Enantioselective Analysis of Secondary Alcohols and Their Esters in Purple and Yellow Passion Fruits

Hedwig Strohalm, Márta Dregus, Astrid Wahl, and Karl-Heinz Engel*

Lehrstuhl für Allgemeine Lebensmitteltechnologie, Technische Universität München, Am Forum 2, D-85350 Freising-Weihenstephan, Germany

The enantiomeric compositions of the acetates, butanoates, hexanoates, and octanoates of the secondary alcohols 2-pentanol, 2-heptanol, and 2-nonanol were determined in yellow (*Passiflora edulis* f. *flavicarpa*) and purple (*Passiflora edulis* Sims) passion fruits. The compounds were isolated by means of simultaneous distillation−extraction. Enantiodifferentiation was performed via multidimensional gas chromatography using heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as chiral stationary phase. The series of homologous 2-alkyl esters, which are typical constituents of purple passion fruits, were shown to be present as nearly optically pure (*R*)-enantiomers. The proportions of the (*S*)-enantiomers varied in different batches and were dependent on the alcohol moieties of the esters. For minor amounts of esters detected in yellow fruits, the (*R*)-enantiomers were also dominating. However, the enantiomeric excesses were significantly lower than in the purple variety. Enantioselective analysis of the free alcohols revealed that 2-heptanol exhibited opposite configurations in purple and yellow passion fruits. A similar phenomenon was observed for 2-pentanol, which was present in the yellow fruits as a nearly racemic mixture. Data determined in extracts obtained by other techniques (liquid–liquid extraction, vacuum headspace technique) showed that the isolation procedure had no significant impact on the enantiomeric ratios.

KEYWORDS: Passion fruits; *Passiflora edulis* f. *flavicarpa*; *Passiflora edulis* Sims; secondary alcohols; 2-alkyl esters; enantioselective analysis; MDGC

INTRODUCTION

Among the 400 species of *Passiflora*, yellow (*Passiflora edulis* f. *flavicarpa*) and purple (*Passiflora edulis* Sims) passion fruits are of major commercial importance owing to their attractive exotic flavor. The volatile constituents of these tropical fruits have been investigated by several research groups over the past decades. The broad spectrum of constituents identified has been described in comprehensive reviews (*1, 2*). The presence of chiral sulfur-containing aroma compounds is a typical feature of yellow passion fruits (*3–5*). Some unusual norterpenoids, such as edulans (*6*) and megastigmatrienes (*7*) have been discussed as essential contributors to the floral character of purple passion fruits. Esters (aliphatic, aromatic, and terpenoid) constitute the predominant class of compounds in both varieties. A characterization of the aromatic profile of an aqueous essence of yellow passion fruit by gas chromatography−olfactometry revealed 2-methylbutyl hexanoate and hexyl hexanoate to be among the most intense odorants (*8*).

A subgroup of esters, short-chain fatty acid esters of odd-numbered secondary alcohols, turned out to be suitable for a differentiation of the two passion fruit varieties. Purple passion fruits contain acetates, butanoates, hexanoates, and octanoates of 2-pentanol, 2-heptanol, and 2-nonanol as prominent constituents; in the yellow variety these esters were not detected (*9*) or were present at only trace levels (*5, 10*). The nonesterified alcohols 2-pentanol and 2-heptanol were detected as minor constituents in both varieties.

Investigations of naturally occurring enantiomeric compositions by capillary gas chromatographic (GC) separation of diastereoisomeric esters of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) revealed that the free secondary alcohols 2-pentanol and 2-heptanol present in yellow fruits mainly consisted of the (*S*)-enantiomer (*11*). In contrast, free 2-heptanol contained in purple passion fruits was almost exclusively present as the (*R*)-enantiomer. 2-Heptanol esterified in the 2-heptyl esters of purple passion fruits was shown to be optically pure and possessed the (*R*)-configuration.

The indirect GC separation applied at that time was based on the derivatization of enantiomers to diastereoisomeric esters. For 2-heptyl esters this required the following workup sequence: (i) liquid–solid chromatography of a passion fruit aroma extract on silica gel to obtain an ester fraction; (ii) alkaline saponification of the ester fraction; (iii) isolation of the liberated 2-heptanol by preparative GC; and (iv) derivatization of the alcohol with (*R*)-(+)-MTPA and subsequent GC separation. In addition to a

* Author to whom correspondence should be addressed (telephone +49 (0)8161 71 4250; fax +49 (0)8161 71 4259; e-mail k.h.engel@wzw.tum.de).

reduced sensitivity, this laborious procedure did not allow the determination of the enantiomeric compositions of individual ester homologues.

