyl 2-hydroxybenzoate	<0.1		<0.1	
(E)-4,8-dimethyl-1,3,7-nonatriene and	0.2	1.1	0.2	0.3	phenethyl acetate	0.1		0.1	0.1
(E)-3-hexenyl acetate	1.6	4.2	0.5	1.3	hexyl octanoate	0.3	<0.1	0.4	0.6
(Z)-3-hexenyl acetate	<0.1	<0.1	0.7	<0.1	3-[(E)-1-propenyl]-α-terpineol P1	<0.1			
3-methyl-2-buten-1-ol	<0.1		<0.1	<0.1	octyl hexanoate	<0.1	<0.1	<0.1	<0.1
(Z)-2-penten-1-ol	<0.1	<0.1	<0.1	<0.1	β-damascenone	<0.1		<0.1	<0.1
propyl hexanoate	<0.1	<0.1		<0.1	7,8-dihydro-β-ionone	0.2		0.1	0.2
2-heptanol		<0.1		<0.1	3-mercaptohexanol	0.1		0.2	0.2
hexyl propanoate	<0.1	<0.1		<0.1	hexanoic acid	<0.1		0.1	<0.1
ethyl (E)-2-hexenoate	<0.1	<0.1		<0.1	p-cymenol-8	<0.1		<0.1	<0.1
hexyl 2-methylpropanoate	<0.1				geraniol	0.3		0.8	0.8
ethyl heptanoate					3-[(E)-1-propenyl]-α-terpineol P2	0.2			

Table 2 (Continued)

compound <sup>a</sup>	area %				compound <sup>a</sup>	area %			
	VHS	DHS	SDE	SDEV		VHS	DHS	SDE	SDEV
(Z)-3-hexenyl octanoate	0.1			<0.1	benzyl hexanoate	0.3		0.1	0.1
benzyl butanoate	0.3		0.1	0.1	hexyl benzoate	0.1			
benzyl alcohol	2.8	<0.1	0.3	0.2	3-phenylpropyl butanoate	0.1			
ethyl 5-hydroxyhexanoate	<0.1				(2-nitroethyl)benzene	0.1			
ethyl 3-phenylpropanoate	0.5				ethyl cinnamate	0.8		0.3	0.3
geranyl butanoate	<0.1				3-(methylthio)hexyl hexanoate	<0.1			<0.1
3-(methylthio)hexanol			<0.1		(E)-3, (E)-5-pseudoionone	0.2		<0.1	<0.1
2-phenylethanol	<0.1		0.1	<0.1	$\gamma$ -decalactone	0.2		0.1	<0.1
3-(methylthio)hexyl butanoate	<0.1			<0.1	eugenol	<0.1		0.1	
$\beta$ -ionone	0.1		0.1	0.2	2-methoxy-4-vinylphenol			0.1	
nerylacetol	0.1				phenethyl hexanoate	0.1			<0.1
3-phenylpropyl acetate	<0.1				nonanoic acid	<0.1			
phenethyl butanoate	<0.1			<0.1	<i>cis</i> - $\gamma$ -jasmin lactone	<0.1			
geranylacetol	0.1				<i>trans</i> - $\gamma$ -jasmin lactone	<0.1			
4-hydroxy-2,5-dimethyl-3(2 <i>H</i> )-furanone	0.1			0.1	<i>trans</i> -isoeugenol			<0.1	
3-phenyl-1-propanol	0.2				$\gamma$ -dodecalactone	<0.1		0.1	<0.1
3-mercaptohexyl hexanoate	<0.1			0.1	hexadecanoic acid			14.6	
octanoic acid			0.2						

<sup>a</sup> Identifications based on mass spectral data and linear retention indices on DB-Wax.

applied depending on the different chiral samples (GC details are given in Table 6).

**Synthesis of Reference Compounds.** Authentic reference compounds were synthesized according to well-established synthetic procedures (Klamann, 1985; *Organikum*, 1996): 3,6-octanedione, nerylacetol, geranylacetol, (*E*)-5- and (*Z*)-5-tangerinol, furaneol acetate, diethyl disulfide, diisopropyl disulfide, methyl 2-methylbutyl disulfide, diisopropyl trisulfide, 1,1-bis(methylthio)-2-methylpropane, *cis*- and *trans*-2-methyl-4-*n*-propyl-1,3-oxathiane, 4-methyl-5-vinylthiazole, 3-mercaptohexanol, 3-mercaptohexyl acetate, 3-mercaptohexyl butanoate, 3-mercaptohexyl pentanoate, 3-mercaptohexyl hexanoate, 3-(methylthio)hexanol, 3-(methylthio)hexyl acetate, 3-(methylthio)hexyl butanoate, 3-(methylthio)hexyl hexanoate, 3-(methylthio)propyl butanoate, 3-(methylthio)propyl hexanoate, ethyl (methylthio)acetate, methyl 3-(methylthio)propanoate, ethyl 3-(methylthio)propanoate, propyl 3-(methylthio)propanoate, butyl 3-(methylthio)propanoate, isobutyl 3-(methylthio)propanoate, *sec*-butyl 3-(methylthio)propanoate, pentyl 3-(methylthio)propanoate, 2-methylbutyl 3-(methylthio)propanoate, 3-methylbutyl 3-(methylthio)propanoate, hexyl 3-(methylthio)propanoate, ethyl 3-(methylthio)-(*Z*)-2-propenoate, propyl 3-(methylthio)-(*E*)-2-propenoate, hexyl 3-(methylthio)-(*E*)-2-propenoate, and 3-(1-hydroxy-3-hexyldithio)hexanol. Compounds were purified by preparative GC or HPLC and verified by spectral data (MS, IR, NMR).

## RESULTS AND DISCUSSION

Numerous reviews on sample preparation and isolation procedures have recently been published (Schreier, 1987; Teranishi and Kint, 1993; Buttery and Ling, 1996; Parliment, 1997; Wampler, 1997), and the influence of these different techniques on the resultant flavor extracts was discussed in detail (Leahy and Reineccius, 1984; Winterhalter et al., 1987; Peppard, 1992; Misharina et al., 1994; Krumbein and Ulrich, 1996; Mistry et al., 1997; Blank, 1997).

The vacuum headspace isolation technique (cryogenic vacuum trapping) was introduced by Joulain in 1986 as a convenient method for trapping the volatile compounds emitted by fragrant flowers. To date, this method has been applied successfully by other authors to a large number of floral species (Joulain, 1993; Brunke et al., 1993; Surburg et al., 1993) as well as to the flavor analysis of strawberries, peaches, and cupuaçu fruits (Fischer and Hammerschmidt, 1992; Fischer et al., 1993, 1995). Recently, Tarantilis and Polissiou (1997) reported on the isolation and identifica-

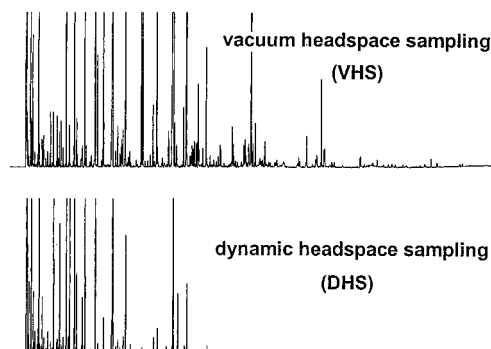


Figure 2. Capillary gas chromatograms of yellow passion fruit volatiles obtained by VHS and DHS.

tion of the aroma components from saffron using the mild vacuum headspace method. For the sake of completeness, however, it should additionally be mentioned that a similar isolation method, used to enrich fruit flavor compounds, had already been described many years ago in the literature under the name "high-vacuum distillation" (Idstein et al., 1984; Idstein and Schreier, 1985a,b).

**Comparison of Sampling Techniques.** Significant differences were found not only in the chemical composition of the resultant extracts but also in their sensory properties. Table 1 shows the sensory assessment of the four passion fruit extracts by a panel of experienced flavorists. The comparison of the four isolation methods showed that the vacuum headspace procedure resulted in the most powerful and typical flavor concentrate representing the true natural delicate and characteristic aroma notes reminiscent in a very typical way of the original fruit flesh. The flavor of the vacuum headspace sample was noted to be of particular sensory interest and was generally recognized as superior to those obtained by classical isolation techniques. Only the odor of the VHS extract was representative of the fruit and was described as fresh, juicy, tropical, grassy, fruity, green, sulfury, and estery and possessing odor notes reminiscent of honey, pineapples, melons, and grapefruits.

Table 2 lists the compounds identified in different passion fruit flavor concentrates along with their GC peak area percents. Each compound listed was identi-

