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ScienceDirect
Food Chemistry 103 (2007) 1032–1043
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

A review of volatile analytical methods for determining the botanical origin of honey

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Received 17 December 2005; received in revised form 20 July 2006; accepted 23 July 2006

Abstract

Aroma is an important quality factor in foods. The aroma of bee honey depends on volatile fraction composition, which is influenced by nectar composition and floral origin. Honey of unifloral origin usually commands higher commercial value, thus the floral determination and certification of unifloral honey plays an important role in quality control. This review concerns investigations made on the volatile fraction of bee honey by gas chromatography/mass spectrometry. Recent advances in extraction methods, results achieved, and comparisons of alternative dependable methods for determining floral origin of bee honey are discussed. We emphasize solid phase micro-extraction gas chromatography (SPME/GC) methodology and present some of the results obtained to date, plus the advantages and drawbacks of SPME/GS in comparison with other methods.

Keywords: HS-SPME/GC–MS; Volatile; Honey; Unifloral

Contents

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0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2006.07.068

1. Introduction

Honey is a natural product produced by *Apis mellifera* bees from the nectar or secretions of plants, and usually contains no additives or preservatives. The chemical composition of honey is highly dependent on the floral origin of the nectar foraged by bees (Roberts, Aureli, Flamini, & Yndestad, 2002). However, honey is often marketed as mixed-flower honey with a blend of flavours. In order to determine the legitimacy of the floral origin of honey, analysis of pollen (melissopalynology), and organoleptic or physico-chemical properties are traditionally employed (Radovic et al., 2001). However, melissopalynology requires a very experienced analyst, is very time consuming, and depends on the expert's ability and judgment (Radovic et al., 2001). Moreover, the chemical compounds present in honey can undergo modifications through time and storage conditions, reducing the dependability of those methods based on quantification of physico-chemical parameters (Iglesias, De Lorenzo, Polo, Martín-Alvarez, & Pueyo, 2004). In this paper, we explore the analysis of the volatile fraction of honey (aroma) as a method for assessing the botanical origin of honey.

The aroma profile is one of the most typical features of a food product, for both organoleptic quality and authenticity (Careri et al., 1994). Owing to the high number of volatile components, the aroma profile represents a "fingerprint" of the product, which could be used to determine its origin (Anklam, 1998; Anklam & Radovic, 2001). It has been pointed out that a careful analysis of the volatile compounds in honey could be a useful tool for characterization of botanical origin (Overton & Manura, 1994). Determination of botanical origin based on aroma profile is particularly dependable for a flavour-rich product such as honey and has led to the development of techniques for measuring its volatile fraction (Radovic et al., 2001).

Aroma compounds are present in honey at very low concentrations as complex mixtures of volatile components of different functionality and relatively low molecular weight. Since gas chromatography/mass spectrometry (GC/MS) combines high separation efficiency and sensitivity and provides qualitative and quantitative data for these compounds, it is usually the technique of choice for aroma profile determination. More recently, electronic noses (sensors) based on mass spectrometry (MSE-nose), piezoelectric effects (zNose™) and electrical resistance (i-PENMOD AIRSENSE Analytics GmbH) have been tested on the volatile fraction of some US (Lammertyn, Veraverbeke, & Irudayaraj, 2004) and European honeys (Ampuero, Bodganov, & Bosset, 2004; Linder & Pöppl, 2003). However, these techniques require the previous removal of sugar and water, both major components of honey (Soria, Martínez-Castro, & Sanz, 2003). Our objective is to review the techniques that have been employed to assess the volatile fraction of honey and identify specific techniques that may be useful for investigators wishing to determine the botanical origin of honey.

2. Solvents

Because of simplicity and the fact that thermolabile compounds do not undergo any change due to heating, solvent extraction has been widely used for honey characterization. Moreover, the low polarity solvents often used for this technique extract neither water nor sugar from honey (Soria et al., 2003). Nevertheless, direct extraction with solvents can solubilize non-volatile compounds and contaminate the GC injection port. In addition, some analytes can be masked by the solvent and elude the GC column inhibiting detection. Major volatile compounds of specific honey sources of three main categories of natural volatiles, such as norisoprenoids, terpenes, and benzene derivatives, have been found through this technique (D'Arcy, Rintoul, Rowland, & Blackman, 1997; Wilkins, Lu, & Tan, 1993). However, aliphatic compounds (Tan, Holland, Wilkins, & Molan, 1988; Tan, Wilkins, Holland, & McGhie, 1989), hydrocarbons (Graddon, Morrison, & Smith, 1979) and products obtained from non-enzymatic browning reaction (Millard reaction) were also detected (D'Arcy et al., 1997; Guyot-Declerck, Chevance, Lermusieau, & Collin, 2000; Iglesias et al., 2004; Soria et al., 2003).

