

# Volatile profile of the headspace fraction of “*assa-peixe*” (*Vernonia* sp.) honeys

## Perfil dos compostos voláteis presentes na fração “headspace” de méis de *assa-peixe*

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### Abstract

The volatile compounds were isolated from the headspace fraction of “*assa-peixe*” honeys by adsorptive column chromatography, eluted with acetone and analysed by GC-FID and GC-MS. Volatile compounds were separated using a polar phase column. Low- and medium-boiling-point volatile compounds predominated in the headspace. A large proportion of 3-penten-2-one ( $80.5 \pm 13.9 \mu\text{g.kg}^{-1}$ ) and benzaldehyde ( $25.9 \pm 4.2 \mu\text{g.kg}^{-1}$ ) was found in the headspace fraction, while 2-penten-1-ol, 3-hexenyl butanoate, octadecane and hexanoic acid ( $<0.01 \mu\text{g.kg}^{-1}$ ) were by far the less abundant volatile compounds. A total of 12 volatile compounds were identified and, among them, 5 compounds were reported as “*assa-peixe*” honey constituents for the first time. Of the 5 new volatile compounds reported, 3-penten-2-one, dodecane, tridecane and benzaldehyde were definitively identified in the headspace fraction of Brazilian “*assa-peixe*” honeys.

**Keywords:** GC-MS; headspace; aroma; “*assa-peixe*” honey.

### Resumo

Os compostos voláteis da fração “*Headspace*” de méis de *assa-peixe* foram adsorvidos pela técnica de cromatografia de adsorção, eluídos com acetona e analisados através da CG/DIC e CG/EM usando coluna polar de sílica fundida. Os compostos voláteis de baixo e médio ponto de ebulição predominaram na fração “*headspace*”. Foi achada uma grande proporção de 3-penten-2-ona ( $80,5 \pm 13,9 \mu\text{g.kg}^{-1}$ ) e benzaldeído ( $25,9 \pm 4,2 \mu\text{g.kg}^{-1}$ ), enquanto o 2-penten-1-ol, o hexanoato de 3-hexenila, o octadecano e o ácido hexanóico ( $<0,01 \mu\text{g.kg}^{-1}$ ) foram compostos minoritários. Um total de doze compostos voláteis foi identificado, sendo cinco descritos pela primeira vez no mel de *assa-peixe*. Destes cinco, a 3-penten-2-ona, o dodecano, o tridecano e o benzaldeído foram definitivamente identificados na fração “*headspace*” dos méis de *assa-peixe* brasileiro.

**Palavras-chave:** CG/EM, “*headspace*”, aroma, mel de *assa-peixe*.

## 1 Introduction

Brazil has a vast number of unifloral honeys with exotic aromas and flavours that are greatly appreciated by consumers. The typical Brazilian “*assa-peixe*” honey is mainly produced in the south-eastern region of Brazil. This unifloral honey has a dark brown colour and characteristic flavour. Information related to the aroma composition of this honey is limited. Recently, however, we reported a study on the identification of volatiles from aqueous “*assa-peixe*” honey solutions (MATOS et al., 2002). In this case, these solutions were directly passed through an adsorptive Porapak trap, eluted with acetone and analysed by gas chromatography (GC) combined with flame ionization detection (FID) and mass spectrometry (MS). A total of 31 compounds were identified in the aqueous extract of the “*assa-peixe*” honey. Alcohols, hydrocarbons, acids, esters and aromatic compounds were the most abundant chemical classes of this aroma concentrate (MATOS et al., 2002).

To date, however, studies of “*assa-peixe*” honey volatile compounds based on the headspace sampling technique have not been reported. In the present study, we have analysed the volatile composition of an “*assa-peixe*” headspace fraction by both GC-FID and GC-MS.

## 2 Materials and methods

### 2.1 Samples

Ten authentic “*assa-peixe*” (*Vernonia* sp.) honey samples were obtained from Brazilian apicultors. The samples were stored in 250 mL plastic (low-density polyethylene) bottles at  $-18 \text{ }^\circ\text{C}$ , under  $\text{N}_2$  atmosphere. Before analysis, the honeys were defrosted at  $40 \text{ }^\circ\text{C}$  and homogenized by gently stirring for 10 minutes. Screening for floral purity was based on pollen analysis (LOUVEAUX; MAURIZIO; WORWOHL, 1978).

### 2.2 Reagents

Acetone was from Merck (Darmstadt, Germany). All other reagents were supplied by Aldrich (Wisconsin, USA).

### 2.3 Isolation of the volatile fraction (MOREIRA; DE MARIA, 2005)

One hundred millilitres of honey aqueous solution ( $20 \text{ g\%, w.v}^{-1}$ ) were placed in a 500 mL heavy-walled Pyrex filtering flask with a side hose-connection (Brand, Wertheim,

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Germany), o.d. 10 mm, at 30 °C, under stirring. Purified nitrogen (0.9-1.0 L/min) was passed through the system during 3 hours and the entrained volatiles were adsorbed on a glass column (0.5 cm i.d. x 18 cm) packed with a Porapak Q adsorbent (50-80 mesh) supplied by Supelco (Philadelphia, USA). The polymer was previously conditioned at 225 °C for 3 hours under N<sub>2</sub> at a flow rate of 0.9-1.0 L/min. The volatiles were subsequently desorbed with acetone (100 mL) and concentrated to 200 µL.