**Table 3. Volatile Compounds Identified in Yellow Passion Fruit Flavor for the First Time**

component <sup>a</sup>	retention index (DB-Wax)	component <sup>a</sup>	retention index (DB-Wax)
Hydrocarbons			
( <i>E</i> )-4,8-dimethyl-1,3,7-nonatriene	1303	( <i>Z</i> )-3, ( <i>E</i> )-6- $\alpha$ -farnesene	1721
( <i>Z</i> )-4,8-dimethyl-1,3,7-nonatriene	1274	( <i>E</i> )-3, ( <i>E</i> )-6- $\alpha$ -farnesene	1749
$\beta$ -pinene	1114	( <i>E</i> )- $\beta$ -farnesene	1666
$\alpha$ -phellandrene	1171	<i>trans</i> - $\alpha$ -bergamotene	1577
$\beta$ -phellandrene	1212	$\beta$ -bisabolene	1729
Alcohols			
3-pentanol	1111	$\beta$ -citronellol	1760
( <i>Z</i> )-2-penten-1-ol	1312	isopulegol	1617
3-heptanol	1290	borneol	1650
1-hepten-3-ol	1341	isomyrcenol	1628
6-methyl-5-hepten-2-ol	1462	<i>cis</i> -isopiperitenol	1745
( <i>E</i> )-3-octen-1-ol	1558	<i>trans</i> -isopiperitenol	1750
( <i>Z</i> )-5-octen-1-ol	1608	8-hydroxymenthol	2165
2-nonanol	1530	<i>p</i> -cymenol-8	1832
3-nonanol	1493	<i>p</i> -1,5-menthadienol-8	1723
1-decanol	1769	<i>p</i> -1,8-menthadienol-4	1668
( <i>E</i> )-3-decenol	1759	<i>p</i> -1(7),2-menthadienol-8 <sup>b</sup>	1778
( <i>Z</i> )-3-decenol	1783	3-[( <i>E</i> )-1-propenyl]- $\alpha$ -terpineol P1 <sup>b</sup>	1803
( <i>Z</i> )-4-decenol	1784	3-[( <i>E</i> )-1-propenyl]- $\alpha$ -terpineol P2 <sup>b</sup>	1846
( <i>Z</i> )-4, ( <i>Z</i> )-7-decadienol-1 <sup>b</sup>	1859	$\beta$ -ionol	1939
( <i>E</i> )-4, ( <i>Z</i> )-7-decadienol-1 <sup>b</sup>	1862	7,8-dihydro- $\beta$ -ionol	1971
nerylacetol [6,10-dimethyl-( <i>Z</i> )-5,9-undecadien-2-ol] <sup>b</sup>	1930	3,4-dehydro- $\beta$ -ionol	2007
geranylacetol [6,10-dimethyl-( <i>E</i> )-5,9-undecadien-2-ol] <sup>b</sup>	1954	cinnamic alcohol	2289
Aldehydes			
3-methyl-2-butenal (prenal)	1206	( <i>E</i> )-2-decenal	1636
( <i>E</i> )-2-hexenal	1215	geranial	1725
( <i>Z</i> )-3-hexenal	1135	neral	1674
( <i>E</i> )-3-hexenal	1140	$\beta$ -cyclocitral	1601
( <i>E</i> )-2, ( <i>E</i> )-4-heptadienal	1482	( <i>E</i> )-cinnamaldehyde	2015
octanal	1297	phenylacetaldehyde	1641
decanal	1489	salicylaldehyde	1670
Ketones			
4-methyl-3-penten-2-one	1129	4-methylacetophenone	1765
2-cyclopenten-1-one	1345	3-ethylacetophenone <sup>b</sup>	1809
cyclohexanone	1285	( <i>E</i> )- $\alpha$ -ionone	1831
3-methyl-2-cyclohexenone-1	1579	( <i>E</i> )- $\beta$ -damascone	1798
3-heptanone	1163	<i>p</i> -3-menthen-2-one (carvenone)	1714
3,6-octanedione <sup>b</sup>	1638	menthone	1449
1-undecenone-5 <sup>b</sup>	1569	pulegone	1644
nerylacetone	1813	camphor	1528
2-tridecanone	1803	piperitone	1714
2-pentadecanone	2031	isopiperitenone	1851
4, ( <i>Z</i> )-6, ( <i>E</i> )-8-megastigmatrienone-3	2193	carvone	1743
acetophenone	1644	verbanone	1624
3-methylacetophenone	1730	verbenone	1696
Esters			
cyclohexyl acetate	1319	isobutyl butanoate	1161
( <i>E</i> )-3-hexenyl acetate	1300	1-methylbutyl butanoate	1217
heptyl acetate	1380	pentyl butanoate	1304
( <i>E</i> )-2-heptenyl acetate	1427	isopentyl butanoate	1263
( <i>Z</i> )-4-heptenyl acetate	1411	cyclopentyl butanoate <sup>b</sup>	1387
octyl acetate	1473	heptyl butanoate	1502
3-octenyl acetate	1466	1-methylhexyl butanoate	1388
( <i>Z</i> )-5-octenyl acetate	1523	decyl butanoate	1807
decyl acetate	1669	geranyl butanoate	1890
styryl acetate	1692	neryl butanoate	1858
2-phenethyl acetate	1796	3-phenylpropyl butanoate	2083
methyl phenylacetate	1758	hexyl ( <i>E</i> )-2-butenate	1548
ethyl phenylacetate	1785	( <i>Z</i> )-3-hexenyl ( <i>E</i> )-2-butenate	1591
citronellyl acetate	1672	benzyl ( <i>E</i> )-2-butenate	2032
furfuryl acetate	1518	isobutyl 3-hydroxybutanoate	1633
( <i>Z</i> )-5-tangerinol [6,10-dimethyl-( <i>Z</i> )-5,9-undecadienyl 2-acetate] <sup>b</sup>	1871	hexyl 3-hydroxybutanoate	1906
( <i>E</i> )-5-tangerinol [6,10-dimethyl-( <i>E</i> )-5,9-undecadienyl 2-acetate] <sup>b</sup>	1903	( <i>Z</i> )-3-hexenyl 3-hydroxybutanoate	1946
hexyl propanoate	1325	ethyl 3-acetoxybutanoate	1570
( <i>Z</i> )-3-hexenyl propanoate	1380	ethyl 3-methylbutanoate	1074
benzyl propanoate	1775	hexyl 2-methylbutanoate	1429
hexyl 2-methylpropanoate	1345	isopentyl 2-methylbutanoate	1276
neryl 2-methylpropanoate	1769	hexyl 3-methylbutanoate	1447
isopropyl butanoate	1039	butyl pentanoate	1306
		( <i>Z</i> )-3-hexenyl pentanoate	1554
		propyl hexanoate	1324

Table 3 (Continued)

component <sup>a</sup>	retention index (DB-Wax)	component <sup>a</sup>	retention index (DB-Wax)
isopropyl hexanoate	1236	1-methylhexyl octanoate	1772
isobutyl hexanoate	1357	ethyl ( <i>Z</i> )-5-octenoate	1473
1-methylbutyl hexanoate	1397	ethyl 3-hydroxyoctanoate	1874
2-methylbutyl hexanoate	1445	hexyl decanoate	1998
hexyl 3-hydroxyhexanoate	2066	diethyl succinate	1682
ethyl 5-hydroxyhexanoate	1865	ethyl benzoate	1650
geranyl hexanoate	2075	hexyl benzoate	2056
neryl hexanoate	2038	benzyl benzoate	2607
1-methylbutyl ( <i>E</i> )-3-hexanoate	1457	methyl ( <i>E</i> )-cinnamate	2077
( <i>Z</i> )-3-hexenyl-( <i>E</i> )-2-hexanoate	1777	ethyl 3-phenylpropanoate	1878
ethyl heptanoate	1342	ethyl salicylate	1786
isopentyl octanoate	1645	hexyl furoate	1998
1-methylbutyl octanoate	1588		
		Lactones	
$\delta$ -hexalactone	1772	<i>trans</i> - $\gamma$ -jasmin lactone	2163
3-methyl- $\delta$ -hexalactone <sup>b</sup>	1780	$\delta$ -jasmin lactone	2226
<i>cis</i> - $\gamma$ -jasmin lactone	2156	<i>trans</i> -marmelolactone	2139
		Miscellaneous Compounds	
perillene	1409	cycloionone (2,5,5,8a-tetramethyl-6,7,8,8a-tetrahydro-5 <i>H</i> -1-benzopyran)	1628
<i>trans</i> -rose oxide	1371	4-hydroxy-2,5-dimethyl-3(2 <i>H</i> )-furanone	2014
<i>cis</i> -edulan	1476	4-methoxy-2,5-dimethyl-3(2 <i>H</i> )-furanone	1576
thymolmethyl ether	1574	furaneol acetate	1968
(1-isopropyl-2-methoxy-4-methylbenzene)		[4-acetoxy-2,5-dimethyl-3(2 <i>H</i> )-furanone]	
carvacrol	2223	<i>trans</i> -edulan <sup>c</sup>	1602
chavicol	2320	thymol	2172
dill ether ( <i>cis</i> -3,9-epoxy- <i>p</i> -menth-1-ene)	1585	1-nitro-2-phenylethane	2095
Riesling acetal	1625		

<sup>a</sup> Identifications based on mass spectral data and linear retention indices on DB-Wax. <sup>b</sup> Identified for the first time in food flavor (with reference to TNO; Nijssen et al., 1996). <sup>c</sup> For yellow passion fruit only one isomer is reported in the literature thus far.

fied by its GC retention index and by its mass spectrum obtained during a GC/MS run.

Significant differences were found in both the qualitative and quantitative compositions of extracts obtained by different isolation procedures. The chromatograms of both headspace samples are shown in Figure 2. The vacuum headspace concentrate contains more high-boiling components. Quantitatively, the major components were hexyl butanoate, hexyl hexanoate, ethyl 3-hydroxybutanoate, and 3(*Z*)-hexenyl hexanoate. Furthermore, this concentrate comprises  $\beta$ -ionone, 2-phenylethyl butyrate, *cis*- and *trans*- $\gamma$ -jasmin lactone, and 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone as well as numerous sulfur components that may play an important role in the characteristic tropical and very pleasant flavor of yellow passion fruits. The composition of the dynamic headspace sample is rather dedicated to the very volatile components. This sample comprised, as expected, more volatile ester compounds including ethyl butanoate, butyl acetate, 2- and 3-methylbutyl acetate, ethyl hexanoate, and hexyl acetate as well as 3(*Z*)-hexenyl acetate. As a result, the latter extract was characterized as largely atypical, lacking in the volatiles responsible for the characteristic aroma of yellow passion fruits. The simultaneous distillation/extraction technique involves the possibility of forming thermally induced artifacts. Therefore, atmospheric SDE produced a falsified aroma, with a slight, cooked jam-like nuance. Consequently, the SDE sample contained, for example, some furan derivatives or hexadecanoic acid, neither of which was present in the headspace extracts in comparable concentrations. Furthermore, losses of high-volatility components have been observed using the simultaneous distillation/extraction technique. Vacuum SDE appears to be a valuable alternative that avoids thermal degradation and formation of thermal artifacts

(Maignial et al., 1992; Pollien and Chaintreau, 1997). Furthermore, the well-known browning reactions observed during the atmospheric extraction are largely suppressed under vacuum. Consequently, the concentrate obtained by vacuum SDE showed more fruity and more exotic flavor characteristics compared with the atmospheric SDE sample.

**Medium-Pressure Adsorption Chromatography on Silica Gel.** As mentioned previously, the vacuum headspace concentrate was recognized to be of particular sensory interest in comparison with other isolation techniques and was therefore elucidated in somewhat more detail. The material prepared by vacuum headspace sampling was next separated into 20 fractions using medium-pressure adsorption chromatography on silica gel with solvent mixtures of gradually increasing polarity (pentane/diethyl ether gradient). These fractions were concentrated to a small volume and subsequently analyzed by capillary gas chromatography (HRGC) and capillary gas chromatography/mass spectrometry (HRGC/MS). Some unknown aroma components were isolated by preparative capillary gas chromatography in microgram amounts to characterize their chemical structures by MS, IR, and <sup>1</sup>H NMR data. Thus, a variety of functionalities including 10 hydrocarbons, 34 alcohols, 26 ketones, 14 aldehydes, 73 esters, 6 lactones, and 15 miscellaneous compounds could be positively identified as constituents of yellow passion fruit flavor for the first time (Table 3).

Among them, unsaturated aliphatic alcohols are important contributors to the overall flavor of yellow passion fruits associated in general with green, floral, and fruity odor impressions.