Column extraction method is an alternative technique for isolating volatile compounds without the use of heat, and combines both solvent and a porous polymer. In order to determine the aroma compounds of Haze (*Rhus succedanea*) honey from Japan, a column extraction method was used by Shimoda, Wu, and Osajima (1996). The honey sample was dissolved in a solution of deionized water and cyclohexanol and passed through a column packed with porous polymer beads (Porapak Q). The adsorbed constituents were then eluted with diethyl ether, concentrated and analyzed by GC/MS. They identified 130 compounds such as alcohols, aldehydes, ketones, esters, acids, hydrocarbons, furanoids and miscellaneous compounds. Moreira, Trugo, Pietroluongo, and De Maria (2002) by using the column extraction method employed by Shimoda et al. (1996) with slight modification, analyzed two Brazilian honeys: Cashew (*Anarcadium occidentale*) and Marmeleiro (*Croton* species). They detected a predominace of hydrocarbons in Cashew honey and significant presence of linalool-related compounds in Marmeleiro honey

Table 1
Volatile compounds of importance in some unifloral honeys

Honey source	Compound	Extraction method	Reference
Chestnut	3-Aminoacetophenone[j], 2-methyldihydrofuranone[h], α-methylbenzyl alcohol[b], 3-hexen-1-ol[2], dimethylstyrene[l]	Purge-and-trap (Tenax™ TA trap)	Radovic et al. (2001)
	Acetophenone[l], 1-phenylethanol[g] and 2-acetophenone[l]	SPME	Piasenzotto et al. (2003); Verzera et al. (2001)
	2-Methylcyclopentanol[b], diethylphenol[g]		Guidotti and Vitali (1998)
	1-Phenylethanol[g], 2-aminoacetophenone[j]	Likens–Nickerson	Guyot et al. (1998)
	Styrene[l]	Purge-and-trap (Tenax™ TA trap)	Radovic et al. (2001)
Heather			
Erica arborea	Benzoic acid[e], decanoic acid[e], high levels of cinnamic acid[e], isophorone[d], 4-(3-oxobut-1-enylidene)-3,5,5-trimethylcyclohexen-2-en-1-one[m] Shikimate-pathway derivatives: 4-metoxibenzaldehyde[c], 4-metoxibenzoic acid[e], methyl vainillate[g]	Likens–Nickerson	Guyot et al. (1999)
Calluna vulgaris	Phenylacetic acid[e], dehydrovomifoliol[m], (4-(3-oxo-1-butynyl)-3,5,5-trimethylcyclohexen-2-en-1-one)[m], higher levels of 3,5,5-trimethylcyclohexene derivatives[m]	Likens–Nickerson	Guyot et al. (1999)
Heather	1-Penten-3-ol[b], 4-methylbicyclo[2,2,2]octan-1-ol[b], phenylacetaldehyde[c]	Purge-and-trap (Tenax™ TA trap)	(Radovic et al., 2001)
	Isophorone[m]	SPME	Soria et al. (2003)
		Solvent	Tan et al. (1989)
Eucalyptus	Acetoin[d], aldimethyldisulphide[k], dimethyltrisulphide[k], alkane[a], nonane[a]	Purge-and-trap (Cryogenic trap)	Bouseta et al. (1992)
	Acetoin[d]	Purge-and-trap (Tenax™ TA trap)	Radovic et al. (2001); Graddon et al. (1979)
		SPME	Pérez et al. (2002); Piasenzotto et al. (2003); Verzera et al. (2001)
	Nonanol[b], nonanal[c], nonanoic acid[e]	SPME	Guidotti and Vitali (1998); Piasenzotto et al. (2003); Verzera et al. (2001)
Lime	2-Pentanone[d], acetoin[d], furfural[i], 4-methylacetophenone[l], methyl isopropylbenzene[l], dimethylstyrene[l]	Purge-and-trap (Tenax™ TA trap)	(Radovic et al., 2001)
	Ethylmethylphenol[g], estragole[l], carvacol[l]	Likens–Nickerson	(Bouseta & Collin, 1995)
	Ethylmethylphenol[g], carvacrol[l], estragole[l]	Likens–Nickerson	(Guyot et al., 1998)
Citrus	1-*p*-Menthen-9-al[c]	SPME	Alissandrakis et al. (2005, 2007)
	Lilac aldehyde[i]	SPME	Alissandrakis et al. (2005, 2007); Piasenzotto et al. (2003); Soria et al. (2003);
	Limonene diol[i], hotrienol[i]	SPME	Guidotti and Vitali, 1998; Verzera et al., 2001
	Methyl anthranilate[l]	SPME	(Alissandrakis et al. (2005, 2007); Piasenzotto et al. (2003); Soria et al. (2003); Verzera et al. (2001))
			Graddon et al. (1979); Serra and Coll (1995)
Lavander	Ethanol[b], 2-methyl-1-propanol[b], 3-methyl-1-butanol[b], 3-methyl-3-buten-1-ol[b], 3,7-dimethyl-1,5,7-octatrien-3-ol (hotrienol)[i], furfuryl alcohol[h], hexanal[c], heptanal[c], 1-hexanol[b], furfural[h], phenylacetaldehyde[c], benzaldehyde[c]	Purge-and-trap (Tenax™ TA trap)	Radovic et al. (2001)
	Hexanal[c], heptanal[c], ethyl propionate[f]	Dynamic Headspace	Bouseta et al. (1992)
Acacia	Acetone[d], furfural[h], benzaldehyde[c]	Purge-and-trap (Tenax™ TA trap)	Radovic et al. (2001)
Rosemary	Acetone[d], 2-pentanone[d], benzaldehyde[c], 4-oxoisophorone[m]	Purge-and-trap (Tenax™ TA trap)	(Radovic et al., 2001)