In the meantime, there has been tremendous progress regarding the direct separation of enantiomers using chiral stationary phases (*12*). The aim of this study was to exploit these analytical techniques and to determine the naturally occurring enantiomeric distributions of chiral secondary alcohols and their individual esters in purple and yellow passion fruits via direct enantio-differentiation using multidimensional gas chromatography and a modified β-cyclodextrin as chiral stationary phase.

MATERIALS AND METHODS

Materials. Purple (*P. edulis* Sims) and yellow passion fruits (*P. edulis* f. *flavicarpa*) from Colombia were purchased from a local market. Yellow passion fruits were also directly obtained from Thailand by air freight. The fruits were stored at 5 °C before analysis.

Chemicals. Authentic reference chemicals were purchased from commercial sources (Aldrich, Steinheim, Germany; Merck, Darmstadt, Germany). (*S*)-2-Pentanol, (*R*)-2-heptanol, and (*S*)-2-nonanol were purchased from Aldrich, Steinheim, Germany. (*S*)-2-Heptanol was obtained from Fluka, Steinheim, Germany. (*R*)-2-Pentanol and (*R*)-2-nonanol were obtained from Wako Pure Chemical Industries, Ltd., Tokyo, Japan. Esters were synthesized from the corresponding acyl chlorides using 4-dimethylaminopyridine as catalyst. The secondary alcohol (20 mmol) was dissolved in pyridine (15 mL), and 4-dimethyl-aminopyridine (250 mg) was added at room temperature. The mixture was cooled in an ice–water bath, and the acyl chloride (20 mmol) was added dropwise. After 3 h of stirring at room temperature, methanol was added. The reaction mixture was poured into a stirred suspension of MTBE and water. The organic layer was separated and washed with HCl (2 N), water, saturated sodium bicarbonate solution, and saturated sodium chloride solution, dried over anhydrous magnesium sulfate, filtered, and concentrated. The obtained pale yellow oil was distilled under reduced pressure to yield the product (yields 65–91%). The identities of the compounds were confirmed by mass spectrometric analyses: 2-pentyl acetate [*m/e* (%)], 43 (100), 87 (33), 70 (21), 55 (14), 42 (8), 41 (6), 71 (4), 45 (4), 115 (3), 61 (3); 2-pentyl butanoate, 71 (100), 43 (46), 70 (23), 89 (15), 115 (12), 55 (11), 41 (10), 42 (8), 88 (8), 72 (5); 2-pentyl hexanoate, 99 (100), 43 (57), 71 (41), 70 (40), 117 (27), 55 (18), 60 (16), 41 (14), 42 (13), 87 (13); 2-pentyl octanoate, 127 (100), 70 (56), 43 (50), 57 (49), 145 (38), 55 (29), 41 (21), 71 (21), 144 (19), 60 (18); 2-heptyl acetate, 43 (100), 87 (37), 56 (19), 98 (13), 41 (11), 55 (11), 57 (9), 70 (9), 69 (8), 58 (7); 2-heptyl butanoate, 71 (100), 43 (40), 57 (28), 115 (22), 56 (20), 98 (17), 41 (16), 89 (13), 70 (12), 55 (11); 2-heptyl hexanoate, 99 (100), 43 (37), 57 (33), 56 (27), 98 (24), 71 (22), 117 (19), 41 (16), 55 (15), 70 (14); 2-heptyl octanoate, 127 (100), 57 (74), 98 (34), 56 (26), 43 (25), 145 (25), 55 (22), 41 (19), 70 (16), 144 (16); 2-nonyl acetate, 43 (100), 87 (41), 55 (18), 56 (17), 41 (13), 70 (12), 69 (12), 97 (12), 84 (11), 126 (10); 2-nonyl butanoate, 71 (100), 43 (38), 115 (19), 41 (14), 55 (14), 56 (14), 57 (12), 70 (12), 89 (12), 126 (11); 2-nonyl hexanoate, 99 (100), 43 (42), 71 (34), 56 (21), 117 (20), 55 (19), 126 (18), 41 (16), 57 (16), 97 (15); 2-nonyl octanoate, 127 (100), 57 (53), 43 (31), 126 (25), 84 (25), 55 (24), 145 (23), 41 (18), 97 (17), 71 (16).