(*E*)-3-Octen-1-ol has been identified in purple passion fruit juice, whereas (*Z*)-4-decenol has been characterized in buchu oil (Nijssen et al., 1996) and has been described

**Table 4. Spectroscopic Data of Novel Flavor Constituents of Yellow Passion Fruits**

compound	spectral data
(Z)4,(Z)7-decadienol-1	MS [ <i>m/z</i> (%)] 67 (100), 79 (97), 41 (81), 68 (55), 55 (55), 81 (47), 93 (33), 39 (32), 29 (30), 95 (30), <b>154 (0.4)</b>
(E)4,(Z)7-decadienol-1	MS [ <i>m/z</i> (%)] 67 (100), 79 (96), 41 (79), 55 (55), 68 (54), 81 (45), 93 (37), 39 (33), 29 (30), 95 (28), <b>154 (0.5)</b>
nerylacetol [6,10-dimethyl-(Z)-5,9-undecadien-2-ol]	MS [ <i>m/z</i> (%)] 69 (100), 41 (59), 109 (41), 67 (28), 43 (25), 45 (25), 55 (19), 153 (17), 82 (14), 81 (14), <b>196 (1)</b> ; <sup>1</sup> H NMR δ 5.142 [ttq, <i>J</i> <sub>1</sub> = 7.3 Hz, <i>J</i> <sub>2</sub> = 1.3 Hz, <i>J</i> <sub>3</sub> = 1.3 Hz, 1 H, CH=C(CH <sub>3</sub> )CH <sub>2</sub> ], 5.118 [mc, 1 H, CH=C(CH <sub>3</sub> ) <sub>2</sub> ], 3.806 (mc, 1 H, CHO), 2.16–1.98 [m, 6 H, CH <sub>2</sub> CH=C(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub> ], 1.695 (q, <i>J</i> = 1.4 Hz, 3 H, CH <sub>3</sub> ), 1.686 (mc, 3 H, CH <sub>3</sub> ), 1.613 (mc, 3 H, CH <sub>3</sub> ), 1.55–1.41 (m, 2 H, OCHCH <sub>2</sub> ), 1.363 (d, <i>J</i> = 4.6 Hz, 1 H, OH), 1.189 (d, <i>J</i> = 6.2 Hz, 3 H, CHCH <sub>3</sub> )
geranylacetol [6,10-dimethyl-(E)-5,9-undecadien-2-ol]	MS [ <i>m/z</i> (%)] 69 (100), 41 (70), 109 (68), 67 (41), 43 (35), 45 (31), 153 (27), 55 (26), 81 (15), 27 (13), <b>196 (3)</b> ; <sup>1</sup> H NMR δ 5.143 [ttq, <i>J</i> <sub>1</sub> = 7.3 Hz, <i>J</i> <sub>2</sub> = 1.3 Hz, <i>J</i> <sub>3</sub> = 1.3 Hz, 1 H, CH=C(CH <sub>3</sub> )CH <sub>2</sub> ], 5.080 [ttq, <i>J</i> <sub>1</sub> = 7.1 Hz, <i>J</i> <sub>2</sub> = 1.4 Hz, <i>J</i> <sub>3</sub> = 1.4 Hz, 1 H, CH=C(CH <sub>3</sub> ) <sub>2</sub> ], 3.810 (mc, 1 H, CHO), 2.16–1.96 [m, 6 H, CH <sub>2</sub> CH=C(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>2</sub> ], 1.680 and 1.601 [mc, 6 H, CH=C(CH <sub>3</sub> ) <sub>2</sub> ], 1.621 [mc, 3 H, CH=C(CH <sub>3</sub> )CH <sub>2</sub> ], 1.57–1.43 (m, 2 H, OCHCH <sub>2</sub> ), 1.372 (d, <i>J</i> = 4.5 Hz, 1 H, OH), 1.193 (d, <i>J</i> = 6.2 Hz, 3 H, CHCH <sub>3</sub> )
<i>p</i> -1(7),2-menthadienol-8	MS [ <i>m/z</i> (%)] 59 (100), 94 (81), 79 (59), 43 (22), 91 (17), 93 (15), 77 (13), 31 (10), 41 (9), 95 (7), <b>152 (0.5)</b>
3-[(E)-1-propenyl]-α-terpineol P1	MS [ <i>m/z</i> (%)] 161 (100), 59 (97), 93 (78), 43 (44), 41 (40), 105 (40), 176 (34), 91 (32), 121 (30), 79 (30)
3-[(E)-1-propenyl]-α-terpineol P2	MS [ <i>m/z</i> (%)] 59 (100), 93 (89), 136 (42), 43 (38), 121 (37), 108 (30), 41 (29), 107 (27), 161 (27), 79 (25), <b>194 (2)</b>
3,6-octanedione	MS [ <i>m/z</i> (%)] 113 (100), 57 (99), 29 (56), 85 (23), 95 (18), 27 (17), 43 (10), 114 (9), 55 (8), 58 (5), <b>142 (2)</b>
1-undecenone-5	MS [ <i>m/z</i> (%)] 43 (100), 113 (86), 55 (74), 83 (43), 85 (34), 98 (32), 41 (20), 57 (17), 29 (16), 27 (10), <b>168 (6)</b>
3-ethylacetophenone	MS [ <i>m/z</i> (%)] 133 (100), <b>148 (38)</b> , 105 (34), 43 (15), 79 (14), 77 (14), 134 (10), 103 (9), 51 (7), 149 (4)
cyclopentyl butanoate	MS [ <i>m/z</i> (%)] 71 (100), 43 (45), 68 (25), 89 (24), 41 (20), 69 (18), 86 (13), 67 (10), 88 (10), 27 (9)
3-methyl-δ-hexalactone	MS [ <i>m/z</i> (%)] 56 (100), 42 (83), 41 (49), 43 (47), 69 (36), 84 (30), 39 (26), 55 (22), 27 (18), 29 (14), <b>128 (4)</b>
(E)-5-tangerinol [6,10-dimethyl-(E)-5,9-undecadienyl 2-acetate]	MS [ <i>m/z</i> (%)] 109 (100), 69 (81), 43 (66), 41 (47), 67 (33), 68 (25), 135 (15), 110 (15), 81 (14), 55 (13); <sup>1</sup> H NMR δ 5.13–5.06 [m, 2 H, CH=C(CH <sub>3</sub> )CH <sub>2</sub> and CH=C(CH <sub>3</sub> ) <sub>2</sub> ], 4.883 (mc, 1 H, CHO), 2.10–1.96 (m, 6 H, CH <sub>2</sub> CH=CCH <sub>2</sub> CH <sub>2</sub> ), 2.029 (s, 3 H, O=CCH <sub>3</sub> ), 1.680 (mc, 3 H, CH <sub>3</sub> ), 1.600 (mc, 3 H, CH <sub>3</sub> ), 1.585 (mc, 3 H, CH <sub>3</sub> ), 1.68–1.56 (m, 1 H, CH/O), 1.499 (dddd, <i>J</i> <sub>1</sub> = 13.7 Hz, <i>J</i> <sub>2</sub> = 8.5 Hz, <i>J</i> <sub>3</sub> = 7.3 Hz, <i>J</i> <sub>4</sub> = 5.3 Hz, 1 H, CH/O), 1.213 (d, <i>J</i> = 6.3 Hz, CHCH <sub>3</sub> )
(Z)-5-tangerinol [6,10-dimethyl-(Z)-5,9-undecadienyl 2-acetate]	MS [ <i>m/z</i> (%)] 69 (100), 109 (73), 43 (58), 41 (45), 68 (25), 67 (25), 110 (16), 93 (16), 178 (15), 135 (15), <b>238 (0.2)</b> ; <sup>1</sup> H NMR δ 5.14–5.06 [m, 2 H, CH=C(CH <sub>3</sub> )CH <sub>2</sub> and CH=C(CH <sub>3</sub> ) <sub>2</sub> ], 4.884 (mc, 1 H, CHO), 2.09–1.96 (m, 6 H, CH <sub>2</sub> CH=CCH <sub>2</sub> CH <sub>2</sub> ), 2.024 (s, 3 H, O=CCH <sub>3</sub> ), 1.67–1.70 (m, 6 H, 2 CH <sub>3</sub> ), 1.609 (mc, 3 H, CH <sub>3</sub> ), 1.620 (dddd, <i>J</i> <sub>1</sub> = 13.7 Hz, <i>J</i> <sub>2</sub> = 8.3 Hz, <i>J</i> <sub>3</sub> = 7.4 Hz, <i>J</i> <sub>4</sub> = 6.7 Hz, 1 H, CH/O), 1.490 (dddd, <i>J</i> <sub>1</sub> = 13.7 Hz, <i>J</i> <sub>2</sub> = 9.1 Hz, <i>J</i> <sub>3</sub> = 6.8 Hz, <i>J</i> <sub>4</sub> = 5.3 Hz, 1 H, CH/O), 1.208 (d, <i>J</i> = 6.3 Hz, CHCH <sub>3</sub> )

as an important aroma component of yali (Kawabata et al., 1994). (Z)-4-Decenol has a fruity melon-like and cucumber-like character with herbaceous, waxy, peely, and earthy aspects. 6-Methyl-5-hepten-2-ol (formed by decomposition of carotenoids) was detected in numerous fruits and spices (Nijssen et al., 1996) and possesses flowery, green, mushroom-like, and honey-like flavor notes. (Z)-5-Octen-1-ol has been found in apples and bananas (Nijssen et al., 1996) and was organoleptically evaluated to possess green, juicy, fruity, sweet, and fresh flavor notes including melon-like, pear-like, mango-like, and cucumber-like odor impressions. Its taste threshold value in water was found to be 2–3 ppb. For this reason, (Z)-5-octen-1-ol is supposed to be of interest to yellow passion fruit flavor chemistry.

In general, aldehydes are common to fruit flavors and are believed to play an important role in many fruits. For this reason it is a surprise that only very few aldehydes have been identified in yellow passion fruits thus far. Our GC/MS analysis enabled 14 aldehydes to be unambiguously identified. Among them, (E)-2-hexenal (green, fruity, juicy, sweet, leafy, apple-like, banana-like), (Z)-3-hexenal (green, herbaceous, leafy, sweet, bean-like, apple-like, tomato-like, passion fruit-like), and (E)-3-hexenal (herbaceous, fatty, aldehyde-like, tomato-like) as well as neral, geranial, β-cyclocitral, and phenylacetaldehyde contribute mainly green, floral, and fruity aroma impressions to yellow passion fruit flavor.