Table 1 (*continued*)

Honey source	Compound	Extraction method	Reference
Haze	Methyl-*p*-anisaldehyde[c], trimetoxibenzene[l], 5-hydroxy-2-methyl-4H-pyran-4-one[n], lilac aldehyde isomer A[i], lilac aldehyde isomer B[i], lilac aldehyde isomer C[i], lilac aldehyde isomer D[i]	Purge-and-trap (Tenax™ TA trap)	(Shimoda et al., 1996)
Cashew	*trans*-Linalool oxide[i], nonacosane[a]	Purge-and-trap (Tenax™ TA trap)	Moreira et al. (2002)
Marmeleiro	Linalool[i], linalool acetate[i]		
Sardinian strawberry-tree	α-Isophoron[m], β-isophoron[m], 4-oxo-isophoron[m]	Purge-and-trap (Carbopack™ trap)5	Bianchi et al. (2005)

[a] Hydrocarbons.
[b] Alcohols.
[c] Aldehydes.
[d] Ketones.
[e] Acids.
[f] Esters.
[g] Phenols (and ethers).
[h] Furans.
[i] Terpenes.
[j] Amines and miscellaneous nitrogen compounds.
[k] Sulfur compounds.
[l] Benzen derivatives.
[m] Norisoprenoides.
[n] Miscellaneous.

(Table 1), which could be used to make a distinction between these two types of honeys. The use of polymers can therefore be helpful to isolate major groups of volatiles, but it is still necessary to use solvents to recover the trapped analytes in the porous matrix, which introduces the limitations of solvents described above.

3. Simultaneous distillation-extraction

Investigative research has been reported on the honey volatiles fractionated by employing a simultaneous steam distillation-extraction method (Bicchi, Belliardo, & Fratinni, 1983; Bouseta & Collin, 1995; D'Arcy et al., 1997; Guyot, Bouseta, Scheirman, & Collin, 1998; Guyot, Scheirman, & Collin, 1999; Nickerson & Likens, 1966). Bicchi et al. (1983), with the intention of avoiding sugar interference, proposed a two-step protocol which included preliminary acetone extraction followed by simultaneous Likens–Nikerson steam distillation and solvent extraction. Since heat treatments can lead to artifacts or Millard reaction products, a modified version of the Likens–Nikerson method was adapted by using vacuum at room temperature (Maignial, Pibarot, Bonetti, Chaintreau, & Marion, 1992; Wilkins et al., 1993).