Isolation of Volatiles by Simultaneous Distillation–Extraction (SDE). Three hundred grams of purple passion fruit pulp or 400 g of yellow passion fruit pulp was homogenized with 750 mL of phosphate buffer solution (pH 6.8) for 1 min. After addition of the internal standard (2-hexanol, 100 μg), the homogenate was pressed through a sieve. The juice was transferred into a 2 L round-bottom flask connected to an SDE apparatus (*13*). Distillation–extraction was performed for 2 h, using 200 mL of a mixture of *n*-pentane and diethyl ether (1:1, v/v) as solvent. The aroma extract was dried over anhydrous sodium sulfate and concentrated at ~40 °C to a final volume of 1 mL using a Vigreux column (30 cm × 2 cm i.d.).

Comparison of Isolation Techniques. Nine hundred grams of purple passion fruit pulp was homogenized with 2250 mL of phosphate buffer solution (pH 6.8) for 1 min. After addition of the internal standard (2-hexanol, 300 μg) and pressing of the homogenate through a sieve, the obtained juice was divided into three aliquots of 1000 mL.

Vacuum Headspace (VHS) Technique. One aliquot was transferred into a 2 L round-bottom flask and connected to a vacuum headspace apparatus (*5*). The flask was brought to a temperature of ~35 °C in a water bath (Gerhardt, type SV 24). Vacuum was applied for 3 h (1–10 mbar; Leybold-Heraus vacuum pump, type D4A). The volatiles were condensed in three cooling traps, which were cooled with ice–water (I and II) and liquid nitrogen (III), respectively. After 3 h, the aqueous condensates were allowed to thaw, combined, and extracted three times with 50 mL of a mixture of *n*-pentane and diethyl ether (1:1, v/v). The combined extracts were dried over anhydrous sodium sulfate and concentrated at ~40 °C to a final volume of 1 mL using a Vigreux column.

Liquid–Liquid Extraction (LLE). One aliquot was transferred into a Kutscher-Steudel liquid–liquid extractor (*14*). Extraction was performed for 24 h using 200 mL of a mixture of *n*-pentane and diethyl ether (1:1, v/v) as solvent.

SDE. One aliquot was subjected to SDE as described above (Isolation of volatiles).

Capillary Gas Chromatography (HRGC-FID). The separations were performed on a Carlo Erba Mega II 8575 series gas chromatograph (Thermo Fisher Scientific, Dreieich, Germany) equipped with a split/splitless injector (215 °C, split ratio 1:10) and a flame ionization detector (FID) operating at 230 °C. The column used was a 60 m × 0.25 mm (i.d.) fused silica capillary column coated with DB-Wax (0.25 μm film thickness; J&W Scientific). The oven temperature was programmed from 40 °C (5 min hold) at 4 °C/min to 230 °C (25 min hold). Carrier gas used was hydrogen at a constant inlet pressure of 105 kPa. Data acquisition was done via Chromcard software (Thermo Fisher Scientific).

Quantification. Quantifications were carried out using 2-hexanol as internal standard (100 μg, stock solution in diethyl ether/ethanol, 4:1, v/v) and taking into account extraction recoveries and FID responses. The recoveries after SDE from buffer solution were determined in model experiments with authentic compounds (100 μg, stock solution in diethyl ether/ethanol, 4:1, v/v). The following average values were obtained: 2-pentyl esters (68%), 2-heptyl esters (75%), 2-nonyl esters (99%). For 2-heptyl hexanoate (2.5 mg) spiked to the fruit homogenate (300 g) the recovery via SDE was 84%. FID response factors were determined with solutions of authentic compounds relative to the internal standard (0.1 μg/μL diethyl ether). The limits of detection and the limits of determination were calculated according to described procedures (*15, 16*), using a series of five dilutions of reference compounds ranging from 0.5 to 10.0 μg/kg.

Gas Chromatography–Mass Spectrometry (GC-MS). Mass spectral data were obtained on a gas chromatograph–mass spectrometer (GC 8000^{TOP} with a Voyager GC-MS, Thermo Fisher Scientific) equipped with a split/splitless injector (220 °C, split ratio 1:10). The separation was performed on a 30 m × 0.25 mm (i.d.) fused silica capillary column coated with DB-WaxEtr (0.5 μm film thickness; J&W Scientific). The oven temperature was programmed from 40 °C (5 min hold) at 4 °C/min to 240 °C (25 min hold). Carrier gas used was helium at a constant inlet pressure of 75 kPa. Ionization energy was set at 70 eV, source temperature at 200 °C, and interface temperature at 240 °C. Data acquisition was done via MassLab software (Thermo Fisher Scientific).

