International Journal of Mass Spectrometry 282 (2009) 45–49

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

# In vivo analysis of palm wine (*Elaeis guineensis*) volatile organic compounds (VOCs) by proton transfer reaction-mass spectrometry☆

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ARTICLE INFO

*Article history:*
Received 16 December 2008
Received in revised form 6 February 2009
Accepted 6 February 2009
Available online 20 February 2009

*Keywords:*
PTR-MS
Palm wine
Volatile organic compound
Fragmentation pattern

ABSTRACT

The in vivo volatile organic compounds (VOCs) release patterns in palm wine was carried out using the PTR-MS. In order to analyze the complex mixtures of VOCs in palm wine, the fragmentation patterns of 14 known aroma compounds of palm wine were also investigated. Results revealed masses *m*/*z* (43, 47, 61, 65, 75, 89 and 93) as the predominant ones measured in-breathe exhaled from the nose, during consumption of palm wine. Further studies of aroma's fragmentation patterns, showed that the *m*/*z* 43 is characteristic of fragment of various compounds, while *m*/*z* 47 is ethanol, *m*/*z* 61 (acetic acid), *m*/*z* 65 (protonated ethanol cluster ions), *m*/*z* 75 (methyl acetate), *m*/*z* 89 (acetoin) and *m*/*z* 93 (2-phenylethanol) respectively. The dynamic release parameters ($I_{max}$ and $t_{max}$) of the 7 masses revealed significant ($P = 0.05$) differences, between maximum intensity ($I_{max}$) and no significant ($P = 0.05$) differences between $t_{max}$ among VOCs respectively.

## 1. Introduction

Palm wine also called palm Toddy or simply Toddy is an alcoholic beverage created from the sap of various species of palm tree. Palm wine is a refreshing beverage enjoyed by people in parts of Africa, Asia and South America [1]. It has a milky flocculent appearance due to its high concentration of yeast which thus serves as a rich dietary source of vitamins of the 'B' complex [2]. For instance an estimated $225 \times 10^6$ L is believed to be consumed in Nigeria annually [3]. From a consumer perspective, the most appealing features of palm wine are its flavor, and nutrition. Food flavor appreciation is one of the first evaluation signals along with food appearance and texture encountered by consumers during eating of food [4]. Furthermore, it is well known that this food's characteristic strongly influences consumer acceptability/preference judgment. Information on the flavor chemistry of natural and synthetic palm wine during production and consumption is increasingly important for the palm wine industries in determining optimal production and maintenance of acceptable flavors.

Also, an objective of palm wine research is to find chemical markers for the wine, which correlate with the sensory assessment of expert wine tasters and which drive consumer preferences. Currently, controls on aroma are still being carried out by human experts, who check and judge the bouquet of wine by perceiving the volatile compounds in the headspace. The need for an automatic technique which can reproduce the sensitivity of the human nose is highly important. One very interesting technique with rather high sensitivity and/or which can be used for this purpose is the proton transfer reaction-mass spectrometry (PTR-MS). PTR-MS is a relatively new MS technique which basically implements $H_3O^+$ as the ionizing agent in a chemical ionization [5]. The advantage of using $H_3O^+$ as the primary ion lies in its non-dissociative proton transfer reaction with most volatile organic compounds, whereas it does not react with any natural components of air. Compared to conventional MS, the non-dissociative character of the proton transfer reactions leads to less complex spectra and opens the opportunity to skip a previous separation of the compounds [5]. Detection limits of a few ppbv allow an analysis of the headspace without any previous concentration step. Therefore, PTR-MS is a very fast and powerful tool for obtaining real-time data for example, in process control and time intensity studies [6]. It has been used for food analysis, especially for the determination of flavor volatiles [6]. Recently, PTR-MS has been used to evaluate volatiles in the head space of extra virgin and rancid olive oils in order to detect oxidative alterations [7] and geographical origin classification [8] of olive oils respectively. PTR-MS has variously been used in the classification of butter and butter oil [9], identification of strawberry cultivars [10] and to pre-

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☆ *Industrial relevance*: This study is of relevance because PTR-MS is a very fast and powerful tool for obtaining real-time data for example in process control and time intensity studies. Also, the PTR-MS has the capability to detect low odor threshold compounds that are perceived by the human nose but are not detected by FID or by any other instrumental detector.

