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Investigation of important odorants of palm wine (*Elaeis guineensis*)

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Abstract

Palm wine (*Elaeis guineensis*) was sensorially evaluated and the key odorants were investigated by means of high resolution gas chromatography–olfactometry and mass spectrometry of solvent extracts as well as headspace samples. A total of 41 compounds were identified, 32 of them previously unknown in palm wine. From these, a total of 13 compounds were quantified by means of stable isotope dilution assays. Quantitation and calculation of the odour-activity values (OAVs) of the 13 key odorants revealed that the earthy-smelling 3-isobutyl-2-methoxypyrazine, the buttery-smelling acetoin, the fruity compounds ethyl hexanoate, 3-methylbutyl acetate and the popcorn-like-smelling 2-acetyl-1-pyrroline are likely to be important odorants of palm wine, with 3-isobutyl-2-methoxypyrazine, acetoin, and 2-acetyl-1-pyrroline being reported here for the first time as aroma constituents of palm wine.

Keywords: Gas chromatography–olfactometry; Stable isotope dilution assay; Palm wine; Aroma

1. Introduction

Palm wine is a whitish, effervescent, alcoholic beverage produced by the spontaneous yeast-lactic fermentation of the sugary sap of palm trees. It is indigenous to the tropical regions where palms grow, such as Africa, Asia and South America (Uzochukwu, Balogh, Tucknot, Lewis, & Ngoddy, 1994). In such areas, the beverage plays an important role in the culture of the people. Palm sap is transparent, with a sugar content of 100–144 g/kg, a pH of 7.0–7.4 and traces of ethanol. Palm wine, the fermented sap, is whitish and has a pH of about 3.6 and alcohol contents of 3.3–4.0%, depending on the stage of fermentation at which the wine is consumed. It contains a heavy suspension of yeast and bacteria, giving the wine a milky-white appearance (Okafor, 1975; Uzochukwu et al., 1994, Uzochukwu, Balogh, Tucknot, Lewis, & Ngoddy, 1999). These microorganisms metabolize the sugars in the fresh wine within 36–48 h and produce various organic acids and alcohols (Bassir, 1962). Many studies have been done in Nigeria and other African countries during the past 20 years on various aspects of palm wine (Esechie, 1979; Okafor, 1978; Van Pee & Swings, 1971) but, so far, very few published studies on the aroma compounds of the wine have been reported and even none on the aroma quantification. Samarajeewa, Adams, and Robinson (1981), reported the major volatiles in spirits prepared by distilling palm wine, obtained from coconut and boras palm (*Borassus flabellifer*). Uzochukwu et al. (1994, 1997, 1999) identified 82 volatile constituents in palm wine and also reported the roles of yeasts and bacteria on the wine volatiles. Also, Jirovetz, Buchbauer, Fleischhacker, and Ngassoum (2001), reported the volatile organic compounds of two different palm wine species from Cameroon. However, the chemical basis of palm wine aroma was not provided in all these studies. Numerous investigations (Buettner, 2004; Buettner & Schieberle, 1998, 2001; Guth, 1996, 1997) in this field have shown that the chemical elucidation of the key odorants responsible for the fine-tuned nasal sensation often require heavy analytical tasks. For instance, the characterization of key aroma compounds of two chardonnay wines

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and fresh hand-squeezed Valencia late oranges, respectively, by Buettner and Schieberle (1998), Buettner (2004) has to be mentioned. About 33 key odorants were characterized in chardonnay wines by means of gas chromatographic–olfactometric techniques, stable isotope dilution assays (SIDA) and aroma reconstitution experiments. This study affords a glimpse into how analytical procedure can lead to fundamental understanding of the chemical basics of wine aroma. To date, more than 80 volatile compounds have been identified in different palm wine varieties (Jirovetz et al., 2001; Uzochukwu et al., 1994). Systematic studies to indicate the odorants responsible for the characteristic bouquet of palm wine have not been reported. Since the aroma compounds that are characteristic for the distinct alcoholic, moody, non-typical floral fruity notes of palm wine are only small fractions of the volatile mixtures, isotope dilution assay (SIDA), as shown earlier (Schieberle & Grosch, 1987), is an accurate method for the quantification of flavour compounds that contribute significantly to the palm wine odour impression. Guth (1996) used SIDA for the quantification of wine lactone in various red and white wines and 42 wine odorants identified by Guth (1997) in Gewuerztraminer and Scheurebe wines were quantified by SIDA. To have an insight into the development of profiles of wine aroma during and after consumption, the modified exhaled odorant measurement (EXOM), which is a new approach for quantifying the degree of in-mouth release of food aroma compounds, was recently reported (Buettner & Schieberle, 2000). This approach allows the study of the release of even low concentration odorants *in vivo*. As a result of scientific investigations into the nutrient and microbial contents of palm wine in Nigeria, the palm wine industry in the country has grown from a peasant one to a modern cottage industry. An estimated 225×10^6 l is believed to be consumed in the country annually (Uraih, Izuagbe, & Ivbievbiokun, 1985). With time, it is expected that demand will surmount supply and there might be a need for a synthetic palm wine to fill the gap. The aim of this study was to investigate: (1) the key odorants of palm wine by high resolution gas chromatography–olfactometry (HRGC/O) of solvent extract and head space analysis and (2) to quantify selected palm wine odorants by means of stable isotope dilution assays.