Multidimensional Gas Chromatography. For enantioselective gas chromatographic analysis an instrumentation of two coupled GC 8000 series gas chromatographs (Thermo Fisher Scientific) with two independent temperature controls and with FID on each GC system was used. The columns were coupled via a moving column stream switching device (MCSS) (*17, 18*). An achiral column for preseparation (precolumn) was installed in the first GC oven and was connected via the MCSS device and a deactivated fused silica transfer capillary (1 m × 0.25 mm i.d.) with the chiral separation column (main column) in the second GC oven. In the first oven two types of achiral precolumns were used: (I) DB-Wax (60 × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific) and (II) DB-5 (60 × 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific). For both columns the temperature program

Secondary Alcohols in Passion Fruit

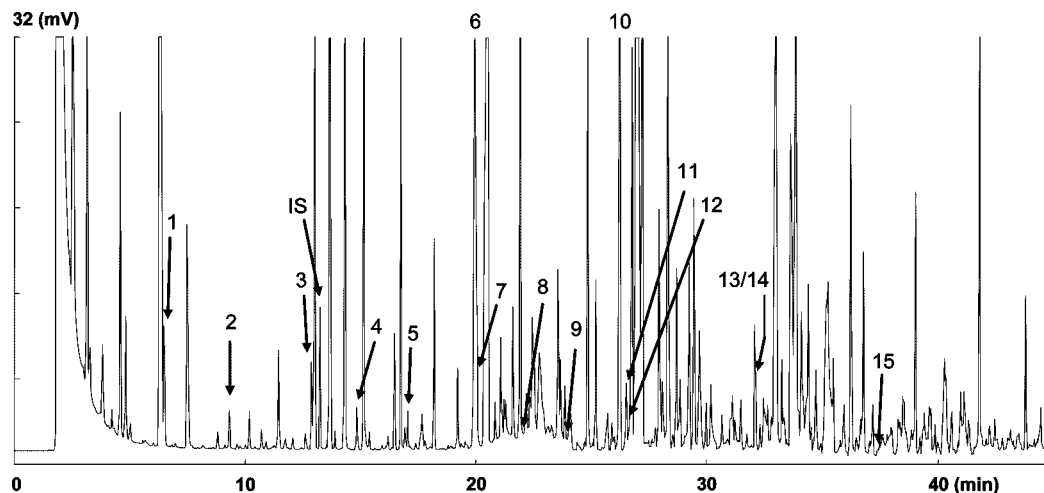*J. Agric. Food Chem.*, Vol. 55, No. 25, 2007 **10341**

Figure 1. Typical gas chromatographic separation of secondary alcohols and their esters isolated from purple passion fruits by SDE [peak numbers correspond to **Table 1**; for GC conditions see Capillary Gas Chromatography (HRGC-FID) under Materials and Methods; IS = internal standard, 2-hexanol].

started at 40 °C (5 min hold) and was programmed at 4 °C/min to 230 °C. In the second GC oven a chiral column was installed coated with 25% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in SE54 (30 m × 0.32 mm i.d., 0.25 μm film thickness). Synthesis of the cyclodextrin derivative and column preparation were carried out in-house (*19*). The temperature of the chiral main column was programmed from 37 °C (10 min hold) to 200 at 2 °C/min. Injection into the precolumn was done in the split mode (215 °C; split ratio 1:15). Carrier gas used was hydrogen at a constant inlet pressure of 165 kPa. The outlet pressure of the column was 98 kPa (measured within the "dome" of the MCSS device via an additional manometer), which also corresponded to the actual inlet pressure for the chiral column in the second GC oven. The FID of the first oven was set at 230 °C and the FID of the second oven at 200 °C. Data acquisition and control of the MCSS device were done via Chromcard software (Thermo Fisher Scientific). MDGC analysis of the volatile compounds was performed via four cut combinations. The cut intervals using DB-Wax as precolumn were as follows: [I] 2-pentanol (12.18–12.35 min), 2-heptanol (20.08–20.21 min); [II] 2-heptyl esters (17.81–17.95 min; 22.93–23.08 min; 29.38–29.57 min; 35.37–35.51 min), 2-nonyl hexanoate (35.37–35.51 min); [III] 2-pentyl butanoate (15.81–15.99 min), 2-pentyl hexanoate (23.14–23.22 min), 2-nonyl acetate (24.86–25.00 min); [IV] 2-pentyl acetate (10.34–10.49 min), 2-pentyl octanoate (29.66–30.16 min), 2-nonyl butanoate (29.66–30.16 min). The limits of detection and determination of the MDGC transfer were calculated according to described procedures (*15, 16*), using a series of five dilutions of reference compounds ranging from 0.5 to 3.0 μg/kg per enantiomer.