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doi:10.1016/j.ijms.2009.02.005

dict the sensory profile of espresso coffee [11]. The role of PTR-MS as a useful on-line monitoring tool for benzene formation in stored beverages [12] has also been reported. Furthermore, the capability of the PTR-MS method to detect low odor threshold compounds that are perceived by the human nose but are not detected by FID or by any other instrumental detector has been reported [13].

In the present study, PTR-MS technique is applied to define the flavor profile of in-breathe exhaled from the nose during consumption of palm wine. To analyze the complex mixtures of volatile compounds in palm wine, basic data on the behavior of individual compounds is essential. Therefore, the fragmentation patterns of 14 individual volatile flavor compounds earlier identified in palm wine [14] were first evaluated.

## 2. Materials and methods

### 2.1. Materials

Three bottled palm wine (4% vol, 1.5 L) were purchased at a wine shop in Nigeria and dispensed into a hundred 45 mL glass-tubes and stored at −20 °C until analysis.

### 2.2. Panelists

Four panelists (two males, two females, ages 22–40 years, non smokers) were from the Technical University of Munich. They exhibited no known illnesses at the time of examination and normal olfactory and gustatory function. In regular weekly training sessions, panelists were tested for their sensory performance with selected suprathreshold aroma solutions prior to participation in the experiments, whereas subjective aroma perception was tested with a defined set of aroma substances.

### 2.3. Proton transfer reaction-mass spectrometry of palm wine

PTR-MS (Ionicon Analytik, Austria) has been described in detail elsewhere [6,12,15]; therefore, only a brief summary will be given here. The key elements of successful operation of PTR-MS are: an intense source of $H_3O^+$ primary ions, giving $10^6$ counts $S^{-1}$ primary ions; a cross-section for proton transfer which ensures unit conversion efficiency of primary ions to secondary ions on every collision; a mass analyzer which selects the ion peaks specific to the trace compounds present in the exhaust gas; and an electron multiplier (EM) detector operated in pulse counting mode with single particle detection efficiency. By optimizing the characteristics of all these parts, we achieved real time sub-ppb detection sensitivity of VOCs in air. As confirmed by the experiments, the formation of spurious chemical compounds due to reactions occurring inside the drift tube could be considered negligible unless the concentration of the compounds under study becomes large. The quantity measured with PTR-MS is usually the intensity of a protonated compound, on the mass of which information is obtained. This does not directly allow the definite identification of the compound itself because there are large numbers of compounds having the same nominal mass. Whenever qualitatively unknown mixtures of compounds have to be investigated, the problem of identification becomes a crucial one. Thus, PTR-MS is primarily a method for on-line monitoring of compounds rather than for gas analysis.

The headspace of a 20 mL palm wine aliquot contained in a temperature stabilized (29 °C) 40 mL vial (Supelco) capped with a Teflon Septum was exchanged with ambient air with a continuous flow of 2 mL min$^{-1}$. This was flushed with nitrogen gas, and transferred through a heated capillary line directly into the reaction chamber. Five repeat measurements were performed. The PTR-MS operated at standard conditions (drift tube voltage: 600 V, pressure: 730 mbar. $U_2$: 150 V, $U_3$: 80 V, SEM: 3050 V, inlet flows: 50 mL min$^{-1}$).

### 2.4. PTR-MS of pure volatile organic compounds (VOCs)

To assign character to the obtained ions from palm wine headspace, PTR-MS spectra of some pure VOCs were first determined. An aqueous solution (2 g L$^{-1}$) of the individual volatile compounds (10 mL) was placed in a glass vial (100 mL). The headspace was drawn at 50 mL min$^{-1}$, 15 mL min$^{-1}$ of which was led into the PTR-MS. Samples were analyzed according to the method described by Lindinger et al. [6], and while employing a constant drift voltage of 600 V. Transmission of the ions through the quadrupole was considered according to the specification of the instrument. Background and transmission corrected spectra were averaged over five cycles. Presented PTR-MS spectra (Table 1) were obtained by normalizing the most abundant mass fragment to an intensity of 100.

