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A short review of headspace extraction and ultrasonic solvent extraction for honey volatiles fingerprinting

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reviews

Summary

Honey volatiles exhibit a potential role in distinguishing honeys as a function of botanical origin, but heating of honey generates artefacts such as compounds of Strecker degradation and Maillard reaction products. This short review is focused on the most recently applied methods for honey volatiles fingerprinting (without generation of thermal artefacts): headspace extraction (dynamic headspace extraction (DHE), headspace solid-phase microextraction (HS-SPME)) and ultrasonic solvent extraction (USE). These methods display a varying degree of selectivity and effectiveness depending upon the compounds involved and the extraction conditions. Recent developments of these methods are discussed, with application examples drawn from the literature as well from our own research. Flavour qualities of the honey are very much dependent on the volatile and semivolatile organic compounds present in both the sample matrix and the headspace aroma. Therefore the use of one single technique is not adequate for reliable honey volatiles profiling, but combined use of headspace extraction and ultrasonic solvent extraction could be a useful tool for the characterization of the honey and identification of its botanical source through typical volatile marker compounds.

Keywords: dynamic headspace extraction (DHE), headspace solid-phase microextraction (HS-SPME), ultrasonic solvent extraction (USE), honey volatiles, marker compounds

Introduction

The Commission of the EU is encouraging the development of harmonized analytical methods of quality verification for different honeys. Assessment of the honey botanical origin is of great importance in food analysis, since authenticity guarantees the quality (Bogdanov et al., 2004).

Unifloral nectar honeys as well as honeydews differ from each other, among other features, in volatile compound composition, which influences remarkably their individual sensory characteristics (Cuevas-Glory et al., 2007). Therefore honey volatiles exhibit a potential role in distinguishing honeys as a function of botanical origin. Their research began in the early 1960's. Honey volatiles may be derived 1) from the plant or nectar, 2) from the transformation of plant compounds by a honeybee or directly generated by honeybee, 3) from heating or handling during honey processing and storage, or 4) from microbial or environmental contamination. Certain specific volatile compounds are characteristic of a given floral origin such as semivolatile methyl anthranilate for citrus honey (Bertelli et al., 2008), 2-hydroxy-5-methylhexan-3-one and 3-hydroxy-5-methylhexan-2-one for eucalyptus honey (de la Fuente et al., 2007) or 2-aminoacetophenone for chestnut honey (Guyot et al., 1998). In other cases (Castro-Vázquez et al., 2009; Soria et al., 2003; D' Arcy et al., 1997), the floral origin is determined by a greater concentration of certain compounds (terpenes, norisoprenoids, benzene compounds, their derivatives, and others) in several honey types in comparison with others. More than 600 organic compounds have been identified in different honey volatile flavours originated from various biosynthetic pathways including aldehydes, ketones, esters, alcohols, hydrocarbons, and sulfur compounds (De Maria, et al., 2003).

The isolation of volatile components from a complex mixture such as honey in order to obtain representative extracts is very difficult. Methods of extracting the honey volatiles may display a varying degree of selectivity and effectiveness depending upon the compounds involved and the extraction conditions (Cuevas-Glory et al., 2007). In addition, heating of honey generates artefacts such as compounds of Strecker degradation and Maillard reaction products due to the effect of heat on honey carbohydrates and amino acids (Alissandrakis et al., 2005; Jerković et al., 2007), Fig. 1. Heating honey at

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temperatures as low as 50 °C leads to the formation of new volatile flavour compounds, and the peak areas of many compounds vary significantly as a result of different heating conditions (Visser et al., 1988).