RESULTS AND DISCUSSION

Quantification. Volatile constituents were isolated from purple and yellow passion fruits by means of SDE. The extracts obtained were analyzed by means of GC-FID and GC-MS. **Figure 1** shows the typical gas chromatographic separation of volatiles isolated from purple passion fruits; the target compounds of this study (secondary alcohols and their esters) are indicated by numbers.

Quantitative data obtained from triplicate analyses of purple and yellow passion fruits are presented in **Table 1**. In purple fruits the complete series of the acetates, butanoates, hexanoates, and octanoates of 2-pentanol, 2-heptanol, and 2-nonanol could be detected. The 2-heptyl esters were dominating with regard to the alcohol moieties, and the butanoates and hexanoates were the major representatives with regard to the fatty acyl moieties. Accordingly, 2-heptyl butanoate and 2-heptyl hexanoate were shown to be prominent ester constituents (3800 and 2400 μg/

Table 1. Secondary Alcohols and Their Esters Isolated from Purple and Yellow Passion Fruits by Means of SDE

| | | | concentrations[a] (μg/kg) | |
| | | | purple passion fruits, | yellow passion |
peak[b]	compound	KI[c] (DB-Wax)	Colombia (batch 1)	fruits, Thailand
	alcohols			
2	2-pentanol	1125	98 ± 19	22 ± 4
5	2-heptanol	1328	68 ± 13	15 ± 11
9	2-nonanol	1525	<2	nd[d]
	esters			
1	2-pentyl acetate	1074	138 ± 37	nd
3	2-pentyl butanoate	1216	459 ± 171	nd
7	2-pentyl hexanoate	1407	438 ± 122	<2
12	2-pentyl octanoate	1605	70 ± 30	nd
4	2-heptyl acetate	1266	215 ± 38	nd
6	2-heptyl butanoate	1401	3800 ± 628	31 ± 13
10	2-heptyl hexanoate	1591	2403 ± 215	36 ± 16
14	2-heptyl octanoate[e]	1788	72 ± 6	nd
8	2-nonyl acetate	1466	<2	nd
11	2-nonyl butanoate	1600	187 ± 20	nd
13	2-nonyl hexanoate[e]	1787	17 ± 3	nd
15	2-nonyl octanoate	1982	<2	nd

[a] Data from triplicate experiments for each batch; mean ± standard error. [b] Numbers correspond to chromatogram peaks shown in **Figure 1**. [c] Kovats retention indices. [d] Below limit of detection (<0.7 μg/kg). [e] Not separated; concentrations estimated on the basis of the ratios of characteristic MS fragments: *m/e* 127 (2-heptyl octanoate) and 99 (2-nonyl hexanoate).

kg, respectively) of purple passion fruits. In contrast, in the yellow variety these two esters were contained only as minor volatiles. Apart from traces of 2-pentyl hexanoate, no other esters of secondary alcohols could be detected. The free secondary alcohols 2-pentanol and 2-heptanol were present in both varieties; in the purple fruits also traces of 2-nonanol were detected.

These quantitative patterns were in agreement with those obtained after isolation of volatiles via LLE and were confirmed in the other batches of purple and yellow passion fruits listed in **Table 2** (data not shown). They are in agreement with previously reported data (*5, 10*) and confirm that esters of secondary alcohols are suitable for a differentiation of the two passion fruit varieties (*9*).

Enantioselective MDGC. Several stationary phases were screened regarding their suitability for enantioselective analysis of the target compounds of this study. The enantiomers of the

10342 *J. Agric. Food Chem.,* Vol. 55, No. 25, 2007

Strohalm et al.

Table 2. Enantiomeric Distributions of Secondary Alcohols and Their Esters from Purple and Yellow Passion Fruits

	enantiomeric distributions (%)									
	purple passion fruits						yellow passion fruits			
	Colombia (batch 1)[a]		Colombia (batch 2)[b]		Colombia (batch 3)[b]		Thailand[a]		Colombia[a]	
compound	R	S	R	S	R	S	R	S	R	S
alcohols										
2-pentanol	70.8	29.2	85.5	14.5	84.3	15.7	49.7	50.3	51.7	48.3
2-heptanol	85.7	14.3	92.5	7.5	72.0	28.0	29.1	70.9	42.5	57.5
2-nonanol	–[c]	–	–	–	–	–	nd[d]	nd	nd	nd
esters										
2-pentyl acetate	>98.0[e]		>95.3		>98.2		nd	nd	nd	nd
2-pentyl butanoate	99.2	0.8	>89.9		92.9	7.1	nd	nd	nd	nd
2-pentyl hexanoate	>98.7		93.5	6.5	96.4	3.6	–	–	89.1	10.9
2-pentyl octanoate	>97.9		98.8	1.2	>98.9		nd	nd	nd	nd
2-heptyl acetate	>98.4		>99.5		>98.6		nd	nd	nd	nd
2-heptyl butanoate	99.4	0.6	99.2	0.8	97.7	2.3	87.2	12.8	>84.6	
2-heptyl hexanoate	99.8	0.2	99.8	0.2	99.3	0.7	93.1	6.9	94.4	5.6
2-heptyl octanoate[f]	>98.8		99.1	0.9	98.6	1.4	nd	nd	nd	nd
2-nonyl acetate	–	–	>96.3		>87.7		nd	nd	nd	nd
2-nonyl butanoate	98.2	1.8	97.5	2.5	96.9	3.1	nd	nd	nd	nd
2-nonyl hexanoate[f]	>98.8		99.1	0.9	98.6	1.4	nd	nd	nd	nd
2-nonyl octanoate	–	–	–	–	–	–	nd	nd	nd	nd