### 2.5. In vivo flavor release analysis

Nose-space analysis aims at sampling and analyzing the air exhaled through the nose while palm wine is being consumed. For this, nose-space air was sampled via the two inlets of a glass nosepiece placed in both nostrils of the assessors. The nosepiece had one outlet for breathing and an orthogonal outlet for sampling. The latter was used to remove the air, without disturbing the assessor's breathing or swallowing pattern. The air was drawn

**Table 1**
Fragmentation patterns for high sensitivity PTR-MS for pure VOCs, determined in aqueous solutions with background subtraction.

| Compound | Intensity of molecular | | Dominant fragments with intensities in (%)[a] |
|---|---|---|---|
| | *m*/*z* | % | |
| Acetic acid | 61 | 90 | 43 (100) |
| Butanoic acid | 89 | 100 | 43 (21), 39 (31), 41 (28), 55 (8), 57 (10), 71 (4) |
| 2-Methyl butanoic acid | 103 | 100 | 43 (32), 39 (25), 41 (23), 57 (25), 73 (12), 85 (6), 106 (4) |
| 3-Methyl butanoic acid | 103 | 100 | 41 (87), 57 (57), 43 (55), 45 (35), 75 (25), 47 (19) |
| Pentanoic acid | 103 | 100 | 57 (31), 41 (26), 39 (19), 75 (10), 43 (7), 85 (6) |
| Ethanol | 47 | 100 | 29 (80), 45 (25), 73 (17) |
| Acetoin | 89 | 100 | 29 (90), 45 (73), 59 (37) |
| 2-Phenyl ethanol | 123 | 0 | 93 (100), 105 (61), 107 (10), 91 (9), 79 (9), 121 (7) |
| 3-Methyl butanal | 87 | 11 | 39 (100), 41 (71), 45 (46), 69 (43) |
| 3-Methylthiolpropanal | 105 | 1 | 49 (100), 79 (53), 61 (43), 95 (27), 57 (24), 45 (21) |
| 2,3-Butandione | 87 | 52 | 59 (100), 43 (27), 60 (9), 69 (7), 85 (4) |
| Gamma-dodecalactone | 199 | 0 | 60 (100), 61 (27), 153 (25), 135 (16), 171 (3) |
| Ethylbutanoate | 117 | 100 | 89 (42), 118 (17), 71 (7), 43 (4) |
| Ethyl-2 methylbutanoate | 131 | 63 | 103, (100), 57 (41), 41 (17), 43 (10), 39 (7) |
| Methyl acetate | 75 | 100 | 49 (9) |

[a] Concentration = 2 g L$^{-1}$ water.