Bouseta and Collin (1995) optimized the Bicchi et al. (1983) method (Fig. 1) by pre-extraction with dichloromethane instead of acetone under an inert atmosphere, followed by an optimized steam distillation extraction. They detected less furan derivatives in the dichloromethane extracts than in the acetone extracts, which are due to non-enzymatic browning reaction. Guyot et al. (1998) using the method modified by Bouseta and Collin (1995) found volatile compounds such as phenols and benzene derivatives (Table 1) characteristics for chestnut and lime

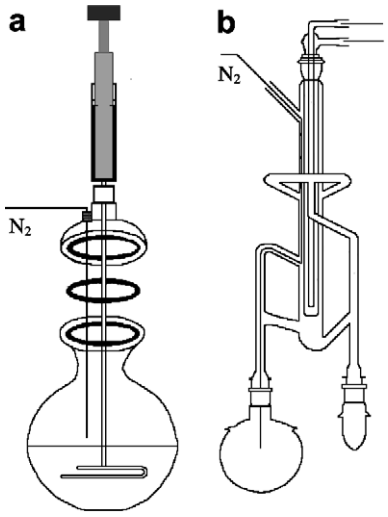

Fig. 1. (a) Pre-extraction apparatus and (b) microextractor for simultaneous steam distillation-solvent extraction (diagram adapted from Bouseta and Collin, 1995).

tree unifloral honeys. Subsequently Guyot et al. (1999), using the same method, identified volatile compounds characteristic for differentiating between honey of different species (*Erica arborea* and *Calluna vulgaris*) from the same family (Ericaceae). They also identified the presence of shikimate-pathway derivatives, such as 4-methoxibenzaldehyde, 4-methoxibenzoic acid, and methyl vanillate.

Solvent extraction methods have been commonly used for honey aroma extraction, whether by means of direct extraction or SDE. As mentioned above, the generation of artifacts is due to exposure of the samples to heat. Alissandrakis, Daferera, Tarantalis, Polissiou, and Harizanis (2003) compared ultrasound-assisted solvent extraction (USE) with SDE in an attempt to develop an easier solvent

extraction method at room temperature. Similar approaches have found success in studies of wine (Cocito, Gaetano, & Delfini, 1995). This method produced lower levels of hotrienol (2,7-dimethyl-1,5,7-octadiene-3-ol) in citrus honey, which is thermally generated by its precursor (*E*)-2,6-dimethyl-6-acetoxy-2,7-octadienal (Wintoch, Morales, Duke, & Schreier, 1993) also found in citrus flowers. The predominance of linalool derivatives found in citrus honey and in citrus blooms can be considered parameters for characterizing this particular honey. Also, better performance of USE and SPME (see Section 6) extraction methods were achieved when four isolation techniques (USE, SPME, Hydrodistillation and microsimultaneous steam distillation-solvent extraction) were evaluated for honey aroma compounds extraction (Alissandrakis, Kibaris, Tarantilis, Harizanis, & Polissiou, 2005, 2007). Even though USE procedure can be considered a good alternative to some other sampling methods, it needs improvement regarding its repeatability. Overall, the results suggest that dichloromethane extraction, under an inert atmosphere, followed by simultaneous steam distillation-dichloromethane extraction appeared to be a useful method for honey aroma characterization.

4. Headspace (HS)

Static headspace (SHS) analysis has not been widely applied to analyze honey volatile fractions because of low concentrations of volatiles in honey and the low recoveries obtained for semi-volatile compounds (Rowland, Blackman, D'Arcy, & Rintoul, 1995). Dynamic HS purge-and-trap (Fig. 2) techniques have the advantage of identification and quantification of a wide range of volatiles and semivolatiles, with sensitivity higher than that of static headspace. Dynamic HS techniques have therefore been used for the characterization of honey of different floral and geographical origin (Bouseta, Collin, & Doufour, 1992; Overton & Manura, 1994; Radovic et al., 2001). Various HS purge-and-trap techniques and modifications have been used for the extraction of honey aroma, but require adding new equipment to the systems of aroma analysis: GC, GC–MS, GC–nose, nose, GC–MSnose and enose.