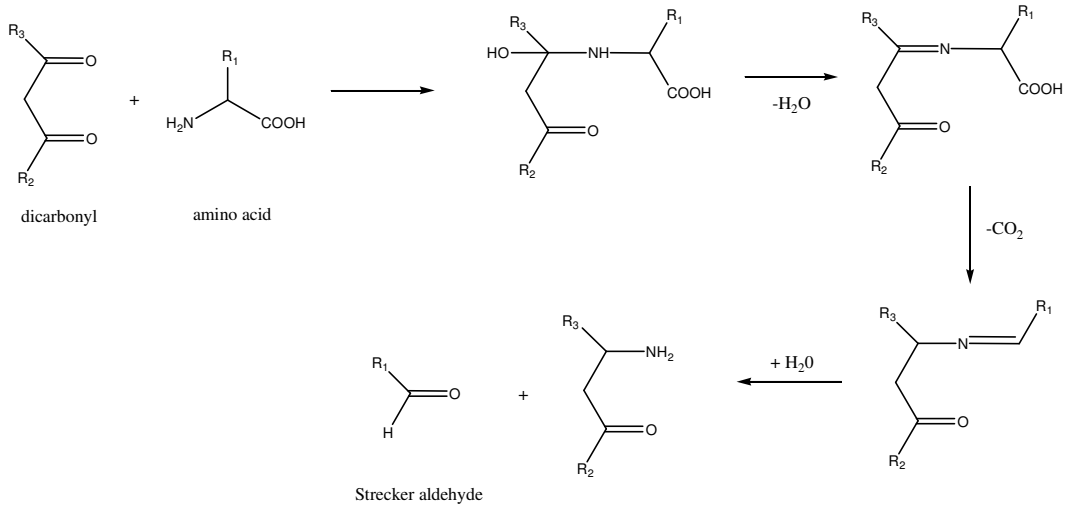

Fig. 1. General scheme of Strecker degradation

Since artefacts can be generated, appropriate methods for honey volatiles isolation should be applied to obtain very representative honey volatile fingerprint. Different methods can be used. Hydrodistillation (HD) and simultaneous steam distillation – solvent extraction (SDE) are the most common techniques for volatiles isolation, but not suitable for reliable honey fingerprinting due to intense promotion of artefacts formation (Alissandrakis et al., 2005; Serra-Bonvehi and Coll, 2003). In 1995 solvent extraction and subsequent steam distillation with simultaneous solvent extraction (Likens-Nickerson methodology) was introduced (Bouseta and Collin, 1995). Nowdays, alternative to these classical methods that may overcome their disadvantages are used (Alissandrakis et al., 2003, Pawliszyn, 1997) such as solid-phase microextraction (SPME) or ultrasonic solvent extraction (USE). The methods of headspace extraction and ultrasonic solvent extraction are focus of this paper. Recent developments of these methods are discussed, with application examples drawn from the literature as well from our own research. This short review can not be fully comprehensive, but selected topics are presented including recent trends.

Headspace methods

Headspace analysis is generally defined as a vapour-phase extraction, involving the partitioning of volatiles between a honey (usually dissolved in saturated water) and the vapour phase above. There are a number of techniques for sampling headspace vapours and introducing them to a GC. A decade ago, there were essentially two techniques that could be described as headspace-GC: static (vapour-phase extraction) and dynamic (purge and trap). Today, there are several more, as techniques have become miniaturized and automated.

Static headspace extraction (SHSE)

The classical static headspace technique include sealed sample in a gas-tight vial, using a closure with a septum. After equilibration at defined temperature headspace vapour is sampled. Numerous instruments are available for its automated application. Most modern headspace-GC instruments employ static sampling with a heated transfer line and they pressurize the sample vial above the capillary column head pressure, which allows for rapid sample transfer and ready equilibration as well for interfacing the sampling device to the GC. The main limitation of classical static headspace-GC is that sensitivity is limited, compared to dynamic techniques, such as purge and trap and to the techniques involving on-line concentration such as SPME. Static headspace analysis has not been widely applied to analyze honey volatiles because of their low concentrations as well as low recoveries obtained for semivolatile compounds (Rowland et al., 1995).