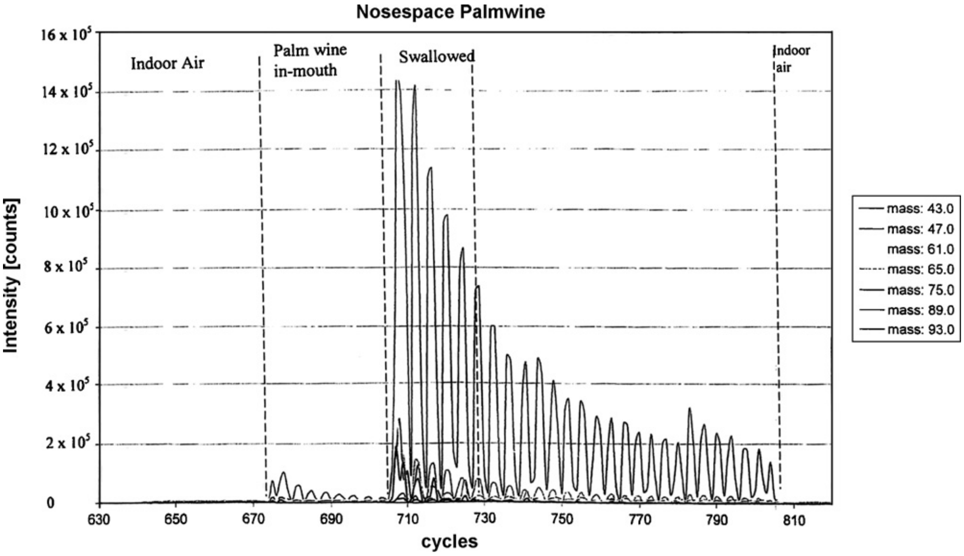

**Fig. 1.** Typical ion traces (*m*/*z*, 43, 47, 61, 65, 75, 89, 93) from PTR nose-space analysis of the consumption of palm wine for a panelist.

at a rate of 50 mL min$^{-1}$, 15 mL min$^{-1}$ of which was drawn into the PTR-MS. The assessor placed his/her nose in the nosepiece with normal breathing for the first 30 s, after which the assessor drank 15 mL of palm wine and retained it in the mouth for another 30 s. Finally, the assessor swallowed the palm wine and continued to breathe into the nosepiece for another 60 s. This was repeated three times for each of the four assessors (3 × 4 = 12 replicates). The release of the predominant masses in the expired air (*m*/*z* 43, 47, 61, 65, 75, 89 and 93) was measured as described by Lindinger et al. [6].

### 2.6. PTR-MS data analysis

Parameters calculated were: the maximum intensity of the released profile ($I_{\max}$) and the time necessary to reach the maximum intensity ($t_{\max}$). Using only the raw data obtained during consumption of palm wine until the swallowed event itself (Fig. 1), the maximum intensity of this time interval represents $I_{\max}$, and the time necessary to reach $I_{\max}$ is termed $t_{\max}$.

## 3. Results and discussion

### 3.1. Fragmentation patterns in palm wine

To analyze the complex mixtures of volatile compounds in palm wine, basic data on the behavior of individual volatile flavor compounds earlier identified in palm wine [14] were first evaluated (Table 1). Generally, a high degree of fragmentation was observed. The protonated molecular ions showed the most abundant intensity for butanoic acid, 2/3-methybutanoic acids, pentanoic acid, the esters (ethylbutanoate and methyl acetate), acetoin and ethanol respectively. 2-Phenyl ethanol split off water and break up to non-specific alkane fragment at $m/z = 93$. Fragmentation of the aldehydes (3-methylbutanal, 3-methythiol propanal) resulted to $m/z = 39$ and 49 as the dominant signals in the PTR-MS spectra of these compounds. On the other hand, the protonated molecular ion showed abundance intensity for two esters, i.e., ethylbutanoate, $m/z = 117$ and methyl acetate, $m/z = 75$ respectively. While the other ester, ethyl-2-methylacetate had $m/z = 103$ as the dominant signal. Fragmentation of the ketones (2,3-butandione and gamma-dodecalactone) led to $m/z = 50$ and 60 as the dominant signals in the PTR-MS spectra of these compounds.

### 3.2. PTR-MS headspace of palm wine

A scan of the volatile compounds from the headspace of palm wine by PTR-MS revealed that the masses (*m*/*z* 43, 47, 61, 65, 75, 89, and 93) were the predominant signals obtained. Although, PTR-MS is a one-dimensional technique, the soft ionization results in either mass + 1 ion or characteristic large product ions. Studies on the breakdown patterns of spectra from the palm wine headspace showed that *m*/*z* 43 is characteristic for fragment of various compounds. While mass (*m*/*z* 47) is probably from ethanol. Mass (*m*/*z* 61) has *been reported to originate from acetic acid, 1-propanol or fragment of ethyl* acetate [16]. On the other hand mass (*m*/*z* 75) is probably derived from methyl acetate, while mass (*m*/*z* 89) has been reported to originate from 2-methyl propionic acid, butyric acid or acetoin [13]. The last mass (*m*/*z* 93), is from 2-phenyl ethanol.