## 2.4 GC analysis

GC/FID: A Carlo Erba (Milan, Italy) 5890 GC equipped with a 50 m x 0.25 mm i.d. LM-120 polar polyethylene glycol capillary column ( $d_f = 0.50 \mu\text{m}$ , L&M, São Paulo, Brazil) and a FID were employed. The injector and detector temperatures were 230 and 240 °C. Helium was used as the carrier gas at a 0.83 mL/min flow rate. The oven temperature was programmed from 50 to 230 °C at 3 °C/min. The split ratio was 1:20. Linear retention indices (LRI) were estimated by using the modified Kövatz method (VAN DEN DOOL; KRATZ, 1963). All honey samples were analysed in two replicates. The estimated composition of the volatiles was carried out by external standardization and means and standard deviations were obtained for all the volatiles quantified.

GC/MS: A Shimadzu (Kyoto, Japan) GC-17A/QP5050 quadrupole mass spectrometer was used. The instrument was operated in the electron ionisation mode at 70 eV, an ion source temperature of 240 °C and a mass range of 20 to 300. Tentative identification of the volatiles was based on the comparison of mass spectra of unknown compounds against the NIST library data of the GC-MS. Where possible, the results were confirmed by comparison with authentic substances.

## 3 Results and discussion

The enrichment of the headspace fraction from the honey aqueous solution samples in the Porapak trap followed by desorption with acetone provided extracts with a slight aroma, but similar to those from the original sample. “*Assa-peixe*” honey volatile compounds isolated from the headspace are listed in Table 1.

**Table 1.** Volatile compounds in “*assa-peixe*” honey.

Compounds	LRI	Concentration ( $\mu\text{g.kg}^{-1}$ , avg $\pm$ SD)
3-penten-2-one <sup>a,b</sup>	-	80.5 $\pm$ 13.9
Dodecane <sup>a,b</sup>	1209	0.9 $\pm$ 0.5
2-penten-1-ol <sup>a,c</sup>	1281	<0.01
Tridecane <sup>a,b</sup>	1303	0.9 $\pm$ 0.3
Tetradecane <sup>b</sup>	1410	0.6 $\pm$ 0.2
2-ethyl-1-hexanol <sup>b</sup>	1499	0.7 $\pm$ 0.3
Pentadecane <sup>b</sup>	1505	0.06 $\pm$ 0.02
Benzaldehyde <sup>a,b</sup>	1524	25.9 $\pm$ 4.2
Hexadecane <sup>b</sup>	1608	0.04 $\pm$ 0.02
3-hexenyl butanoate <sup>c</sup>	1658	<0.01
Octadecane <sup>b</sup>	1811	<0.01
Hexanoic acid <sup>b</sup>	1838	<0.01

LRI - Linear Retention Index Avg - Average SD - Standard Deviation; <sup>a</sup>Not yet reported as volatile constituent of “*assa-peixe*” honey, <sup>b</sup>Definitive identification: via mass spectra + reference compound; and <sup>c</sup>Tentative identification: based only on mass spectra data.

As expected, low- and medium-boiling-point volatile compounds predominated in the headspace fraction. Although external standardizations were used, no recovery method or GC standardization corrections were applied, and thus, the values presented are estimated values. A total of 12 volatiles were identified and, among them, 5 compounds were reported as “*assa-peixe*” honey constituents for the first time. A large proportion of 3-penten-2-one (80.5  $\pm$  13.9  $\mu\text{g.kg}^{-1}$ ) and benzaldehyde (25.9  $\pm$  4.2  $\mu\text{g.kg}^{-1}$ ) was found in the headspace fraction, while 2-penten-1-ol, 3-hexenyl butanoate, octadecane and hexanoic acid (<0.01  $\mu\text{g.kg}^{-1}$ ) were by far the less abundant volatile compounds. Both 2-penten-1-ol and 3-hexenyl butanoate were only tentatively identified in the headspace fraction. The first compound has been recently reported in Brazilian Cambará honey (MOREIRA; DE MARIA, 2005). The remaining volatile compounds (83%) were considered definitively identified by comparison of retention times and mass spectral data with those of authentic reference compounds.

The headspace fraction exhibited a different qualitative and quantitative volatile pattern in comparison to that from “*assa-peixe*” honey aqueous extract (MATOS et al., 2002), where a predominance of medium- and high-boiling-point volatile compounds was observed. On the other hand, tetradecane, 2-ethyl-1-hexanol, pentadecane, hexadecane, 3-hexenyl butanoate, octadecane and hexanoic acid were found in both aroma concentrates.

A review of the literature revealed that benzaldehyde, the second most abundant volatile component of the “*assa-peixe*” honey headspace fraction, was also a major volatile compound in the headspace fraction of Brazilian “*cambará*” (*Gochmatia velutina*) honey (37  $\mu\text{g kg}^{-1}$ ) (MOREIRA; DE MARIA, 2005). Furthermore, benzaldehyde has already been identified in haze (*Rhus succedanea*) honey from Japan (SHIMODA; WU; OSAJIMA, 1996) and in lavender (*Lavandula augustifolia*) and eucalyptus (*Eucalyptus* sp.) (BOUSETA; SCHEIRMAN; COLLINS, 1996) honeys from different European countries.

## 4 Conclusion

Among the 12 volatile compounds found in the headspace fraction analysed in this study, 3-penten-2-one, dodecane, tridecane and benzaldehyde were definitively identified for the first time as “*assa-peixe*” honey constituents.

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