Dynamic headspace extraction (DHSE)

Dynamic headspace sampling involves the passing of carrier gas through a honey solution, followed by trapping of the purged volatiles on a sorbent (trap) and desorption onto a GC (classical purge-and-trap technique). Dynamic headspace extraction coupled with GC-MS was introduced to the honey analysis by Bouseta et al. (1992). A solution of honey in water was directly purged with nitrogen at 70 ^{o}C to a cold trap. The qualitative and quantitative composition of the honey volatiles of various floral honeys was found to be different. The results allowed classification of *Lavandula*, *Abies*, *Eucalyptus*, *Taraxacum* and *Brassica* honeys. Radovic et al. (2001) found 110 compounds (and several markers for determination of botanical and geographical origin) in the honeys of different floral origin, such as chestnut, heather, eucalyptus, lime, rosemary, sunflower, lavender, rape and acacia by GC-MS analysis of the headspace purged at 45 ^{o}C on Tenax TA trap. That method proved to be inadequate for the extraction of volatile compounds from strawberry-tree honey samples, since a remarkable carry-over effect for α-isophorone was observed (Bianchi et al., 2005). A different trapping material was then evaluated by testing the performance of Carbopack^{TM} B. Using this trap, only a negligible carryover effect was observed. A total of 28 aroma compounds from 10 Sardinian strawberry-tree honey samples were identified by DHSE followed by GC-MS, but only norisoprenoid compounds such as α-isophorone, β-isophorone and 4-oxoisophorone, were recognized as specific floral origin markers of the strawberry-tree honey (Bianchi et al., 2005). Investigation of the aroma compounds from headspace of cambará honey aqueous solution was performed by DHSE at 30 ^{o}C (Moreira and De Maria, 2005) using Porapak Q trap. The volatile fraction of 22 commercial honeys of different botanical sources (eucalyptus, thyme, citrus, rosemary, heather, lavander, multiflower and honeydew) was characterised by DHSE followed by GC-MS (Soria et al., 2008). Compounds detected include volatiles derived from the floral nectar of honeydew source such as terpenes, furan derivatives from honey processing and storage and other compounds whose origin could be related to microbial or environmental contamination. The honey marker compounds typical for certain floral origins determined by dynamic headspace GC-MS are presented in Table 1.

Table 1. The honey marker compounds typical for certain floral origins determined by dynamic headspace GC-MS.

BOTANICAL ORIGIN	MARKER COMPOUND		REFERENCE
	Presence of	Absence of	
acacia	both *cis*-linalool oxide and heptanal	both phenylacetaldehyde and dimethyl disulphide	Radovic et al., 2001
lavander	heptanal	4-oxoisophorone	Radovic et al., 2001
rape	dimethyl disulphide	2-methyl-proan-1-ol	Radovic et al., 2001
sunflower	α-pinene or 3-methyl-butan-2-ol	both heptanal and 4-oxoiosphorone	Radovic et al., 2001
chestnut	2-methyldihydrofuranone or α-methylbenzyl alcohol or both hex-3-en-1-ol and dimethylstyrene	-	Radovic et al., 2001
rosemary	-	2-acetylfuran	Radovic et al., 2001
strawberry-tree	α-isophorone, β-isophorone, 4-oxoisophorone	-	Bianchi et al., 2005
heather	α-isophorone, 4-oxoisophorone	-	Soria et al., 2008
citrus	isomers of lilac aldehydes	-	Soria et al., 2008

The DHSE method was proposed as a valid alternative to pollen analysis for floral source detection, especially for honeys like strawberry-tree honey, characterized by low pollen content. For dynamic headspace-GC techniques, publication trends continue to rise, but perhaps not at the rapid rate of HS-SPME. Dynamic headspace extraction of honey volatiles seems to be a promising approach to the determination of the honey botanical origin, also suitable for routine analyses. Selection of the operating conditions (honey concentration, purging temperature and time as well as trap) is crucial for

reliable profiling by DHSE. However extraction conditions should be further optimised in order to extract more semivolatiles. The medium-low-volatility and relatively high polarity of terpenes and their derivatives could be responsible for the limitations observed in their purge and trap fraction (Soria et al., 2008). This technique affords as main advantages a high sensitivity for fractionation of high-volatile compounds, the absence of extended heating times and the reproducibility associated to a totally automated system.