[a] Triplicate isolation of volatiles and triplicate analyses of enantiomers. [b] Single isolation of volatiles and triplicate analyses of enantiomers. [c] Below limit of determination (for alcohols, <0.7 µg/kg; for esters, <1.2 µg/kg). [d] Below limit of detection (<0.4 µg/kg). [e] Ratios "greater than (>)" were calculated using the peak area corresponding to the limit of determination for the second enantiomer. [f] Coelution of the enantiomers.

homologous series (C_5-C_{10}) of secondary alcohols had been separated on octakis(2,3-di-*O*-butyl-6-*O*-*tert*-butyldimethylsilyl)-γ-cyclodextrin (*20*). Short-chain esters of 2-pentanol and 2-heptanol could be enantiodifferentiated using heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin and heptakis(2,3,6-tri-*O*-pentyl)-β-cyclodextrin (Lipodex C) as chiral stationary phases (*21, 22*). The recently developed modified cyclodextrins bearing acetal functions as side chains also showed unusually high separation factors for the short-chain 2-alkyl esters. However, the separation factors decreased significantly with increasing lengths of the acyl and alcohol moieties (*23*). Finally, heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin was selected as chiral stationary phase. Using this modified cyclodextrin, known, for example, from the enantioseparation of chiral sulfur-containing passion fruit volatiles (*24*), baseline separations could be achieved for the enantiomers of the complete series of 2-alkyl esters. **Figure 2a** shows exemplarily the enantioseparation of the 2-heptyl esters. The order of elution of the enantiomers was assigned by co-injection of optically pure reference compounds.

MDGC was employed to transfer the secondary alcohols and their esters from the achiral precolumn to the chiral main column. Taking into account the elution behavior of the target compounds, four cut combinations (see Materials and Methods) were designed, which enabled the transfer and enantiodifferentiation of the complete set of alcohol and ester homologues. The only compounds for which overlapping on both precolumns and on the chiral stationary phase could not be avoided were 2-heptyl octanoate and 2-nonyl hexanoate.

Exemplarily, **Figure 2b** shows the enantiodifferentiation of 2-heptyl esters in purple passion fruits obtained after transfer from a DB-Wax as achiral precolumn. To confirm the results and to rule out the transfer of coeluting compounds, MDGC analyses were repeated for the same extract using DB-5 as a precolumn of opposite polarity (**Figure 2c**). Comparison of the two chromatograms demonstrates that both approaches delivered the same results and revealed the four 2-heptyl ester homologues to be present in purple passion fruits nearly exclusively as (*R*)-enantiomers.

The enantiomeric distributions of secondary alcohols and their esters determined in extracts obtained by SDE from purple and yellow passion fruits are listed in **Table 2**. Data originate from investigations of batches of different origins and from triplicate analyses of enantiomers of each extract. In purple passion fruits esters of secondary alcohols are present as nearly optically pure (*R*)-enantiomers. For the quantitatively dominating 2-heptyl butanoate and 2-heptyl hexanoate, the average proportion of the (*R*)-enantiomer determined in two batches from Colombia was 99.6%. The maximum proportions of (*S*)-configured esters were 2.3 and 0.7%, respectively. For the ester homologues occurring at lower concentrations, in most cases the amounts detected for the (*S*)-enantiomers were below the limits of determination. In these cases, quantification of the minor enantiomer and accurate calculation of an enantiomeric ratio were not possible (*25*). Data obtained for the 2-pentyl esters indicate that for esters containing this alcohol moiety the excess of the (*R*)-enantiomer was slightly less pronounced.

For 2-heptyl butanoate, 2-heptyl hexanoate, and 2-pentyl hexanoate detected as minor constituents in yellow passion fruits, also the (*R*)-enantiomers were dominating. However, the enantiomeric excesses were significantly lower than in the purple variety.