### 3.3. The in-mouth situation

The intensities of those compounds in the breathe of the test persons were observed to change significantly after the consumption of palm wine are shown as dependent on time in Figs. 1 and 2b respectively. There are seven distinctly different groups of compounds as revealed by their masses. The most prominent of the compounds is ethanol (mass 47), followed by acetic acid (mass 61), fragment of many compounds (mass 43), protonated ethanol cluster ions (mass 65), acetoin (mass 89), methyl acetate (mass 75) and 2-phenyl ethanol (mass 93). These compounds rise to a maximum intensity shortly after ingestion of palm wine and decline to normal baseline values within the next 60 s (Fig. 1). In the case of methyl acetate (mass 75), 2-phenyl ethanol (mass 93) and acetoin (mass 89) peak concentrations obtained from the breathe of the test persons were extremely low being <1.0 × $10^5$ ppb respectively, while ethanol (mass 47) reached a peak concentration of approximately 14 × $10^5$ ppb in the breath of one of the test person (Fig. 2a). Recent investigations [17] showed that the amounts of odorants present in a food material or aroma solution can be considerably reduced in the food during consumption or mastication in the oral cavity. Thus, the observed low intensities of 2-phenyl ethanol (mass 93) and methyl acetate (mass 75) in the breathe exhaled from the nose during consumption of palm wine could have been induced by adsorption or resorptive effects of the mouth mucosa or enzymic degradation of the odorants or, generally, interactions of odorants with salivary constituents. For instance, Buettner and

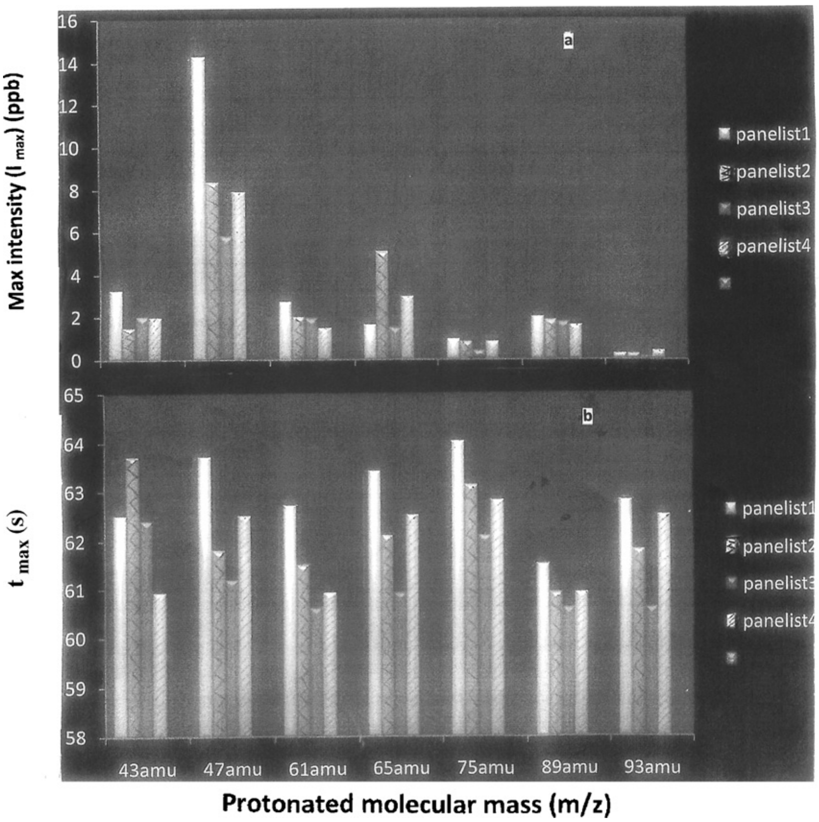

**Fig. 2.** Analysis of total PTR-release profiles from the consumption of palm wine (data is of four panelists, average of three determinations: (a) maximum intensity, (b) time until maximum intensity is reached.