Headspace solid-phase microextraction (HS-SPME)

SPME has been developed as a rapid and solvent-free technique. This technique uses a fine fused silica fibre with a polymeric coating to extract organic compounds from their matrix and directly transfer them into the injector of a GC for thermal desorption and analysis (Zhang and Pawliszyn, 1995). It was first applied on several Italian unifloral honeys by Guidotti and Vitali (1998) using PDMS fiber. The SPME extraction of honey volatiles was further developed by Verzera et al. (2001) by using a PDMS/DVB coated fibre. Unique chromatographic profiles were obtained for each type of the unifloral honeys studied (*Eucalyptus*, *Citrus*, *Hedysarum*, and *Castanea*). In another work CAR/PDMS and PDMS/DVB coated fibers were used (Perez et al., 2002). The first fibre was shown to be able to extract more high-volatile compounds. *Citrus*, *Eucalyptus*, *Rosmarinus*, *Lavandula* and *Thymus* honeys were studied and a total of 35 components were detected. A triple phase DVB/CAR/PDMS SPME fibre was also tested for analysis of honey volatiles (Ruoff, 2003). Available SPME commercial fibres are summarized in Table 2.

Table 2. SPME commercial fibres.

Stationary phase	Recommended use
PDMS: polydimethylsiloxane	for volatiles (non-polar fibre)
PA: polyacrilate	for polar semivolatile compounds (polar fibre)
CW/DBV: carbowax/divinylbenzene	for alcohols and volatiles (polar fibre)
PDMS/DVB: polydimethylsiloxane/divinylbenzene	for volatile compounds, amines and nitroaromatics (non-polar fibre)
DVB/CAR/PDMS: divinylbenzene/carboxene/polydimethylsiloxane	for odours

In general, honey volatile extraction is best achieved when the polarity of the fibre matches the polarity of the volatiles (i.e. non-polar fibres for non-polar compounds, and polar fibres for polar compounds). Table 3 presents several honey marker compounds typical for certain floral origins determined by HS-SPME followed by GC-MS.

Table 3. The honey marker compounds typical for certain floral origins determined by HS-SPME followed by GC-MS.

BOTANICAL ORIGIN	MARKER COMPOUND	FIBRE	REFERENCE
citrus	lilac aldehydes, methyl anthranilate	DVB/CAR/PDMS	Alissandrakis et al., 2007
	methyl anthranilate p-menth-1(7),8(10)-dien-9-ol	PA	Soria et al., 2009
	lilac aldehyde lilac alcohols	CAR/PDMS	Soria et al., 2009
lavander	benzaldehyde, hexan-1-ol, phenylacetaldehyde	CAR/PDMS	Soria et al.,2009
chesnut	2-aminoacetophenone 1-phenylethanol	CAR/PDMS	Soria et al.,2009
Paliurus	lilac aldehydes	PDMS/DVB DVB/CAR/PDMS	Jerković et al., 2009
Amorpha	2-phenylethanol	PDMS/DVB	Jerković et al., 2009

In SPME, the amount of compounds extracted on to the fibre depends not only on the polarity and thickness of the stationary phase, but also on the extraction time and the concentration of the volatiles in the honey. Extraction of volatiles is improved by agitation, addition of salt to the sample, changing the pH, and increasing temperature (Kataoka et al., 2000; Pawliszyn, 1999). The main advantages of SPME are simplicity, high sensitivity, small sample volume, and lower cost per analysis. Other significant aspects of SPME techniques are reproducibility, repeatability, fibre stability, and the possibility of quantitative determinations (Pawliszyn, 1997). Therefore the number of published papers on HS-SPME for honey volatiles profiling is constantly rising.

Ultrasonic solvent extraction (USE)

It is known that the use of ultrasound-assisted extraction aids extraction by significantly reducing extraction times in comparison with traditional methods (exp. shake-flask extraction). The mechanical effect of ultrasound provides a greater penetration of solvent into matrix, *via* cavitation effects, and improves the extraction. Alissandrakis et al. (2003) firstly applied an ultrasound water bath as a mean of extracting honey volatiles in the solvent (mixture of pentane and diethyl ether). This technique does not require heat, thus no thermally generated artefacts are formed. In this regard, evaluation of four isolation techniques (hydrodistillation (HD), mirco-simultaneous steam distillation-solvent extraction (MSDE), ultrasound-assisted extraction (USE) and solid-phase microextraction (SPME) for honey aroma compounds was investigated (Alissandrakis et al., 2005). The drawbacks of the drastic conditions of HD and MSDE that lead to the formation of artefacts and degradation of sensitive compounds were avoided by USE. In addition, the comparison of hydrodistillation (HD) and ultrasonic solvent extraction (USE) for the isolation of volatile compounds from two unifloral honeys of *Robinia pseudoacacia* L. and *Castanea sativa* L. was performed (Jerković et al., 2007). USE method gave the more representative profile in comparison to HD isolates that contained many thermally derived artefacts (especially phenylacetaldehyde), Table 4. In addition, USE enabled extraction of low molecular weight semivolatiles (especially benzoic, vanillic and phenylacetic acids) that can not be extracted by HD.