In purple passion fruits the free secondary alcohols 2-pentanol and 2-heptanol were mainly present as (*R*)-enantiomers. However, the enantiomeric excess was less pronounced than for the esters. On the other hand, in yellow passion fruits the (*S*)-enantiomer dominated for 2-heptanol, and for 2-pentanol nearly racemic mixtures were found.

Comparison of Isolation Techniques. Various techniques have been shown to be suitable for the isolation of volatiles from passion fruits (*26*). In this study SDE was chosen because preliminary tests demonstrated that this technique provided high recoveries of the target compounds (on average 30% more than with LLE). To rule out changes of the naturally occurring enantiomeric distributions due to the thermal treatment involved in the SDE approach, the results were compared with those obtained by isolation of the volatiles by the gentler VHS technique and by LLE. As shown in **Table 3** for the esters
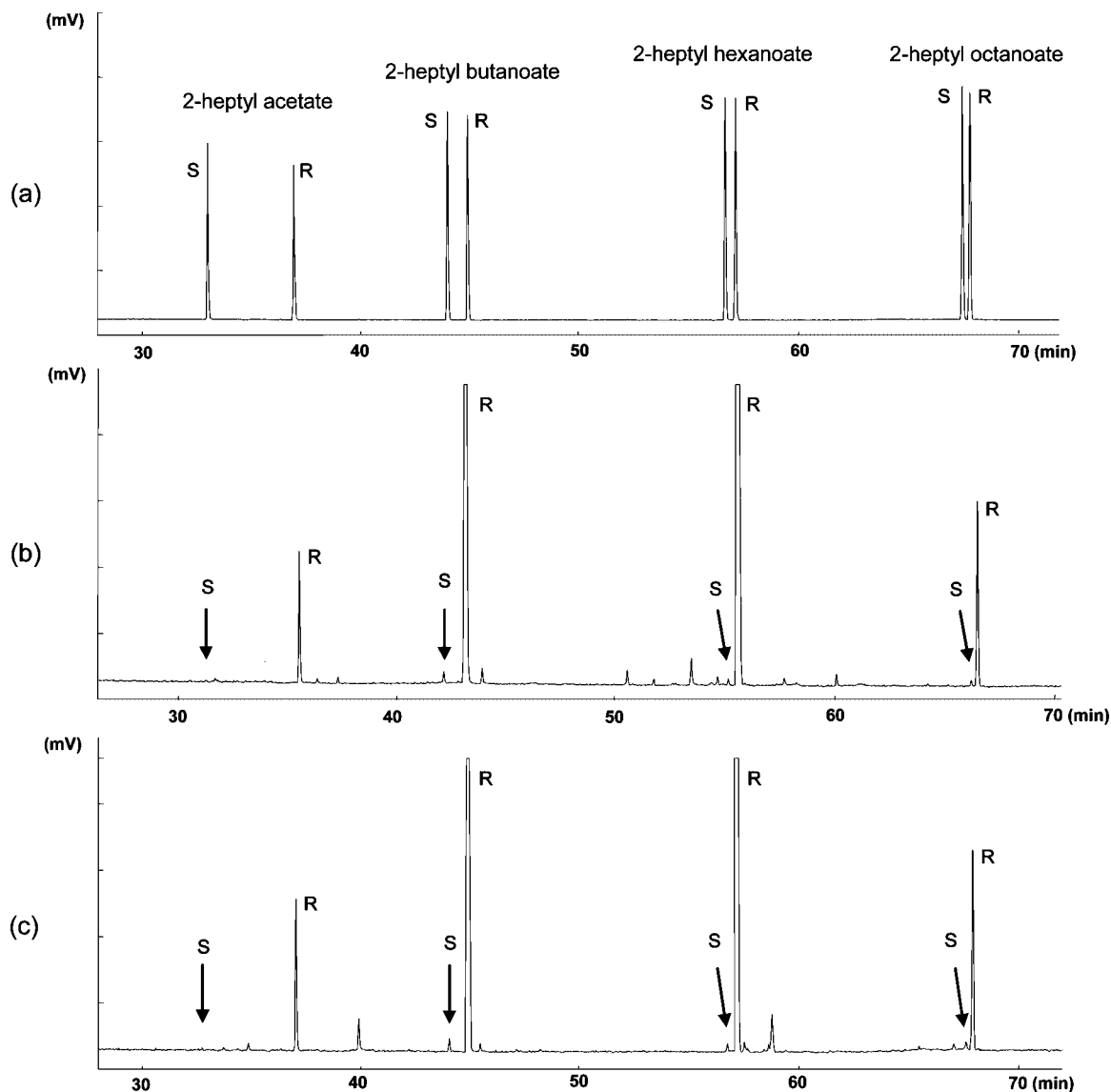
Secondary Alcohols in Passion Fruit