2. Materials and methods

2.1. Materials

Three samples of bottled palm wine (*Elaeis guineensis*) (1.5 l) were purchased at a wine shop in Nigeria and dispensed into 45 ml glass-tubes and stored at −20 °C until analysis.

2.2. Chemicals

The following odorants were obtained from the suppliers shown.

Methyl butanoate, 99%; 2-/3-methyl-1-butanol, 98%; ethyl hexanoate, 97%; acetoin, 98%; ethyl lactate, 99%; 3-methylthio-1-propanal (methional) 98%; 3-isobutyl-2-methoxypyrazine, 70%; 3-methylbutyl acetate, 99%; linalool, 98%; butanoic acid, 97%; 2-/3-methylbutanoic acid, 98%; 2-methoxyphenol, 98%; 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, 98%; 3-methylpentanoic acid, 98%; ethyl cinnamate, 98%; diethyl succinate, 99+%; phenylacetic acid, 99%; 2-/3-methylbutanal, 98%; ethyl 2-methylbutanoate, 99%; hexyl methylbutanoate, 97%; methylpropanoic acid, 98%; (*E*,*E*)-2,4-nonadienal, 99%; 1-hexanol, 98%; 3-mercapto-2-methylpentanone, 98%; γ-dodecalactone, 98%; ethyl pentanoate, 99%; 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolone), 98% (Aldrich, Steinheim, Germany), 2,3-butandione, 99%; 2-acetylpyridine, 99+%; pentanoic acid, 98%; (Fluka, Neu-Ulm, Germany), 2-ethyl-3,5-dimethylpyrazine, 98%; 2-phenylethanol, 99% (Acros Organics, New Jersey, USA), acetic acid 99%; 4-methoxy methylphenol, 98% and vanillin, 98% (Merck, Darmstadt, Germany) β-damascenone, 98% (Symrise, Holzminden, Germany). The compounds were freshly distilled prior to analysis. Chemical and sensory purity were checked by high resolution gas hromatography–olfactometry (HRGC/O), as well as high resolution gas chromatography–mass spectrometry (HRGC/MS).

2.3. Syntheses

The following compounds were synthesized according to the literature cited in brackets: (*Z*)-Octa-1,5-dien-3-one (Ullrich & Grosch, 1988), 2-acetyl-1-pyrroline (Buttery, Ling, & Juliano, 1982).

2.4. Stable isotope labelled standards

The following labelled internal standards were synthesized according to literature: [$^{13}C_4$]-acetoin (intermediate product on the synthetic path to [$^{13}C_4$]-diacetyl according to (Schieberle & Hofmann, 1997)), 2-[$^2H_{2-5}$] acetyl-1-pyrroline (Schieberle & Grosch, 1987), ($^2H_3$)-3-methylbutanol (Semmelroch & Grosch, 1996), 2-phenyl (1,1 $^2H_2$) ethanol (Semmelroch & Grosch, 1996), 2-($^2H_3$)-methoxyphenol (Schieberle, 1993), 3-methyl (3,4-$^2H_2$) butylacetate (Schieberle, 1993), (2,2,2-$^2H_3$) ethyl hexanoate (Schieberle, 1993), ($^2H_3$)-vanillin (Semmelroch, Laskawy, Blank, & Grosch, 1995), ($^{13}C_2$) phenylacetic acid (Aldrich, Steinheim, Germany), tetrahydrolinalool (Sen & Grosch, 1991), 2-[2,2,2-$^2H_3$] ethyl-3,5-dimethylpyrazine (Cerny & Grosch, 1993), 3-isobutyl-2-[$^2H_3$]methoxypyrazine (Semmelroch & Grosch, 1996), [$^2H_2$]-methylpropanoic acid (Zimmermann, 2001).