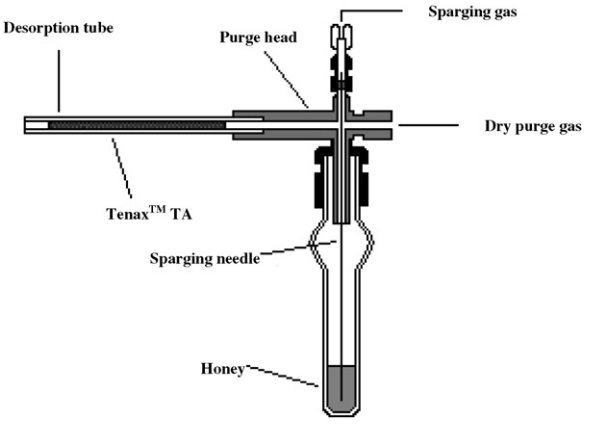

Fig. 2. Purge and trap device (diagram adapted from Overton and Manura, 1994).

By purging with a stream of nitrogen gas, the volatile compounds from a honey matrix and concentrating them into a cooled trap, Bouseta et al. (1992) were able to extract and analyze the aroma of 84 varieties of honey from different countries. They identified a variety of analytes belonging to seven major groups: aldehydes, ketones, cyclic compounds, alcohols, esters, hydrocarbons and chlorinated compounds. Although some aldehydes and alcohols reflect product quality and are a consequence of microbiological activity, heat exposure, and honey aging, it was possible to identify other compounds such as linear aldehydes as characteristic honey compounds associated with certain floral origins. Extensive details of honey aroma compounds identified by these authors and indicative of floral origin are given in Table 1. A modified GC/MS system enabling dynamic HS sampling with on-line cryofocusing and cold on-column injection of liquid samples (Barcarolo & Casson, 1997) was tested during analysis of acacia honey aroma using HS sampling as well as on-column injection of a solvent extract. With this new HS sampling configuration, liquid injections are also achievable in the same system without changes to the hardware configuration. By using this method it was possible to detect a wide range of highly volatile and semivolatile compounds of acacia honey aroma. This represents potential time saving on changing from one sampling method to another and a good alternative for achieving a better characterization of the honey. However, polar compounds were not identified because capillary columns with polyethyleneglycol (PEG) or other water sensitive phases are not recommended for use with liquid injections due to the risk of GC column deterioration by water contamination.

Adsorbent traps such as Tenax™ have been used to isolate the volatile compounds purged from five honeys from North American floral sources such as thistle, tulip polar, sourwood, mountain laurel, and tupelo (Overton & Manura, 1994). The compounds were separated from the adsorbent resins by thermal desorption, and then carried into the GC column to be analyzed in a GC–MS system. The GC oven temperature was maintained at −40 °C during the extraction and desorption steps. They found numerous mono- and sesqui-terpenoid compounds, as well as flavors such as benzaldehyde, furfural, isovareladehyde and phenyl acetaldehyde. A similar technique with slight modifications has been used to analyze honey of nine different botanical origins from eight different countries (Radovic et al., 2001). The compounds isolated in the adsorbent resin were thermally desorbed and concentrated in a cold trap device attached to the injector end port. They reported finding volatile compounds such as aldehydes, ketones, and short-chain alcohols in unifloral honeys. Bianchi, Careri, and Musci

(2005) by using Carbopack™ as an absorbent resin and following a similar procedure described by Radovic et al. (2001), were able to identify mainly norisoprenoids compounds as characteristic markers in Sardinian strawberry-tree honey. Adsorbent traps showed good potential for differentiating characteristic compounds in the honeys studied (Table 1), suggesting that this technique can be useful for elucidating honey based on the volatile fraction.

Headspace SPDE (solid phase dynamic extraction) is a novel solvent free technique that utilizes a hollow needle with an internal coating of a polymer. The volatile compounds are concentrated on the absorbent film (polymer) by passing the gas through the device with repeated aspiration/ejection motions of the syringe plunger. Compared to HS-SPME, the system HS-SPDE is mechanically more resistant and has the potential advantage of increasing the amount of absorbing polymer, as well as the surface area available for absorbing volatile compounds. This method has successfully been applied for HS-Sampling of food matrices, giving good repeatability and intermediate precision for a group of compounds characterizing the matrices analyzed (Bicchi, Cordero, Liberto, Rubiolo, & Sgorbini, 2004). However, since this method is relatively new, further investigations are needed in order to understand the effects of parameters such as temperature, type of fibre, agitation and number of aspiration cycles on the recovery of analytes. Ampuero et al. (2004) compared the SPDE extraction method with SHS and SPME extraction methods on an electronic nose for characterizing unifloral honeys. The results of this investigation are described in the following section of this review.