As shown in Table 3 the major part of volatiles identified for the first time as constituents of yellow passion fruit flavor consisted of esters. Qualitatively, the newly identified ester compounds were mainly characterized by a number of various acetates, butanoates, and hexanoates. Both alkenyl alkanooates and alkyl alkenoates were detected. The dominant alkenyl groups are hexenyl, heptenyl, and octenyl with double bonds in different positions. Furthermore, hydroxyalkanoates and esters of the terpene alcohols geraniol, nerol, and citronellol have also been identified in yellow passion fruit flavor for the first time.

(E)-α-Ionone and (E)-β-damascone have been shown to be present in the vacuum headspace concentrate of yellow passion fruits in trace quantities. Since the odor threshold level of both ketones is very low, it is likely that both intensely odorous ketones contribute to the unique flavor of the yellow variety.

Moreover, compounds not previously reported in yellow passion fruit flavor include *cis*- and *trans*-γ-jasmin lactone, δ-jasmin lactone, *trans*-marmelolactone, cycloionone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (mesifuran), and 4-acetoxy-2,5-dimethyl-3(2*H*)-furanone. Most of these compounds had previously been found in other natural products. For example, γ- and δ-jasmin lactone had been identified in various fruits by our research group (Werkhoff et al., 1993b). Both the character and

strength of these constituents make them important components in the overall passion fruit flavor profile.  $\gamma$ -Jasmin lactone provides sweet, creamy, flowery, and tropical fruit-like notes and produces peach-like, apricot-like, and coconut-like flavors.  $\delta$ -Jasmin lactone is mainly associated with creamy, milky, buttery, fruity, sweet, and floral odor impressions and is additionally characterized as peach-like, apricot-like, coconut-like, and cumarin-like. Marmelolactone had been first reported in quince by Tsuneya et al. (1980) and later in the bound fraction of peach flavor by Krammer et al. (1991). Marmelolactone possesses a strongly fruity, floral, and quince-like aroma and is also associated with creamy, buttery, and coconut-like odor notes.

This paper also reports the first identification of cycloionone in yellow passion fruit flavor. Cycloionone was detected for the first time in cognac (Nijssen et al., 1996). This component appears to be a potent flavoring with fruity, sweet, floral, and woody aroma characteristics.

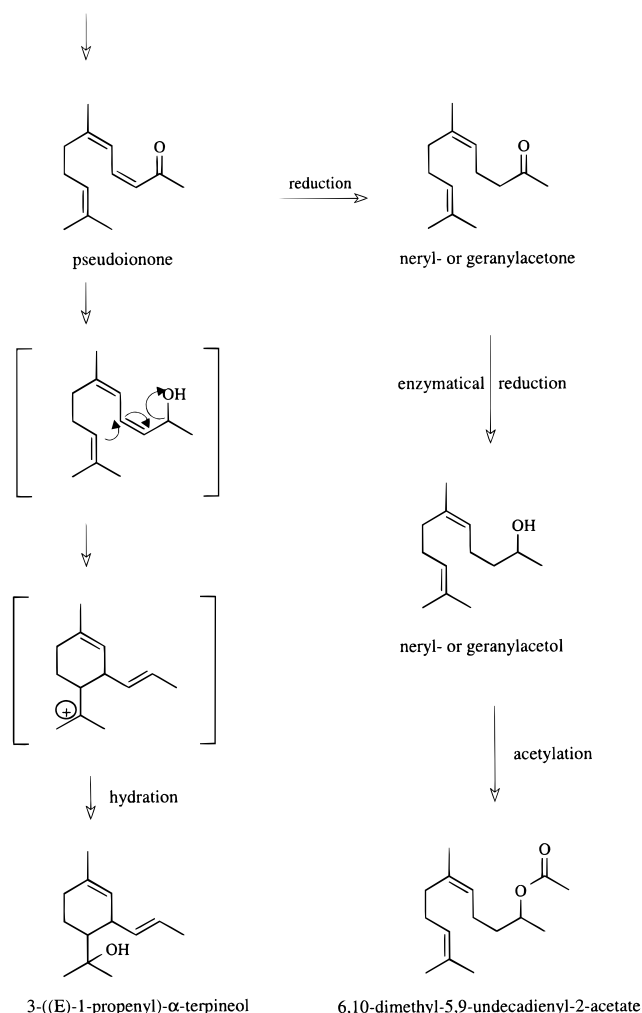
The organoleptic properties of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol) and 4-methoxy-2,5-dimethyl-3(2*H*)-furanone (mesifuran) as well as their importance for fruit flavors are well documented in the literature. Both compounds probably influence the aroma profile of passion fruit flavor due to their low odor thresholds. In addition, 4-acetoxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol acetate) could be identified in yellow passion fruit flavor. To our knowledge, Furaneol acetate has been positively identified in a solvent extract of ripe wild strawberries (Polesello et al., 1993). Further occurrence was recently mentioned in the odorants of two meat-like flavorings. Furaneol acetate was described by the authors as savory and caramel-like and was found to contribute significantly to the savory aroma (Blank et al., 1994). Furaneol acetate was synthesized in our laboratory by acetylation of the corresponding alcohol with acetic anhydride in pyridine. Furaneol acetate produces a typical roasty, caramel-like, chocolate-like, and vanilla-like sensory impression. In particular, it possesses a characteristic creamy, milky, and malty note but is also reminiscent of strawberries.

Most of the above-mentioned components had previously been found in other natural products. Fourteen constituents of yellow passion fruits, however, have not been reported in the literature in relation to flavor chemistry (Nijssen et al., 1996). They are new natural components. The spectroscopic data of all these novel flavor components are compiled in Table 4. The identification of these compounds was accomplished by comparing their mass spectra and GC retention indices with those of authentic compounds available in our chromatographic and spectroscopic libraries.

Among aliphatic alcohols, we have identified four unsaturated C<sub>10</sub> components in yellow passion fruit flavor for the first time. Unsaturated alcohols have relatively low threshold values, and therefore compounds such as 3-decenol-1 or 4,7-decadienol-1, which have a vegetable-like odor with a green, leafy note, may play an important part in the fruity characteristics of passion fruit flavor. (*E*)- and (*Z*)-3-decenol are not listed in the TNO compilation thus far. However, for the sake of completeness, it should be mentioned that (*E*)- and (*Z*)-3-decenol have already been identified as volatile constituents of Chinese pear yali (Kawabata et al., 1994) and that one isomer of 3-decenol has been characterized

### Scheme 1. Postulated Mechanism of the Formation of Neryl-/Geranylacetol, (*Z*)-5- and (*E*)-5-Tangerinol as well as 3-[(*E*)-1-Propenyl]- $\alpha$ -terpineol

Biodegradation of carbocyclic isoprenoids  
(Wahlberg and Enzell, 1987; Croteau and Karp, 1991)



in soft mold-ripened cheese (Adda et al., 1989) as well as in French bread dough (Frasse et al., 1993).

It is worth mentioning that nerylacetol and geranylacetol and the corresponding acetates could not be found in the literature as having been identified in food flavors thus far. However, geranylacetol has already been reported in the literature as a carotenoid metabolite in the headspace of tea roses (Kaiser, 1988; Eugster and Märki-Fischer, 1991). Geranylacetol was enriched from yellow passion fruit flavor by preparative capillary gas chromatography in microgram amounts and was structurally elucidated by NMR, mass, and IR spectra. To confirm their structures, neryl- and geranylacetol as well as the corresponding acetates were synthesized according to procedures described in the literature by reducing geranylacetone with NaBH<sub>4</sub> and subsequent esterification of the corresponding alcohols by acetic anhydride. <sup>1</sup>H NMR and MS data of nerylacetol, geranylacetol and both 6,10-dimethyl-5,9-undecadienyl 2-acetates [(*E*)-5 and (*Z*)-5-tangerinols] are summarized in Table 4. The possible formation pathway of the newly identified components is illustrated in Scheme 1. Both alcohols and esters may arise by enzymatical reduction of neryl- and geranylacetone—which are well-known constituents of numerous natural flavors or essential



**Table 5. Volatile Sulfur-Containing Constituents Identified in VHS Concentrate of Yellow Passion Fruits**

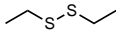
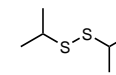
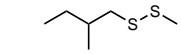
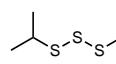

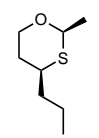
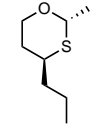