The concentrations of the labeled internal standards and the response factors (FID) were determined gaschromatographically using methyl octanoate as the internal standard, as described recently (Buettner & Schieberle, 2001). The calibration factors for the labelled compounds were

calculated as reported by Sen and Grosch (1991), and are given in Table 1.

2.5. Panellists

Panellists were non-pregnant volunteers (non-smokers) of the Technical University of Munich, exhibiting no known illnesses at the time of examination and with normal olfactory and gustatory function. Subjective aroma perception was normal in the past and at the time of examination. The panellists had a normal salivary flow and were selected for their excellent oral hygiene. Ten assessors (five males, five females) were recruited and trained in preceding weekly training sessions in recognizing about 150 selected, orthonasally and retronasally, about 150 selected odorants at different odorant concentrations, according to their odour qualities. Participation in these sessions was at least for 1 year prior to participation in the actual sensory experiments.

2.6. Isolation of palm wine volatiles

Palm wine (100 ml) was extracted with dichloromethane (100 ml) three times, total volume (300 ml), the combined extract dried over Na_2SO_4 and finally concentrated to 100 ml by distilling off the solvent over a Vigreux Column at 45 °C. The volatile fraction was isolated by high vacuum distillation using the apparatus described recently (Engel, Bahr, & Schieberle, 1999).The distillate was concentrated at 45 °C to 400 μl by using a small size Vigreux Column (Schieberle & Hofmann, 1997); 1 μl of this extract was used for the sniffing experiments.

2.7. Static headspace analysis-gas chromatography/olfactometry (SH-HRGC/O)

Palm wine (15 ml) was equilibrated in a septum-sealed vessel (100 ml total volume) for 30 min at room temperature. Using fresh 15 ml portions of wine in each experiment, decreasing headspace volumes (10–0.32 ml) were taken off by means of gas-tight syringes, then cryofocussed on a fused silica trap (TCT-PTI-system 4001; Chrompack, Mühlheim, Germany) and finally injected onto a fused silica column DB-5 (SE-54; 30 m × 0.32 mm i.d., 0.25 μm FD, J & W Scientific, Folsom, USA) (Schieberle, 1993). After injection, the temperature of the oven was held at 0 °C for 2 min, then raised at 6 °C/min to 200 °C. At the end of the column, the effluent was split 1:1 (by vol.) onto two uncoated but deactivated fused silica capillaries (50 cm × 0.32 mm) leading to a FID and a sniffing port. The perceived odours were attributed to the odorants identified in the preceding experiments using the solvent extract, by means of odour quality, odour intensity, and retention index. Identification was further based on mass spectrometric identification as described below.

2.8. High resolution gas chromatography–olfactometry

Application of the samples was performed by the cool-on-column injection technique at 35 °C (solvent extract sample). The odorants were screened in parallels by three panellists by sniffing the effluent after one- or two-dimensional gas chromatographic separation. Sniffing analysis was repeated twice by each panellist. All detected odorants were identified by comparison with reference substances on the basis of the following criteria: retention index (RI) on two stationary phases of different polarity (FFAP, SE-54), mass spectra obtained by MS (EI) and MS (CI), and odour quality, as well as odour intensity, perceived at the sniffing port. The one- or two-dimensional gas chromatography system (TD/HRGC) consisted of a mega 2 gas chromatograph (Fisons Instruments, Mainz- Kastel, Germany) as the precolumn system in tandem with a Fisons GC 5160 as the main column system. The following fused silica

Table 1
Mass traces and calibration factors of potent odorants in palm wine used for the quantitation by SIDA

Number	Odorant[a]	Mass traces (*m/z*)		Calibration factors[b]
		Unlabelled	Labelled	
1	3-Methylbutanol	71	74	0.88
2	Ethyl hexanoate	145	148	1.0
3	Acetoin	87	89	1.0
4	2-Acetyl-1-pyrroline	112	114–117	0.98
	2-Acetylpyridine	122	125	0.97
5	2-Ethyl-3,5-dimethyl pyrazine	137	140	1.0
7	3-Isobutyl-2-methoxy pyrazine	167	170	0.95
8	3-Methylbutyl acetate	131	133	0.79
9	Linalool[c]	137	141	1.61
10	Methylpropanoic acid	75	79	1.0
11	2-Methoxyphenol	125	128	1.0
12	2-Phenylethanol	105	107	1.0
13	Phenylacetic acid	137	139	1.0

[a] Compounds were determined using the respective stable isotope labeled standards by means of an ion trap detector ITD-800 (Finnigan, Bremen, Germany) running in the CI-mode with methanol as reagent gas.
[b] The calibration factor was determined as reported previously (Guth & Grosch, 1993).
[c] For quantification of linalool the internal standard tetrahydrolinalool was used as described in the experimental section.

columns were used: DB-FFAP (30 m × 0.32 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, USA) and DB-5 (SE-54; 30 m × 0.32 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, USA). The gas chromatographic conditions were the same as described previously (Buettner & Schieberle, 2001).