Schieberle [18] reported that the adsorbed residue played an important role in the persistence of desirable or undesirable aroma in the mouth or in the nasal cavity. According to the present investigation, some of the adsorbed compounds were probably degraded by the enzymic activity of the saliva, thereby reducing their persistence as well as the overall intensity of the 'after smell' of these compounds, whereas, others might not be affected at all. It is worthy of note that the reason for the significant variation observed in the aroma breath profile of panelist 1 in respect of compounds with masses; 43, 47, 61 and 89 respectively, (Fig. 2a), cannot be determined unambiguously. Finally, the time until maximum intensity is reached $t_{\max}$ is presented in Figs. 2b and 3b respectively. The $t_{\max}$ was higher for five compounds, with masses 75, 43, 47, 65 and 93, respectively. However, this effect was not significant ($P = 0.05$)

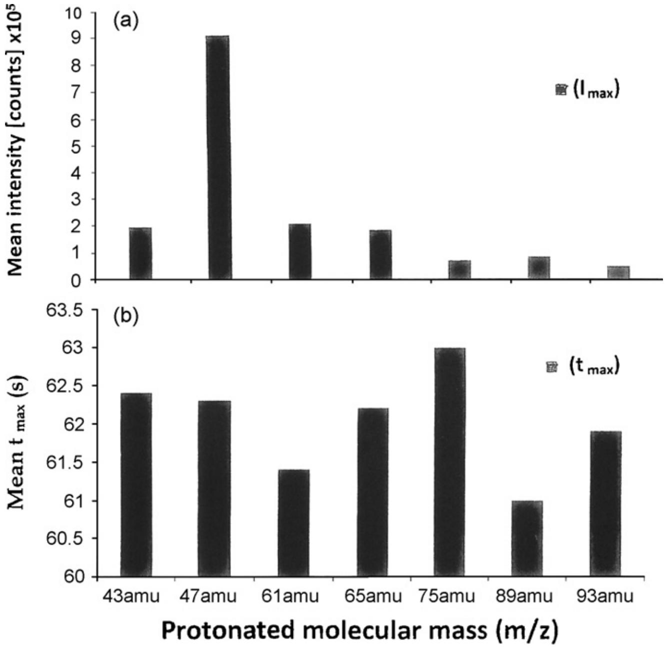

**Fig. 3.** Mean of total PTR-release profile from consumption of palm wine (data is of four panelists, average of three determinations: (a) maximum intensity, (b) time until maximum intensity is reached).

The variations observed for the pulses between panelists (Fig. 2a) indicated that the differences in the salivary activity of different humans exist, possibly resulting in a varying aroma perception. The dynamic release parameters ($I_{\max}$ and $t_{\max}$) of the 7 masses (Figs. 2 and 3) respectively revealed that while maximum intensity ($I_{\max}$) differed significantly ($P = 0.05$) among the compounds, there was no significant ($P = 0.05$) differences in $t_{\max}$ (which showed the persistence of a flavor compound).

## 4. Conclusion

The intensities of compounds in the breathe exhaled from test persons were observed to change significantly with time after the consumption of palm wine. Seven distinctly different groups of compounds with masses $m/z$ (43, 47, 61, 65, 75, 89 and 93) were the predominant ones. The most prominent of the compound is ethanol (mass 47), followed by acetic acid (mass 61), fragment of many compounds (mass 43), protonated ethanol cluster ions (mass 65), acetoin (mass 89), methyl acetate (mass 75) and 2-phenyl ethanol (mass 93). The dynamic release parameters ($I_{\max}$ and $t_{\max}$) of the seven masses revealed significant ($P = 0.05$) differences, between maximum intensity ($I_{\max}$) and no significant ($P = 0.05$) differences between $t_{\max}$. Finally, the coupling of PTR-MS with GC/MS would be necessary to allow for the quantization of the VOCs that contribute to a single PTR-MS ion signal.

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