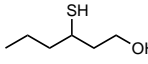
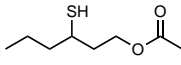
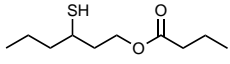
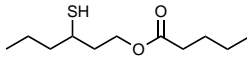
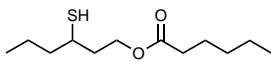
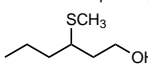
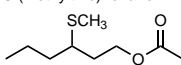
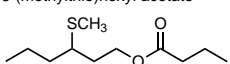
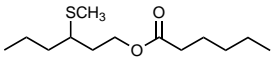
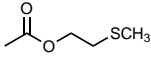
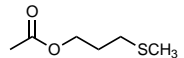
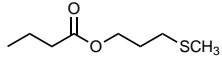
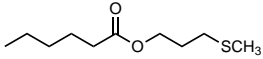
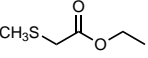
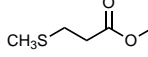
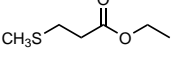
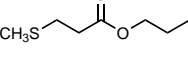
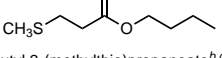
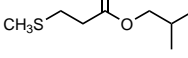
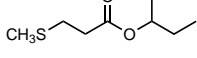
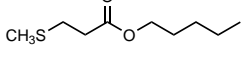
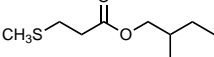
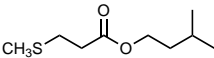
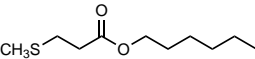
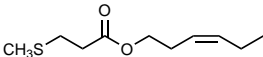
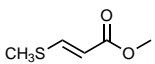
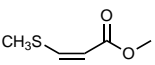
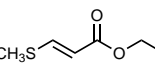
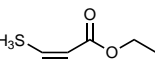
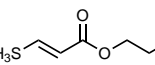
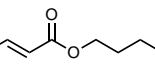
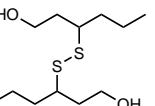
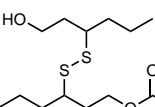
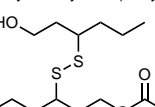
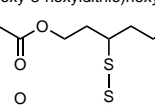
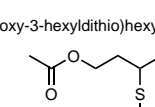
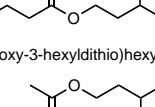
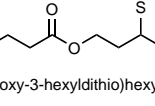
compound/chemical structure <sup>a</sup>	RI (DB-Wax)	previously reported in natural products	odor description <sup>d</sup>	MS data, m/z (%)
 diethyl disulfide <sup>b</sup>	1209	Nijssen et al. (1996) Weenen et al. (1996) Wong and Tie (1995) Park et al. (1994)	sulfury, rubbery, carbide-like, sweet	MS ref <sup>e</sup>
 diisopropyl disulfide <sup>b</sup>	1252	Nijssen et al. (1996) Werkhoff et al. (1993a) Yukawa et al. (1994)	sulfury, oniony, roasted onion, tropical fruits, durian-like	MS ref <sup>e</sup>
 methyl 2-methylbutyl disulfide <sup>b</sup>	1364	Werkhoff et al. (1993a)	sulfury, rubbery, onion-like	43 (100), <b>150 (38)</b> , 71 (28), 80 (22), 41 (22), 29 (18), 55 (11), 27 (9), 45 (9), 39 (8)
 diisopropyl trisulfide <sup>b</sup>	1516	Nijssen et al. (1996)	oniony, leek-like, durian-like, sulfury, roasted onion, metallic	MS ref <sup>e</sup>
 1,1-bis(methylthio)-2-methylpropane <sup>b,c</sup>	1415		roasty, coffee-like, shiitake, oniony, metallic	55 (100), 103 (96), <b>150 (48)</b> , 107 (25), 45 (15), 61 (13), 39 (8), 29 (7), 47 (7), 41 (7)
 <i>cis</i> -2-methyl-4- <i>n</i> -propyl-1,3-oxathiane	1514	Nijssen et al. (1996) Weber et al. (1995)	fruity, tropical fruit note, green, passion fruits	Winter et al. (1976)
 <i>trans</i> -2-methyl-4- <i>n</i> -propyl-1,3-oxathiane	1549	Nijssen et al. (1996) Weber et al. (1995)	sulfury, tropical fruit note, herbaceous, mango, passion fruits, guave	Winter et al. (1976)
 4-methyl-5-vinylthiazole	1512	Nijssen et al. (1996) Benn and Peppard (1996) Fischer et al. (1995) Wong and Khoo (1993) Krammer et al. (1997)	fatty, roasty, nutty, roasted peanut, bread crust-like, popcorn, cocoa	MS ref <sup>e</sup>
 3-mercaptohexanol	1836	Engel and Tressl (1991) Weber et al. (1994)	juicy, tropical fruits, grapefruit, black currant, bucco, mango, guave	Engel and Tressl (1991)
 3-mercaptohexyl acetate	1697	Engel and Tressl (1991) Weber et al. (1995) Tominaga et al. (1996)	grapefruit, black currant, bucco, mango, passion fruit, guave	Engel and Tressl (1991)
 3-mercaptohexyl butanoate	1860	Engel and Tressl (1991) Weber et al. (1995)	fruity, grapefruit, black currant, bucco, tropical fruits, mango	Engel and Tressl (1991)
 3-mercaptohexyl pentanoate <sup>b,c</sup>	1955		tropical fruit, passion fruit, bucco	see Figure 3
 3-mercaptohexyl hexanoate	2046	Engel and Tressl (1991)	grapefruit, black currant, bucco, tropical fruits, passion fruits, guave	Engel and Tressl (1991)
 3-(methylthio)hexanol	1878	Nijssen et al. (1996) Engel and Tressl (1991)	fruity, juicy, melon-like, black currant, passion fruits, guave	Winter et al. (1976)
 3-(methylthio)hexyl acetate	1766	Engel and Tressl (1991)	fatty, fruity, sweet, mango, passion fruits, guave, durian-like	Engel and Tressl (1991)
 3-(methylthio)hexyl butanoate	1918	Engel and Tressl (1991)	sulfury, fruity, caramel-like, rhubarb, carbide-like, caraway-like	Engel and Tressl (1991)

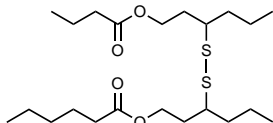
Table 5 (Continued)

compound/chemical structure <sup>a</sup>	RI (DB-Wax)	previously reported in natural products	odor description <sup>d</sup>	MS data, m/z (%)
 3-(methylthio)hexyl hexanoate	2115	Engel and Tressl (1991)	faintly fruity, green, tropical fruit note	Engel and Tressl (1991)
 2-(methylthio)ethyl acetate <sup>b</sup>	1487	Nijssen et al. (1996) Wang et al. (1996) Wyllie et al. (1994)	sulfury, rotten, creamy, cauliflower, kohlrabi	Homatidou et al. (1989)
 3-(methylthio)propyl acetate <sup>b</sup> (methionyl acetate)	1609	Nijssen et al. (1996) Anocibar and Bertrand (1995) Taniguchi et al. (1995) Wyllie et al. (1994) Kawabata et al. (1994)	herbaceous, mushroom-like, cabbage, asparagus, potato	MS ref <sup>e</sup>
 3-(methylthio)propyl butanoate <sup>b,c</sup> (methionyl butanoate)	1769		sulfury, cheese-like, mushroom-like	88 (100), 73 (66), 43 (46), 71 (34), 41 (32), 61 (27), 75 (14), 27 (13), 89 (11), 46 (8), <b>176 (7)</b>
 3-(methylthio)propyl hexanoate <sup>b,c</sup> (methionyl hexanoate)	1973		tropical fruit note, methional-like, canned pineapple	88 (100), 73 (36), 43 (21), 41 (20), 61 (15), 89 (13), <b>204 (11)</b> , 99 (9), 71 (9), 75 (7)
 ethyl (methylthio)acetate <sup>b</sup>	1439	Nijssen et al. (1996) Wong et al. (1995) Kawabata et al. (1994) Wong and Ong (1993) Umano et al. (1992)	fruity, sweet, juicy, radish-like	MS ref <sup>e</sup>
 methyl 3-(methylthio)propanoate <sup>b</sup>	1525	Nijssen et al. (1996) Farine et al. (1996) Wong and Wong (1995) Wang et al. (1996) Wyllie et al. (1994)	sulfury, tropical fruit note, radish-like, cabbage	MS ref <sup>e</sup>
 ethyl 3-(methylthio)propanoate	1560	Nijssen et al. (1996) Wyllie et al. (1994)	fruity, herbaceous, sulfury, milk-like, cheese-like	MS ref <sup>e</sup>
 propyl 3-(methylthio)propanoate <sup>b,c</sup>	1649		vegetable-like, mushroom-like, cabbage	74 (100), 61 (73), 75 (55), <b>162 (51)</b> , 27 (40), 41 (36), 103 (33), 43 (27), 47 (25), 45 (21)
 butyl 3-(methylthio)propanoate <sup>b,c</sup>	1748		sulfury, rubbery, fruity, mushroom-like, kohlrabi, radish-like	74 (100), 61 (84), 120 (53), 75 (50), <b>176 (44)</b> , 41 (42), 29 (34), 103 (30), 27 (23), 119 (21)
 isobutyl 3-(methylthio)propanoate <sup>b,c</sup>	1681		rubbery, fruity, mushroom-like	61 (100), 74 (72), 120 (61), 57 (56), 75 (48), 41 (48), 103 (47), 29 (34), <b>176 (25)</b> , 119 (22)
 sec-butyl 3-(methylthio)propanoate <sup>b,c</sup>	1647		sulfury, not fruity, weak cabbage character	61 (100), 103 (68), 74 (53), 120 (52), 75 (51), 41 (36), <b>176 (34)</b> , 29 (26), 57 (19), 27 (17)
 pentyl 3-(methylthio)propanoate <sup>b,c</sup>	1858		fruity, somewhat pineapple-like, milk-like, green, radish-like	74 (100), 61 (83), 120 (72), 43 (69), 41 (39), 75 (38), <b>190 (29)</b> , 55 (29), 103 (26), 27 (23)
 2-methylbutyl 3-(methylthio)propanoate <sup>b,c</sup>	1792		faintly fruity	120 (100), 61 (95), 43 (94), 74 (60), 71 (53), 75 (43), <b>190 (40)</b> , 103 (40), 55 (30), 119 (29)
 3-methylbutyl 3-(methylthio)propanoate <sup>b,c</sup>	1792		fruity, estery aroma, pineapple-like	43 (100), 120 (72), 61 (62), 74 (47), 103 (44), 71 (41), 70 (39), 75 (32), 55 (31), <b>190 (28)</b>
 hexyl 3-(methylthio)propanoate <sup>b,c</sup>	1958		fruity, tropical fruits, mango, passion fruits, guave, geranium-like	120 (100), 43 (98), 61 (78), 74 (74), 41 (41), <b>204 (40)</b> , 75 (39), 103 (33), 27 (29), 119 (25)
 (Z)-3-hexenyl 3-(methylthio)propanoate <sup>b,c</sup>	2000		sulfury, sweet, green, carbide-like, leek-like	82 (100), 67 (69), 61 (65), 55 (50), 41 (44), 75 (36), 27 (31), 120 (19), 103 (16), <b>202 (16)</b>