2.9. Aroma extract dilution analysis

The FD factors of the odour-active compounds were determined by AEDA (Schieberle, 1995) of the following dilution series: the original palm wine extract (400 μl) from 600 ml of palm wine was diluted with dichloromethane (1 + 1) until no odorant of palm wine was detectable by sniffing of the highest dilution. HRGC/O was performed with aliquots (0.5 μl), using capillary DB-FFAP. In total, three experienced sniffers were used to perform the AEDA experiments. Their response to the individual compounds did not differ by >2 FD factors.

2.10. Identification of volatile compounds

Compounds were identified by comparison with the reference substances on the basis of the following criteria: retention index (RI) on two stationary phases of different polarities, mass spectra obtained by MS (EI) and MS (CI), and odour quality, as well as odour intensity, perceived at the sniffing port. Odour intensity was checked by GC/O and by comparing the FID signal caused by a defined amount of each reference aroma compound.

2.11. High resolution gas chromatography–mass spectrometry

The odorants were analysed by two-dimensional gas chromatography (TD/HRGC) as described above. MS analyses were performed with an ITD-800 (Fison Instruments, Mainz-Kastel, Germany) running in the CI-mode with methanol as the reagent gas. The following fused silica columns were used: DB-FFAP (30 m × 0.32 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, USA) in combination with DB-5 (SE-54; 30 m × 0.32 mm i.d., film thickness 0.25 μm, J&W Scientific, Folsom, USA). The gas chromatographic and mass spectrometric conditions were the same as described previously (Buettner & Schieberle, 2001).

2.12. Quantitation by stable isotope dilution assays (SIDA)

Quantitation using the respective stable isotope labeled standards was performed as described earlier (Buettner & Schieberle, 2001).

2.13. Sensory evaluation

Sensory analyses were performed in a sensory panel room at 21 °C at three different sessions. Samples (100 ml) were freshly opened and singly presented to the sensory panel for orthonasal evaluation in covered glass vessels (capacity 45 ml) as described recently (Guth & Grosch, 1993). For retronasal evaluation, 25 ml aliquots of the sample were taken into the oral cavity, kept for 10 s with closed lips and rinsed carefully within the oral cavity, then swallowed. The overall intensity of single predefined odour qualities was rated by the panellists. Panellists were always asked to score odour intensities on a linear scale from 0.0 (not perceivable) to 9.0 (very intense). The results obtained in three different sessions were averaged and plotted as histograms. The values obtained in different sessions and for the different assessors differed by not more than 10%.

2.14. Retronasal sensory evaluation

Freshly opened palm wine (100 ml), was singly presented to panellists at three different sessions for retronasal evaluation. Palm wine aliquots of 25 ml were taken into the oral cavity, kept for 10 s with closed lips and rinsed carefully within the oral cavity, then swallowed.

2.15. Determination of odour thresholds

Odour thresholds (orthonasal and retronasal) were determined in water by using the triangle test (Schieberle & Hofmann, 1997). The samples were presented in order of increasing concentrations in 1:1 dilution steps. Determinations were performed in three separate sessions and values were averaged.

3. Results and discussion

3.1. Orthonasal and retronasal sensory evaluation of palm wine

Sensory evaluation of palm wine revealed very peculiar aroma profiles which are characteristic for this type of beverage (cf. Fig. 1). In orthonasal mode, the profile was dominated by the acidic and yeast-like character of the palm wine, with additional citrussy and fruity notes, while also some slight nutty impressions were perceivable. During retronasal evaluation, the overall character changed significantly to citrussy and fruity, while the acidic and yeast-like qualities were comparatively lower. There was a strong increase recognized by the sensory panel in the nutty impression. Apart from that, also a popcorn-like aspect was perceived.