Table 4. Major thermally derived volatiles from *Pseudoacacia robinia* L. and *Castanea sativa* L. honeys (Jerković et al., 2007).

	Pseudoacacia robinia				***Castanea sativa***			
	HD		**USE**		**HD**		**USE**	
Compound	**Av.**	**δ**	**Av.**	**δ**	**Av.**	**δ**	**Av.**	**δ**
cis-linalool oxide	2.23	0.75	-	-	0.40	0.17	-	-
2-furancarboxaldehyde (furfural)	3.27	1.26	-	-	2.73	0.95	0.30	0.10
trans-linalool oxide	1.20	0.46	-	-	0.07	0.23	-	-
hotrienol	1.60	0.20	0.57	0.25	0.43	0.15	0.03	0.06
phenylacetaldehyde	66.53	1.72	-	-	12.80	2.50	-	-

Volatile compounds of unifloral *Salvia officinalis* L. honey have been investigated (Jerković et al., 2006) by USE. *Salvia* honey could be distinguished on the basis of the high percentage of benzoic acid (6.4–14.8 %), and especially phenylacetic acid (5.7–18.4 %). Minor, but floral-origin important volatiles were identified such as shikimate pathway derivatives, "degraded-carotenoid-like" structures (3,5,5-trimethylcyclohex-2-ene derivatives) and 2,6,6-trimethylcyclohex-2-ene derivatives. The samples of unifloral *Paliurus spina-christi* honey were also analysed (Jerković et al., 2009) by means of USE. Although the main components of USE extracts were higher saturated aliphatic hydrocarbons, higher aliphatic alcohols and acids, they can not be considered reliable biomarkers due to their probable origin from bee wax or bee cuticle. Although present in small quantities, the more reliable markers in the extracts were benzene derivatives (particularly 4-hydroxy-3,5-dimethylbenzaldehyde, 4-hydroxybenzoic acid and 4-methoxybenzoic acid) along with lower aliphatic acids (butanoic, hexanoic, octanoic and nonanoic). 2-Phenylethanol (10.5–16.8 %) and

methyl syringate (5.8–8.2 %) were the major compounds of ultrasonic solvent extracts of *Amorpha fruticosa* honey samples (Jerković et al., 2009), with an array of small percentages of linalool, benzene and benzoic acid derivatives, aliphatic hydrocarbons and alcohols, furan derivatives and others.

The use of water bath ultrasound as a means of extracting honey volatile and semi-volatile compounds seems to be a promising technique. Furthermore, the whole procedure that was developed is quite rapid, easy to be carried out and does not necessitate special equipment. The most important advantage of the USE, compared to the headspace system, is that it enables the extraction of compounds of molecular weight up to 220 that could contribute to the determination of the honey botanical origin. Optimisation of the procedure is required for quantitative and qualitative evaluation of honey volatiles.

Conclusions

Volatile compound identification with the purpose of assessing the botanical origin of honey has the potential to be an extremely useful strategy. The analysis results on the honey aroma compounds are significantly dependent on the applied isolation techniques with large differences among the obtained headspace extract and ultrasonic solvent extract profiles. Further development of USE can be directed toward the application of different solvents. Flavour qualities of the honey are very much dependent on the volatile and semivolatile organic compounds present in both the sample matrix and the headspace aroma. Therefore the use of one single technique is not adequate for reliable honey volatiles profiling, but combined use of HSE and USE could be a useful tool for the characterization of the honey and identification of the botanical source (through typical marker compounds). In addition, aroma profile analysis should be combined with other methods for the determination of other constituents.

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