**Table 5 (Continued)**

compound/chemical structure <sup>a</sup>	RI (DB-Wax)	previously reported in natural products	odor description <sup>d</sup>	MS data, m/z (%)
 methyl 3-(methylthio)-(E)-2-propenoate <sup>b</sup>	1675	Nijssen et al. (1996)	cheesy, yogurt-like, pineapple-like, weak caramel character	Takeoka et al. (1991)
 methyl 3-(methylthio)-(Z)-2-propenoate <sup>b</sup>	1786	Nijssen et al. (1996)	sulfury, faintly cabbage-like, cauliflower	Takeoka et al. (1991)
 ethyl 3-(methylthio)-(E)-2-propenoate <sup>b</sup>	1733	Nijssen et al. (1996) Kawabata et al. (1994) Takeoka et al. (1992)	sulfury, sweet, metallic	Takeoka et al. (1991)
 ethyl 3-(methylthio)-(Z)-2-propenoate <sup>b</sup>	1837	Kawabata et al. (1994) Nijssen et al. (1996)	not fruity or pleasant, rotten, sour	Takeoka et al. (1991)
 propyl 3-(methylthio)-(E)-2-propenoate <sup>b,c</sup>	1827		faintly fruity, sweet	101 (100), 103 (42), <b>160 (25)</b> , 118 (25), 73 (24), 27 (21), 45 (20), 100 (15), 43 (15), 58 (14)
 hexyl 3-(methylthio)-(E)-2-propenoate <sup>b,c</sup>	2154		sulfury, fruity, green, estery, rotten, faintly passion fruit-like	101 (100), 118 (67), 103 (41), 43 (33), 41 (19), 27 (19), 73 (18), 100 (17), 29 (17), 45 (15), <b>202 (15)</b>
 3-(1-hydroxy-3-hexyldithio)hexano <sup>b,c</sup>	> 2600			55 (100), 83 (96), 148 (30), 41 (25), <b>266 (20)</b> , 101 (14), 115 (13), 43 (11), 31 (9), 29 (7)
 3-(1-hydroxy-3-hexyldithio)hexyl acetate <sup>b,c</sup>	> 2600			see Figure 5
 3-(1-hydroxy-3-hexyldithio)hexyl butanoate <sup>b,c</sup>	> 2600			83 (100), 55 (69), 71 (31), 43 (28), 148 (25), 171 (22), 41 (21), 89 (12), 115 (9), <b>336 (6)</b>
 3-(1-acetoxy-3-hexyldithio)hexyl acetate <sup>b,c</sup>	> 2600			83 (100), 55 (52), 43 (42), 143 (30), 148 (11), 41 (9), 32 (8), 84 (7), <b>350 (6)</b> , 61 (4)
 3-(1-acetoxy-3-hexyldithio)hexyl butanoate <sup>b,c</sup>	> 2600			83 (100), 55 (57), 71 (25), 43 (22), 171 (13), 41 (13), 143 (11), 89 (10), 148 (9), <b>378 (0.5)</b>
 3-(1-acetoxy-3-hexyldithio)hexyl hexanoate <sup>b,c</sup>	> 2600			83 (100), 55 (43), 43 (39), 99 (21), 143 (18), 199 (17), 148 (14), 41 (14), 117 (13), <b>406 (2)</b>
 3-(1-butyryloxy-3-hexyldithio)hexyl butanoate <sup>b,c</sup>	> 2600			83 (100), 71 (57), 55 (48), 43 (42), 171 (26), 41 (21), 89 (19), 148 (10), 27 (9), <b>406 (1)</b>

**Table 5 (Continued)**

compound/chemical structure <sup>a</sup>	RI (DB-Wax)	previously reported in natural products	odor description <sup>d</sup>	MS data, m/z (%)
	> 2600			83 (100), 55 (44), 43 (34), 71 (33), 99 (17), 171 (17), 41 (17), 199 (15), 117 (11), <b>434 (1)</b>

3-(1-butylthioxy-3-hexyldithio)hexyl hexanoate<sup>b,c</sup>

<sup>a</sup> Identifications based on mass spectral data and linear retention indices on DB-Wax. <sup>b</sup> Identified in yellow passion fruit flavor for the first time. <sup>c</sup> Not previously reported in nature. <sup>d</sup> The descriptive analysis was done with an aqueous solution of each compound at a level of 1–10 ppm. <sup>e</sup> *Registry of Mass Spectral Data*; Wiley: New York.

oils derived from carotenoids via pseudoionone (Wahlberg and Enzell, 1987; Croteau and Karp, 1991)—and the following transformation into the corresponding acetates by means of transacylases.

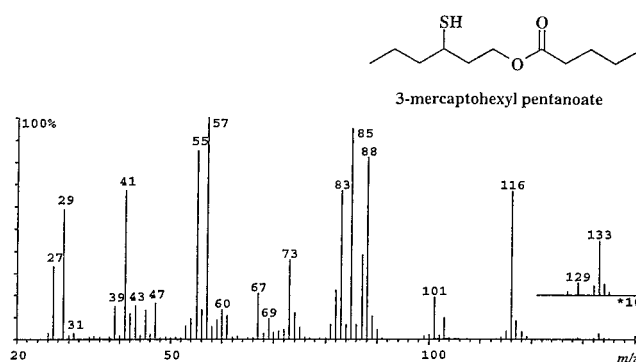
Neryl/geranylacetol and 6,10-dimethyl-5,9-undecadienyl 2-acetates obtained by synthesis were purified by preparative HPLC prior to sensory evaluation. Geranylacetol was shown to have a sweet, fruity, and floral flavor note and was additionally associated with honey-like and peach-like odor impressions, while the organoleptic properties of nerylacetol are characterized as fruity, sweet, floral, mango-like, and rose-like. (*Z*)-5-Tangerinol provides sweet, floral, and woody notes and is mainly associated with a citrus-like odor impression. (*E*)-5-Tangerinol also matches well with citrus oils and exhibits floral, fresh, and woody notes with a slightly soapy accent.

Both isomers of 3-[(*E*)-1-propenyl]- $\alpha$ -terpineol were also isolated from the fruit flavor concentrate by preparative GC and characterized by spectroscopic investigations. 3-[(*E*)-1-propenyl]- $\alpha$ -terpineol could be formed from pseudoionone by ring closure and subsequent hydration of the intermediate shown in Scheme 1.

In addition, 3,6-octanedione, 1-undecenone-5,3-ethylacetophenone, cyclopentyl butanoate, and 3-methyl- $\delta$ -hexalactone have not previously been reported in fruits or any other food flavor. Only *p*-1(7),2-menthadienol-8 has been mentioned in the literature arising from the acid-catalyzed degradation of citral (Kimura et al., 1983; Peacock and Kuneman, 1985). 3,6-Octanedione possessing a slightly lovage-like character was synthesized in our laboratory by the reaction of succinic acid dichloride with methylmeldrum acid and subsequent acidic decarboxylation.

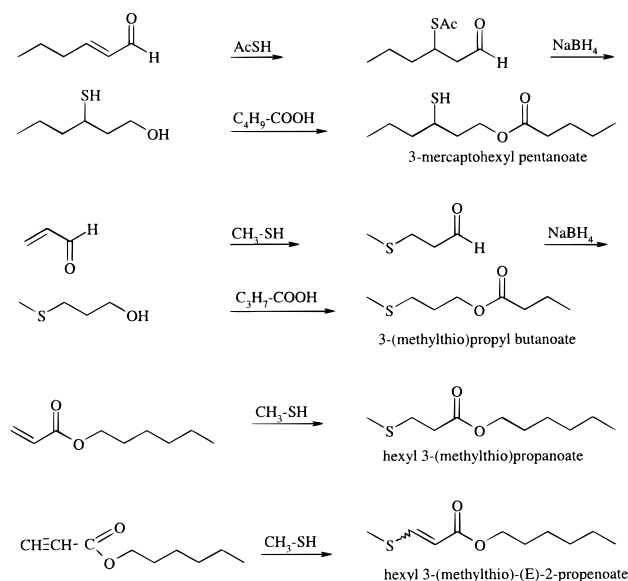
**Characterization of Sulfur-Containing Compounds.** Analysis of the passion fruit VHS aroma fractions using flame photometric detection revealed numerous sulfur-containing components at very low concentrations often masked in the FID trace. Subsequently, these minor sulfur volatiles were enriched by preparative MDGC and again analyzed by FPD as well as by GC/MS. More than 100 sulfur volatiles were detected using the sulfur selective mode. However, in total, only 47 sulfur-containing flavor components were identified in yellow passion fruits (Table 5). Of these compounds, 35 have not previously been reported in yellow passion fruits and 23 have never been described in food flavors at all.

Sulfur-containing molecules are organoleptically by far the most interesting components in yellow passion fruits. Recent work by Engel and Tressl (1991) revealed the sensory importance of 3-mercaptohexanol and 3-(methylthio)hexanol as well as the acetates, butanoates, and hexanoates of these sulfur-substituted alcohols for

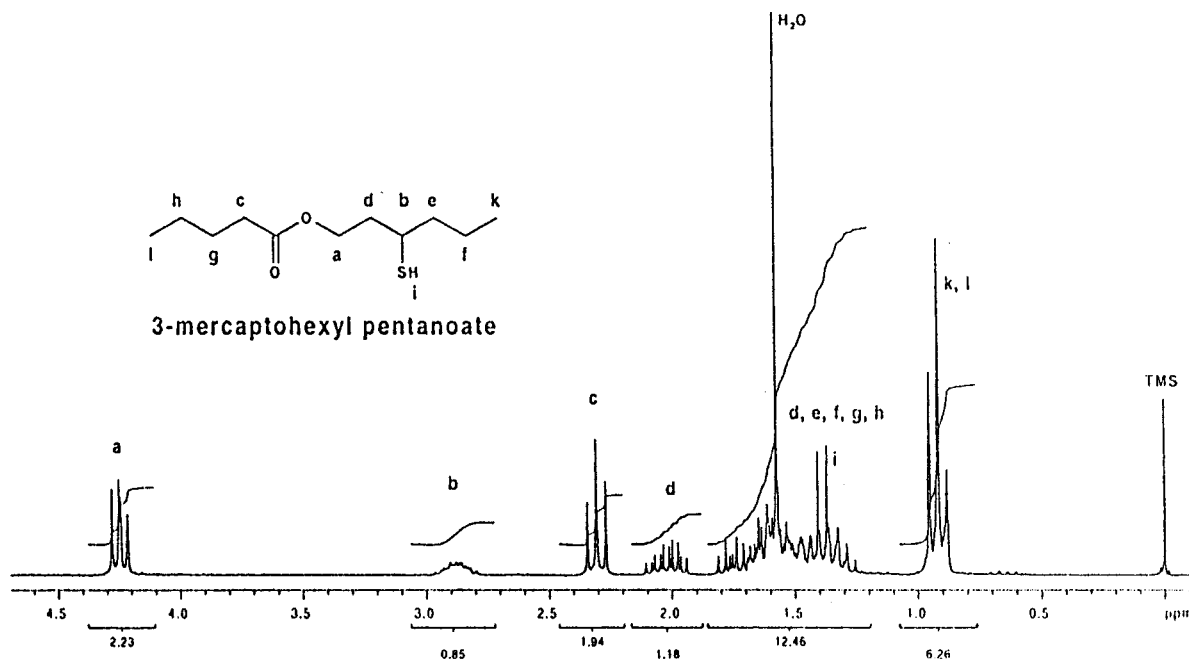


**Figure 3.** Mass spectrum of 3-mercaptohexyl pentanoate.

**Scheme 2. General Synthetic Routes for the Preparation of Sulfur-Containing Passion Fruit Flavor Components**



the aroma of yellow passion fruits. Due to their high odor values and their olfactive profiles, these sulfur substances are expected to be key ingredients of the yellow variety (Table 5). They are especially distinguished by their high intensity and long-lasting character. In addition to 3-mercaptohexyl- and 3-(methylthio)hexyl acetates, butanoates, and hexanoates, we were able to detect 3-mercaptohexyl pentanoate in yellow passion fruit flavor. This component was synthesized from 3-mercaptohexanol (prepared by addition of thioacetic acid to *trans*-2-hexenal and subsequent reduction to alcohol with NaBH<sub>4</sub>) and pentanoic acid (Scheme 2). The mass spectrum of 3-mercaptohexyl pentanoate is presented in Figure 3. Its structure was



**Figure 4.**  $^1\text{H}$  NMR spectrum of 3-mercaptohexyl pentanoate.

further confirmed by means of  $^1\text{H}$  NMR spectroscopy (Figure 4). 3-Mercaptohexyl pentanoate is tropical fruit-like, possesses a sulfury passion fruit character, and has a bucco aroma note. The taste threshold in water is in the range of 10 ppb.