3.2. Identification of the potent palm wine odorants in solvent extracts

To elucidate the molecular principles for the observed aroma impressions, the potent odorants of palm wine were first isolated by means of solvent extraction, high vacuum distillation and concentration procedures, and were subse-

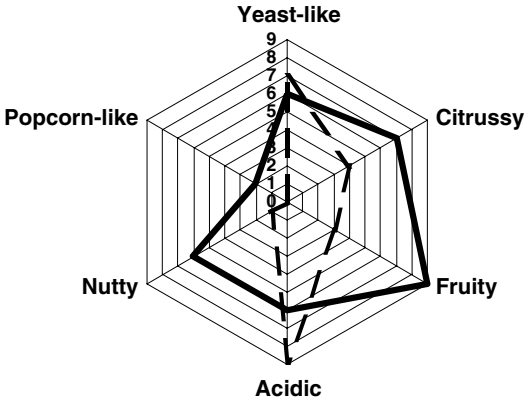

Fig. 1. Orthonasal (dotted line) versus retronasal (solid line) sensory evaluation of the main aroma attributes of palm wine.

quently analysed by means of gas chromatography–olfactometry, as well as mass spectrometry. This approach led to the detection and unequivocal identification of a total of 41 potent odorants in palm wine (cf. Fig. 2). These compounds, together with their odour qualities and retention indices are given in Table 2. The flavour compounds revealed malty (nos. 1, 2 and 7), fruity (nos. 3, 5, 6, 8, 14, 18, 37 and 38), smoky-phenolic (nos. 30 and 41), buttery (nos. 4 and 9), sweaty (nos. 21, 22, 23, 26 and 33), popcorn-like (nos. 11 and 19), cooked-potato-like (nos. 16 and 27) and gravy-meaty (no. 28) odour qualities. Also, diverse sweet, vanilla- or honey-like/flowery compounds were detected (nos. 20, 29, 31, 32, 34, 35, 37, 39 and 40). It is noteworthy that aroma compounds nos. 11 and 28 with popcorn-like and gravy-meaty notes failed to yield any signal at the flame ionization detector. This indicated very low odour thresholds for both compounds. The two odour-active compounds were later characterized by co-injection with the reference compounds on different HRGC stationary phases (cf. Table 2) and comparing their odour qualities and their odour potencies at the sniffing port, and were identified as the popcorn-like-smelling 2-acetyl-1-pyrroline and 3-mercapto-2-methyl pentanone, respectively. 3-Mercapto-2-methylpentanone with its characteristic gravy-meaty odour quality, the popcorn-like-smelling compounds 2-acetyl-1-pyrroline and 2-acetylpyridine, and most of the remaining odour-active compounds were identified for the first time in palm wine (*E. guineensis*). However, some of the esters, namely ethyl pentanoate (no. 6), ethyl hexanoate (no. 8), ethyl lactate (no. 10) and 3-methylbutyl acetate (no. 18) were recently identified in palm wine (Uzochukwu et al., 1994; Jirovetz et al., 2001).

3.3. Aroma extract dilution analysis

Screening for the most potent odorants in the palm wine extract by means of AEDA revealed the buttery-smelling acetoin, the popcorn-like-smelling 2-acetyl-1-pyrroline, and the earthy-smelling 3-isobutyl-2-methoxypyrazine as those compounds with the highest FD factor (256), followed by the banana-like-smelling 3-methylbutyl acetate, the sweaty-smelling methylpropanoic acid, and the