J. Agric. Food Chem., Vol. 55, No. 25, 2007   **10343**

Figure 2. Stereodifferentiation of 2-heptyl esters on heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin as chiral stationary phase; for GC conditions see Multidimensional Gas Chromatography under Materials and Methods: (**a**) racemic reference compounds; (**b**) compounds from SDE extract of purple passion fruits after transfer from DB-Wax; (**c**) compounds from SDE extract of purple passion fruits after transfer from DB-5.

Table 3. Enantiomeric Distributions of Secondary Alcohols and Their Esters Isolated from Purple Passion Fruits by Different Isolation Techniques

| | enantiomeric distributions[a] (%) | | | | | |
| | SDE[b] | | LLE[c] | | VHS[d] | |
compound	*R*	*S*	*R*	*S*	*R*	*S*
2-pentanol	60.2	39.8	58.6	41.4	59.2	40.8
2-heptanol	84.6	15.4	85.1	14.9	84.2	15.8
2-heptyl butanoate	99.4	0.6	99.4	0.6	99.4	0.6
2-heptyl hexanoate	99.8	0.2	99.8	0.2	99.8	0.2

[a] Triplicate analyses of enantiomers. [b] Simultaneous distillation−extraction. [c] Liquid–liquid extraction. [d] Vacuum headspace.

2-heptyl butanoate and 2-heptyl hexanoate and for the secondary alcohols 2-pentanol and 2-heptanol, the isolation procedure applied had no significant impact on the enantiomeric ratios.

Biogenetic Aspects. Investigations of the enantiomeric compositions via MDGC confirmed the predictions previously made on the basis of GC separations of diastereoisomeric derivatives of 2-heptanol (*11*): 2-Heptyl acetate, butanoate, hexanoate, and octanoate contained as typical volatile constitu-

ents in purple passion fruits possess (*R*)-configuration. The possibility to investigate the homologous series of individual esters revealed that this result is also valid for esters containing 2-pentanol and 2-nonanol as alcohol moieties. The sensitivity of the methodology also revealed that the esters are not optically pure. The proportions of (*S*)-enantiomers varied in different batches and were dependent on the alcohol moieties of the esters. The opposite configurations of free 2-heptanol in purple and yellow passion fruits could be confirmed. In addition, a similar phenomenon could be demonstrated for 2-pentanol.

The 2-alkyl esters may be biosynthesized from the corresponding alkan-2-ols by the action of alcohol acyltransferases (*27*). At first glance, the high concentrations of nearly optically pure (*R*)-2-alkyl esters in purple passion fruits suggest pronounced (*R*)-enantioselectivities of these enzymes. On the other hand, the only low amounts of (*S*)-configured free alkan-2-ols indicate that such esterifications may not proceed via "in vivo kinetic resolutions", that is, nearly selective esterification of one enantiomer and accumulation of the antipode as nonused substrate. The data suggest that the alkan-2-ols may already be present mainly as (*R*)-enantiomers. Stereospecific reductions of the corresponding alkan-2-ones, which have also been described

10344 *J. Agric. Food Chem.,* Vol. 55, No. 25, 2007

Strohalm et al.

as passion fruit constituents (*1, 2*), might be one pathway leading to optically enriched secondary alcohols (*11*).

In yellow passion fruits the obtained data sets on quantities and enantiomeric compositions of secondary alcohols and the esters detected as minor constituents suggest low activities of acyl transferases exhibiting preference for (*R*)-alkan-2-ols and starting from nearly racemic mixtures of the secondary alcohols as substrates.

The data elaborated indicate significant metabolic differences between passion fruits and bananas, another tropical fruit that contains secondary alcohols and the corresponding esters as typical volatile constituents. In bananas, consistently high excesses of the (*S*)-enantiomers of 2-pentanol and 2-heptanol and their acetates and butanoates have been described (*21, 22, 28, 29*). According to the reported sensory evaluations, the (*S*)-esters of secondary alcohols exhibit characteristic fruity, estery notes (*30*).

At this point assignments of the actual roles and the stereospecificities of enzymes involved in the biosynthesis of secondary alcohols and their esters remain speculative. However, the data elaborated in this study provide additional valuable details, and the analytical approach presented may serve as a useful tool assisting in the elucidation of pathways by molecular biological investigations.

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