3-(Methylthio)propyl acetate (methionyl acetate) possesses herbaceous odor impressions and a typical vegetable-like character and has already been described in the literature in numerous flavor systems (Nijssen et al., 1996). In addition, methionyl butanoate and methionyl hexanoate have been identified in the vacuum headspace extract of the yellow variety. None of these compounds had been reported in food flavor before. Both substances were synthesized by the reaction of methionol (obtained by reaction of acrolein and methanethiol and subsequent reduction with  $\text{NaBH}_4$ ) and butanoic acid or hexanoic acid (Scheme 2) to elucidate their sensorial properties (Table 5) and to verify their chemical structures. The taste thresholds of methionyl butanoate and methionyl hexanoate in water were determined to be 10–20 and 500 ppb, respectively.

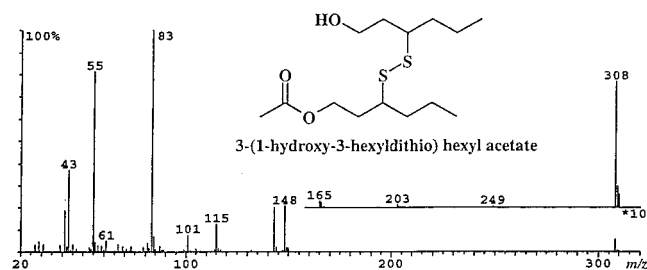
Among the sulfur compounds, methyl and ethyl 3-(methylthio)propanoate dominated in the vacuum headspace extract of yellow passion fruits. Both components have previously been found in several food systems, for example, in pineapple flavor (Nijssen et al., 1996). Furthermore, the ethyl ester has already been found in passion fruits. The taste threshold values of methyl and ethyl 3-(methylthio)propanoate are approximately 150 and 300 ppb in water, respectively. Our work revealed the occurrence of further 3-(methylthio) esters of propanoic acid in the yellow passion fruit variety. The spectrum of esters comprised the whole series of propyl, butyl, isobutyl, *sec*-butyl, pentyl, 2-methylbutyl, 3-methylbutyl, hexyl, and (*Z*)-3-hexenyl esters of 3-(methylthio)propanoic acid. These flavor constituents have not previously been reported in nature. To confirm their structures, we have synthesized these compounds by addition of methanethiol to the corresponding acrylic acid alkyl esters (Scheme 2).

The aroma properties of the 3-(methylthio) esters of propanoic acid are not very interesting with the excep-

tion of the hexyl derivative. In general, the 3-(methylthio)propanoic acid esters have a sulfury, vegetable-like odor, and only hexyl 3-(methylthio)propanoate with its fruity and geranium-like odor note may contribute to the overall olfactory impression of the analyzed fruits. Moreover, threshold values of 3-(methylthio) esters of propanoic acid in water are significantly higher compared with other sulfur compounds. For example, butyl 3-(methylthio)propanoate has a taste threshold in water of 1–2 ppm.

According to the TNO compilation, methyl and ethyl esters of 3-(methylthio)-2-propenoic acid have been identified in pineapple flavor. In addition, ethyl 3-(methylthio)-(*E*)-2-propenoate and the corresponding *Z* isomer were found in both peels and pulps of Chinese pear yali (Kawabata et al., 1994). Six derivatives of 3-(methylthio)-2-propenoic acid were identified among the volatile sulfur components in yellow passion fruits, and two of them, the propyl and hexyl ester, are new compounds. The structure of hexyl 3-(methylthio)-(*E*)-2-propenoate was confirmed by synthesis [*E* configuration assigned by spectroscopic methods]. Alkyl 3-(methylthio)-2-propenoates were obtained by addition of methanethiol to the corresponding propargylic acid alkyl esters (Scheme 2). MS data of hexyl 3-(methylthio)-(*E*)-2-propenoate are given in Table 5. The olfactory evaluation of hexyl 3-(methylthio)-(*E*)-2-propenoate is also listed in Table 5 together with the organoleptic properties of all other sulfur-containing components identified in yellow passion fruit flavor.

Hypothetical biogenetic pathways for the formation of sulfur esters have been discussed in the literature. According to these hypotheses 3-(methylthio)propanol, 3-(methylthio)propanoic acid, and 3-(methylthio)-(*E*)-2-propenoic acid are primarily important intermediates for the formation of sulfur-containing volatiles in fruits. The amino acid methionine is described as a common precursor of 3-(methylthio)propanol and 3-(methylthio)propanoic acid formed by a series of biochemical transformations during the process of ripening in the fruit (Wyllie and Leach, 1990, 1992; Homatidou et al., 1992; Wyllie et al., 1994, 1995). Furthermore, the presence



**Figure 5.** Mass spectrum of 3-(1-hydroxy-3-hexyldithio)hexyl acetate.

of esters of 3-(methylthio)propanoic acid and 3-(methylthio)-(*E*)-2-propenoic acid in fruit flavors and their biogenetic formation was recently discussed by Takeoka et al. (1991).

Finally, we report several newly identified disulfides which are not common in food volatiles and are described here for the first time. Their structures are presented in Table 5: symmetrical and asymmetrical disulfides. They may be formed by dimerization or oxidation of several thiols, namely 3-mercaptohexanol and 3-mercaptohexyl acetate, butanoate, and hexanoate. Due to our careful, mild, and gentle isolation procedure we do not assume that our mercaptans present in the fresh fruit were oxidized during extraction and concentration. However, in many cases, it is rather difficult to distinguish genuine flavorants from artifacts formed by sampling techniques or subsequent chromatographic analysis, and as a result the origin of certain flavor substances remains unclear. All unknown dimeric sulfur components were isolated by preparative capillary GC. Thus, the structures of the new sulfur compounds were not only deduced from their MS fragmentations but additionally established by NMR data, as there was enough material to isolate them for NMR experiments. Due to their high molecular weights newly identified disulfides do not possess strong aromas and consequently do not contribute to the overall olfactory impression of yellow passion fruit flavor. As an example, Figure 5 presents the mass spectrum of 3-(1-hydroxy-3-hexyldithio)hexyl acetate. Further mass spectral data of newly identified sulfur compounds in yellow passion fruit flavor are summarized in Table 5.

In conclusion, the flavor of yellow passion fruits is determined by numerous volatile components belonging to different classes of organic compounds. According to our investigations, however, the attractive tropical flavor note of ripe yellow passion fruits is mainly attributed to the presence of trace levels of sulfur volatiles in combination with other compounds contributing fruity, estery, floral, and green aroma impressions.

**Enantioselective MDGC.** Optically active flavor compounds have been gathering importance over the past decade. Many of the flavor components of yellow passion fruits are chiral, and thus they can be present in isolated flavor concentrates in one or two enantiomeric forms and in various proportions. It is well-known from the literature that the sensory properties of aroma compounds can depend on their configurations; that is, enantiomers may not only differ in their odor quality but they can also show tremendous differences in their odor thresholds (Boelens et al., 1993; Koppenhöfer et al., 1994; Bernreuther et al., 1997). One stereoisomer may be many times more potent than its mirror image. In natural systems one enantiomer may predominate because enzymes are very often stereospe-

cific. As a consequence, the analysis of chiral compounds in natural flavors has grown in importance and focuses on understanding the sensory impact of individual enantiomers. Nowadays, cyclodextrin stationary phase-coated capillary columns are used extensively in chiral GC analysis (König, 1992; Schreier et al., 1995). Multidimensional gas chromatography is a very important tool and has been demonstrated as a successful method for the direct stereodifferentiation of chiral volatiles, since it affords the direct determination of enantiomeric excess of one or more components in a flavor mixture when a conventional (nonchiral) capillary column is suitably coupled to one coated with an O-alkylated or -acylated  $\alpha$ -,  $\beta$ -, or  $\gamma$ -cyclodextrin phase (Mosandl et al., 1989; Bernreuther et al., 1989; Mosandl et al., 1990; Bernreuther and Schreier, 1991; Full et al., 1993).

We investigated the enantiomeric composition of some passion fruit flavor compounds summarized in Table 6 by enantioselective MDGC using different chiral cyclodextrin columns. Several authors have already studied the enantiomeric distribution of  $\gamma$ -decalactone,  $\gamma$ -dodecalactone, linalool,  $\alpha$ -terpineol, 1-terpinen-4-ol, and limonene in various natural products (Bicchi et al., 1995; Mosandl, 1995; Werkhoff et al., 1993b). Generally speaking, our results are in good agreement with literature data (Bernreuther et al., 1989, 1990; Nitz et al., 1989, 1991; Casabianca et al., 1995; Bernreuther and Schreier, 1991).

Enantioselective GC analysis of *cis*- and *trans*-rose oxides has recently been reported in yellow passion fruit flavor by our research group for the first time. Analogous to our results with essential oils, the levorotatory *cis* form as well as the levorotatory *trans* form of rose oxides was always clearly predominating in yellow passion fruit flavor (Werkhoff et al., 1993b). Equally, the chiroselective GC analysis of *cis*- $\gamma$ -jasmin lactone was quite recently described in peach flavor as well as in yellow passion fruit flavor, revealing the prevalence of the (+)-(*R*)-antipode (Fischer et al., 1993; Werkhoff et al., 1993b).

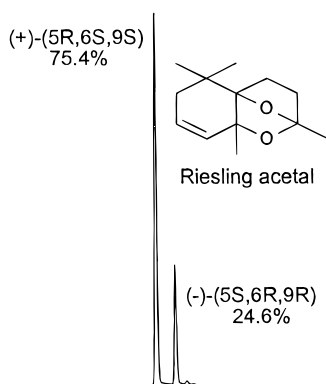
Diastereomeric theaspiranes and theaspiroenes have been identified in many natural products including yellow and purple passion fruits. The syntheses of four theaspirane enantiomers, their sensory properties, and their enantiodifferentiation in a number of natural sources have been reported by Schmidt et al. (1992). Enantiomerically pure theaspiroenes were isolated from quince fruit and structurally assigned by CD spectroscopy (Full et al., 1993). In the latter paper theaspiranes and theaspiroenes were found to exist in a distinct enantiomeric ratio in purple passion fruits whereby the 2*S* isomers predominated. In the case of yellow passion fruit flavor, however, nearly racemic distributions were now observed for theaspirane A and theaspiroenes A and B with the exception of theaspirane B (30:70 ratio) (Table 6).