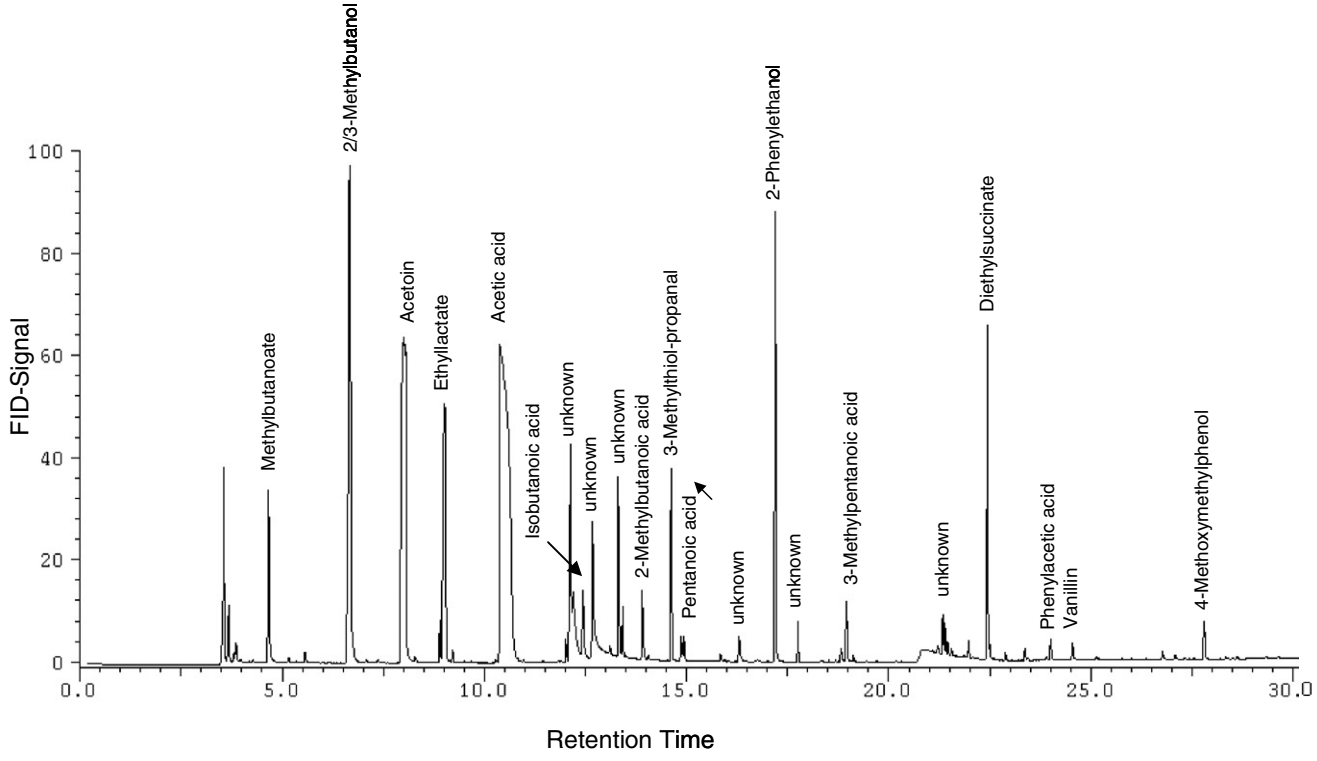

Fig. 2. Characteristic gas chromatogram of solvent extracted palm wine (*Elaeis guineensis*).

Table 2
Most odour-active volatiles in solvent extracted palm wine

Number	Odorant[a]	Odour quality[b]	FD	Retention index		Previously identified in *E.guinensis*[c]
				FFAP	SE-54	
1	2-Methylbutanal	Malty	32	912	663	
2	3-Methylbutanal	Malty	32	927	652	
3	Methylbutanoate	Sweet-fruity	16	981	723	
4	2,3-Butandione	Buttery	32	993	592	1
5	Ethyl 2-methylbutanoate	Fruity	32	1040	852	1
6	Ethyl pentanoate	Sweet-fruity	16	1067	900	
7	2-/3-Methylbutanol	Malty	64	1213	738	10
8	Ethyl hexanoate	Fruity	64	1226	1001	1, 10
9	Acetoin	Buttery	256	1275	nd	
10	Ethyl lactate	Phenolic/smoky	8	1321	nd	1
11	2-Acetyl-1-pyrroline[d]	Popcorn-like	256	1323	922	
12	1-Hexanol	Carrot-like	4	1356	872	
13	Acetic acid	Sweaty	16	1428	600	1, 10
14	Hexyl-3-methylbutanoate	Fruity	16	1430	1244	
15	2-Ethyl-3,5-dimethylpyrazine	Earthy	64	1451	1083	
16	3-Methylthio-1-propanal	Cooked-potato	32	1460	nd	
17	3-Isobutyl-2-methoxypyrazine	Earthy	256	1517	1175	
18	3-Methylbutyl acetate	Banana-like	128	1527	878	10
19	2-Acetylpyridine	Popcorn-like	64	1552	1031	
20	Linalool	Fresh-flowery	64	1540	1103	10
21	Methyl propanoic acid	Sweaty	128	1563	nd	
22	Butanoic acid	Sweaty-buttery	8	1619	821	
23	2-/3-Methylbutanoic acid	Sweaty	32	1661	875	
24	(*Z*)-1,5-Octadien-3-one	Geranium leaf-like	4	1676	984	
25	(*E*,*E*)-2,4-Nonadienal	Fatty	8	1718	1215	
26	Pentanoic acid	Sweaty	16	1720	911	
27	3-Methylthio-1-propanol	Cooked-potato	32	1723	905	
28	3-Mercapto-2-methylpentanone[d]	Gravy-meaty	16	1742	883	
29	β-Damascenone	Flowery	16	1801	1389	
30	2-Methoxyphenol	Smoky	64	1842	1089	
31	2-Phenylethanol	Honey-like	128	1911	1117	10
32	4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	Caramel-like	16	2038	1070	
33	3-Methylpentanoic acid	Sweaty	8	2040	nd	
34	2-Ethyl-4-hydroxy-5-dimethyl-3(2*H*)-furanone	Caramel-like	16	2095	1159	
35	Ethylcinnamate	Sweet	8	2167	1469	
36	3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone	Spicy, savory-like	8	2190	1110	
37	Diethylsuccinate	Sweet, pineapple-like	4	2390	nd	
38	γ-Dodecalactone	Fruity	32	2424	1497	
39	Phenylacetic acid	Honey-like	64	2577	1262	
40	Vanillin[d]	Vanilla-like	16	2601	1404	
41	4-Methoxy-2-methylphenol	Phenolic	8	2639	nd	

nd: Not determined.

[a] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on different stationary phases as given in the table, mass spectra obtained by MS (EI) and MS (CI), and odour quality as well as odour intensity perceived at the sniffing port.

[b] Odour quality perceived at the sniffing port.

[c] Reported in the literature as volatile compounds of palm wine (*E. guineensis*) in Jirovetz et al. (2001) and Uzochukwu et al. (1994).

[d] The MS signals were too weak for an unequivocal interpretation. The compounds were identified on the basis of the remaining criteria given in footnote a.

honey-like-smelling 2-phenylethanol. Very olfactorily diverse compounds were perceived with FD 64, such as the malty-smelling methylbutanols, the fruity ethyl hexanoate, linalool with flowery odour impression, and many more. It is interesting to note that, in agreement with the sensory diversity of odour impressions in the palm wine (see sensory evaluation of palm wine), also a broad chemical diversity was perceived, with odorants ranging from nutty-earthy-popcorn-like impressions, to fruity-flowery and citrussy aroma qualities.

3.4. Static headspace analysis/olfactometry (SHO)

The application of static headspace/olfactometry (SHO) revealed 18 odorants as contributing to the "top-note" of the palm wine (cf. Table 3). These odour-active compounds were also detected as potent odorants in the solvent extract, and were comprised predominantly of the ester fraction of the palm wine aroma constituents. Generally, most compounds exhibited fruity, flowery and sweet impressions (11 compounds). It is also interesting to note that the fruity

Table 3
Static headspace analysis/olfactometry (SHO/O) of palm wine

Number	Odorant[a]	Odour quality[b]	Retention index SE-54
1	2,3-Butandione	Buttery	592
2	Acetoin	Buttery	718
3	Butanoic acid	Sweaty-buttery	821
4	Ethyl-2-methyl butanoate	Fruity	852
5	2-/3-Methylbutanol	Malty	873
6	3-Methylbutyl acetate	Banana-like	878
7	Ethyl pentanoate	Sweet-fruity	900
8	3-Methylthio-1-propanal	Cooked potato-like	905
9	2-Acetyl-l-pyrroline	Popcorn-like	922
10	Methyl butanoate	Sweety-fruity	982
11	Linalool[c]	Fresh-flowery	1102
12	3-Hydroxy-4,5-dimethyl-2(5*H*)-furanone	Spicy, savory-like	1109
13	Ethyl hexanoate	Fruity	1115
14	2-Phenylethanol	Honey-like	1117
15	Hexyl-3-methylbutanoate	Fruity	1244
16	Phenylacetic acid	Honey-like	1262
17	β-Damascenone	Flowery	1389
18	Vanillin[c]	Vanilla-like	1404

[a] The compounds were identified by comparing their mass spectra, retention index, and odour quality with reference compounds.
[b] Odour quality as perceived at the sniffing port.
[c] MS signals were too weak for an unequivocal interpretation. The compounds were identified on the basis of the remaining criteria in footnote a.

smelling esters, methyl butanoate, ethyl pentanoate and ethyl hexanoate, as well as acetoin, produced the most intense odour qualities (data not shown). Apart from that, no further odorants were detected by SHO.

3.5. Quantitation of potent odorants

The volatile profile of palm wine is largely dominated by alcoholic substances such as ethanol, 2-/3-methylbutanol and 2-phenylethanol, as well as acetic acid. Apart from those, major peaks were due to methyl butanoate, acetoin, diethyl succinate, ethyl lactate, and several acids, such as isobutanoic acid, 2-methylbutanoic acid, 3-methylpentanoic acid, phenylacetic acid and pentanoic acid, coeluting with methional. There were also some unidentified peaks which were not further studied as they were completely odourless. Due to the complexity of the volatile fraction and the large differences in concentration, volatility and reactivity of the odorants, it is not possible in most cases to quantify the odorants precisely by using conventional methods (Schieberle, 1995). However, precise quantitative measurements of the palm wine odorants were performed by the stable isotope dilution assay (SIDA). Thirteen odorants were quantified (Table 4). Compounds were selected that were previously unknown in palm wine and were palpable due to their odour intensities during gas chromatographic–olfactometric evaluation, both from the solvent extracts, as well as from headspace analysis (data not shown). Special focus was laid on those compounds which were presumably associated with the nutty-popcorn-like odour qualities observed during retronasal evaluation. It was found that acetoin and 3-methylbutanol were the most abundant aroma compounds, followed by 2-phenyl ethanol and to lesser extents methylpropanoic acid and phenylacetic acid. Odorants presenting extremely low concentrations were 2-methoxyphenol (guaiacol), 2-ethyl-3,5-dimethyl pyrazine and 2-acetyl pyridine. With intermediate concentrations in the range of about 10–100 μg/l, the compounds linalool, 2-acetyl-1-pyrroline, 3-isobutyl-2-methoxypyr-