For ethyl 3-hydroxybutanoate and ethyl 3-hydroxyhexanoate enantiomeric ratios of (+)-(*S*) 75.8%:(-)-(*R*) 24.2% and (+)-(*S*) 84.7%:(-)-(*R*) 15.3% were determined and are consistent with the results observed for other fruits. In previous studies of the flavor composition of tropical fruits, ethyl 3-hydroxy-substituted esters had mainly the *S* configuration in yellow passion fruit, whereas in purple passion fruit the *R* configuration predominated (Tressl and Engel, 1984; Tressl et al., 1988). In general, however, both (*R*)- and (*S*)-antipodes

**Table 6. Enantioselective Multidimensional GC Analysis of Yellow Passion Fruit Constituents**

compound	column <sup>a</sup>	separation factor $\alpha^b$ /resolution $R_s$	order of elution/natural distribution
3-mercaptohexanol	C	1.030/2.85	(+)-(S):(-)-(R) = 90.4%:9.6% (VHS), 93.0%:7.0% (SDEV)
3-(methylthio)hexanol	A	1.012/1.96	(-)-(R):(+)-(S) = 5%:95%
3-mercaptohexyl acetate	C	1.019/2.10	(-)-(R):(+)-(S) = 9.5%:90.5% (VHS), 9.1%:90.9% (SDEV)
linalool	F	1.040/3.92	(-)-(R):(+)-(S) = 49.3%:50.7%
$\alpha$ -terpineol	F	1.045/5.30	(+)-(R):(-)-(S) = 50.4%:49.6%
1-terpinen-4-ol	F	1.024/1.77	(+)-(S):(-)-(R) = 70.6%:29.4%
limonene	F	1.053/4.41	(-)-(S):(+)-(R) = 34.0%:66.0%
<i>cis</i> -rose oxide	D	1.023/3.36	(-)-(2 <i>S</i> ,4 <i>R</i> ):(+)-(2 <i>R</i> ,4 <i>S</i> ) = 99.5%:0.5%
<i>trans</i> -rose oxide	E	1.017/3.53	(+)-(2 <i>S</i> ,4 <i>S</i> ):(-)-(2 <i>R</i> ,4 <i>R</i> ) = 0.5%:99.5%
theaspirane A	A	1.086/4.90	(-)-(2 <i>R</i> ,5 <i>R</i> ):(+)-(2 <i>S</i> ,5 <i>S</i> ) = 57.8%:42.2%
theaspirane B	A	1.050/4.20	(+)-(2 <i>S</i> ,5 <i>R</i> ):(-)-(2 <i>R</i> ,5 <i>S</i> ) = 31.0%:69.0%
theaspirone A	A	1.032/5.23	(+)-(2 <i>R</i> ,5 <i>R</i> ):(-)-(2 <i>S</i> ,5 <i>S</i> ) = 42.9%:57.1%
theaspirone B	A	1.019/2.94	(+)-(2 <i>S</i> ,5 <i>R</i> ):(-)-(2 <i>R</i> ,5 <i>S</i> ) = 50.3%:49.7%
$\gamma$ -decalactone	B	1.023/3.64	(+)-(R):(-)-(S) = 93.2%:6.8%
$\gamma$ -dodecalactone	B	1.010/2.24	(+)-(R):(-)-(S) = 99.3%:0.7%
$\gamma$ -jasmin lactone	B	1.023/4.25	(+)-(R):(-)-(S) = 95.9%:4.1%
Riesling acetal	A	1.057/4.24	(+)-(5 <i>R</i> ,6 <i>S</i> ,9 <i>S</i> ):(-)-(5 <i>S</i> ,6 <i>R</i> ,9 <i>R</i> ) = 75.4%:24.6%
2-methylbutanol	F	1.020/1.50	(+)-(R):(-)-(S) = 8.5%:91.5%
ethyl 3-hydroxybutanoate	F	1.033/2.80	(+)-(S):(-)-(R) = 75.8%:24.2%
ethyl 3-hydroxyhexanoate	F	1.020/2.80	(+)-(S):(-)-(R) = 84.7%:15.3%

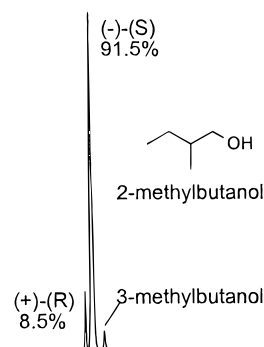
<sup>a</sup> Column A, 50 m  $\times$  0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) heptakis(2,3,6-tri-*O*-methyl)- $\beta$ -cyclodextrin/polysiloxan (CS-Chromatographie Service GmbH); column B, 25 m  $\times$  0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) heptakis(3-*O*-acetyl-2,6-di-*O*-pentyl)- $\beta$ -cyclodextrin/Lipodex D (Macherey-Nagel); column C, 25 m  $\times$  0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) octakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)- $\gamma$ -cyclodextrin/70% OV1701 (Mega); column D, 25 m  $\times$  0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) octakis(2,3,6-tri-*O*-methyl)- $\gamma$ -cyclodextrin (Macherey-Nagel); column E, 30 m  $\times$  0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) octakis(3-*O*-trifluoroacetyl-2,6-di-*O*-pentyl)- $\gamma$ -cyclodextrin (Astec); column F, 25 m  $\times$  0.25 mm ( $d_f = 0.25 \mu\text{m}$ ) heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)- $\beta$ -cyclodextrin/70% PS 086 (Mega). <sup>b</sup> Temperature programmed analysis/carrier gas helium.

**Figure 6.** Determination of the natural enantiomeric composition of Riesling acetal in yellow passion fruit flavor.

of 3-hydroxyesters have been detected in natural products (Engel et al., 1989; Suarez et al., 1993; Torrado et al., 1995; Allegrone and Barbeni, 1992).

However, it should be mentioned that the determination of the enantiomeric composition of Riesling acetal in yellow passion fruit flavor is described here for the first time. The enantiomeric distribution of Riesling acetal was recently reported in various natural sources by Dollmann et al. (1995). In all samples examined almost racemic proportions of Riesling acetal enantiomers have been detected. Contrary to these results we found that the dextrorotatory form of Riesling acetal predominated in yellow passion fruit flavor. The chiroselective analysis revealed an enantiomeric ratio of (+)-(5*R*,6*S*,9*S*):(-)-(5*S*,6*R*,9*R*) = 75.4%:24.6% (Figure 6).

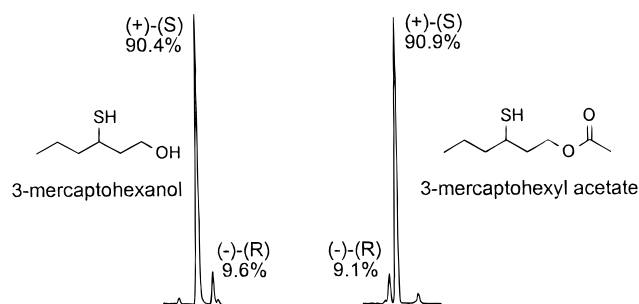
Considering the enantiomeric ratio of 2-methylbutanol in yellow passion fruit flavor, a predominance of the (*S*)-antipode was observed; an enantiomeric excess of 83% *S* was found (Figure 7). This result is worthwhile to emphasize because 2-methylbutanol has previously been found in nature only at high optical purity in favor of the *S* configuration. Meanwhile, we have investigated the enantiomeric composition of 2-methylbutanol in numerous fruit and vegetable flavors by

**Figure 7.** Chiroselective analysis of 2-methylbutanol in yellow passion fruit flavor.

enantio-MDGC including MS detection and found a wide range of enantiomeric ratios including nearly racemic distribution (Werkhoff and Krammer, unpublished results). This surprising phenomenon will be the subject of continuing investigations. Obviously, different progenitors for 2-methylbutanol are possible (Rowan et al., 1996).

As sulfur-containing volatiles are among the most powerful compounds responsible for the characteristic tropical fruit note, the determination of the natural enantiomeric distribution of these trace components was of specific interest. Octakis(2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl)- $\gamma$ -cyclodextrin was used as the chiral stationary phase for the stereodifferentiation of 3-mercaptohexanol and 3-mercaptohexyl acetate. As representative examples, in Figure 8 the MDGC enantioseparation of 3-mercaptohexanol and 3-mercaptohexyl acetate are illustrated. As shown in Figure 8, the stereodifferentiation was very effective in the case of 3-mercaptohexanol; lower separation factors were found for 3-(methylthio)hexanol and 3-mercaptohexyl acetate (Table 6).

In all cases, sulfur-containing passion fruit volatiles were found to be present at high optical purity favoring the *S* configured enantiomer. These results are in good



**Figure 8.** Determination of the natural enantiomeric distribution of 3-mercaptohexanol and 3-mercaptohexyl acetate in yellow passion fruit flavor.

accordance with previously published data where also the *S* configured stereoisomers were always clearly dominant (Weber et al., 1994, 1995). Unfortunately, the separation efficiency of our commercially available chiral capillary column was not sufficient to resolve enantiomers of 3-mercaptohexyl- and 3-(methylthio)hexyl butanoates and hexanoates. The syntheses and the sensory properties of 3-mercaptohexanol and 3-(methylthio)hexanol have already been described by Heusinger and Mosandl (1984), whereas the structures and the sensorial properties of 3-mercaptohexyl- and 3-(methylthio)hexyl alkanooates were given by Weber et al. (1992). It could be demonstrated that odor quality is again strongly influenced by chirality. The naturally occurring *S* configured enantiomers of 3-mercaptohexanol and 3-(methylthio)hexanol possess the interesting exotic and tropical fruit notes, while the *R* forms were distinctly weaker, showing only sulfury and herbaceous odor impressions. In conformity with Engel and Tressl (1991), the mercapto esters were reported to be more powerful than the corresponding methylthio compounds; as expected, the aroma intensities decreased from acetates to hexanoates. Surprisingly, only the *R* configured stereoisomers of 3-mercaptohexyl acetate and -butanoate possess attractive tropical fruity aroma notes, while the naturally dominating *S* forms show insignificant sulfury, herbaceous, and oniony flavor characteristics. On the other hand, *S* configured stereoisomers of methylthio esters are more intensive compared with *R* enantiomers, having in all cases significant sulfury and faintly fruity aroma impressions.

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