Table 4
Concentrations and odour-activity values (OVA's) of potent odorants of palm wine

Number	Odorants	Concentration (μg/l)[a]	Odour threshold in water (μg/l)[b]		OAVs[c]	
			o	r	o	r
1	3-Methylbutanol	18,300	1000	250	18	73
2	Ethyl hexanoate	52.2	0.5	0.5	104	104
3	Acetoin	663,500	800	nd	830	nd
4	2-Acetyl-1-pyrroline	11.4	0.1	nd	114	nd
5	2-Acetylpyridine	0.32	19	nd	<1	nd
6	2-Ethyl-3,5-dimethylpyrazine	0.47	0.16	0.08	3	6
7	3-Isobutyl-2-methoxypyrazine	12.0	0.005	nd	2400	nd
8	3-Methylbutyl acetate	61.7	0.88	3	70	21
9	Linalool	11.2	6	1.5	2	8
10	Methylpropanoic acid	1680	8100	1000	<1	2
11	2-Methoxyphenol	0.28	3	0.75	<1	<1
12	2-Phenylethanol	5880	1000	45	6	131
13	Phenylacetic acid	417	10,000	nd	<1	nd

nd: Not determined.
[a] Data are mean values of at least duplicates.
[b] Odour thresholds as reported in the literature (Rychlik et al., 1998).
[c] The OAVs (o: orthonasal; r: retronasal) were calculated by dividing the concentration of the odorants by their ortho- and retronasal thresholds in water.

azine, ethyl hexanoate and 3-methylbutyl acetate were found.

The high concentration of acetoin in the palm wine was not unexpected as palm wine is known to contain the yeast Kloeckera apiculata (Van Pee & Swings, 1971) which has the ability to produce large amounts of acetoin (Romano, Suzzi, Zironi, & Comi, 1993). Quantitative data for the other compounds determined here have previously not been available.

3.6. Calculation of odour activity values (OAVs)

To estimate the respective contribution of the odorants to the palm wine aroma profile, the OAVs of the odorants were calculated on their nasal and retronasal odour thresholds in water (cf. Table 4). The OAVs indicated that 3-isobutyl-2-methoxypyrazine, acetoin, and to a lesser extent 2-acetyl-1-pyrroline, ethyl hexanoate and 3-methylbutyl acetate, contributed intensely to the palm wine aroma. Interestingly, odorant compounds with high concentrations in the palm wine, such as 3-methylbutanol, 2-pheny1ethanol, 2-ethyl-3,5-dimethyl pyrazine and linalool, still exceeded their orthonasal odour threshold values, but gave relatively low orthonasal OAV's so that their contributions to the overall orthonasal aroma impression of palm wine can be assumed to be low. Nevertheless, it has to be noted that the retronasal OAVs especially of 3-methylbutanol and 2-phenylethanol, are comparatively higher than their corresponding orthonasal ones, so that they might not play such an important role when sniffing the palm wine, but might be of more impact when consuming the wine. Retronasally perceived odorants, in addition to taste substances, are involved in the whole flavour impression during the palm wine consumption. A series of interaction phenomena, such as additive, synergistic or suppressive effects, are well-documented, so that the presented OAVs do not allow a direct prediction of the odorant's contribution to the palm wine aroma sensations. However, they can be regarded as the key to further investigations and sensory experiments in this field of research.

3.7. Conclusions

A total of 41 odour-active compounds were identified in palm wine, many of them previously unidentified in palm wine. Quantitation and odour activity values (OAVs) of 13 key odorants of palm wine revealed that earthy-smelling 3-isobutyl-2-methoxypyrazine, buttery-smelling acetoin, fruity ethylhexanoate, 3-methylbutylacetate and popcorn-smelling 2-acetyl-1-pyrroline were the most potent odorants of those quantified in the palm wine sample.

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