5. Electronic nose

The analysis of the volatile compounds of honey described above has been achieved using GC or GC/MS on different compounds. A different strategy used by Ampuero et al. (2004) in order to characterize the origin of honey based on the volatile fraction was achieved with a fast sensitive electronic nose (MS–nose). Different sampling techniques such as solid phase micro-extraction (SPME), inside needle dynamic extraction (INDEX) (described above as solid phase dynamic extraction (SPDE)), and static head space (SHS) were compared in several Swiss honeys derived from dandelion, lime, acacia, chestnut, fir and rape. They analyzed the volatile fraction as a profile or "fingerprint", avoiding any separation into individual compounds. The response of the detector (specific ionic masses) was then processed by using a statistical program (principal components and discriminating factor). The electronic nose, in combination with the SPME sampling method, showed 98% correct classification of the model samples. A good correlation was also found between sensory analysis and nose analysis for classification of the test samples, proving its effectiveness in achieving the characterization of honey samples by their botanical origin.

Another fast sensitive and non-destructive electronic nose (zNose™) was tested by Lammertyn et al. (2004), on honeys (buckwheat, clover, orange blossom, black locust, mint and carrot) from different geographical origins. The volatile compounds were sampled with a needle (provided by the zNose™) inserted through the septa of the vials from a SHS system. Since the zNose™ is a combination sensor based detection and regular GC analyser, the data resulting from the zNose™ measurements were approached in two different ways: first, by comparing the different peaks and peak areas considering only the positives values of the first derivative plot (chromatogram approach); and second, the positive and negative values of the first derivative profile were considered and treated as spectral data, analysing the full frequency spectrum of each sample. The data were statistically processed by using both principal component analysis (PCA) and canonical discriminant analysis (CDA). The aroma "fingerprints" were sufficiently specific to discriminate between honeys from different floral origin and between honeys and plain sugar.

An artificial neural network tool named ACMD (approximation and classification of medical data), recently developed by Linder and Pöppl (2003), was utilized in order to demonstrate its capabilities in the field of food quality by classifying signals from an electronic nose smelling different types of honeys (mixed honey, fir, buckwheat, and three mixed honeys smelling of cesspit, oil and disinfectant). They used a portable electronic nose from WMA Airsense Analysentechnic GmbH, Shwerin, Germany. The headspace of the samples was measured by 10 different semiconducter gas sensors. A change in their electrical resistance produced 10 signals as a response to the existence of volatile molecules. The signals obtained from the samples and an additional signal from a gas flow (standard) was performed by three different artificial neural networks, resulting in a neural network ACDM more accurate for classification of the honeys according to their floral origin.

This technique analyzes the volatile fraction as a whole, without identifying each of the volatile compounds that constitute the food aroma. Methods based on electronic noses have proved to be simple and sensitive alternatives for fast aroma fingerprinting in foods (Ampuero & Bosset, 2003; Berna, Lammertyn, Buysens, Di Natale, & Nicola, 2005; Maignial et al., 1992; Saevels et al., 2003). However, more extensive studies are still required in order to confirm the reliability of honey characterization methods based on this technology.

6. Solid-phase microextraction

Extraction techniques such as liquid–liquid extraction and steam distillation extraction methods require consumption of expensive and toxic organic solvents, time, and the need for solvent disposal (Pawliszyn & Arthur, 1990). Dynamic solid-phase extraction can eliminate solvents entirely, however this technique

Table 2
SPME commercial fibres (Supelco, 2001)

Stationary phase/coating thickness	Recommended use	Characteristics
PDMS: polydimethylsiloxane (7, 30, 100 μm)	GC/HPLC	Non-polar, for volatile compounds
PA: polyacrilate (85 μm)	GC/HPLC	Polar, for polar semivolatile compounds
CW/DBV: carbowax/divinylbenzene (65 μm)	GC	Polar, for alcohols and volatile compounds
PDMS/DVB: polydimethylsiloxane/divinylbenzene (65 μm)	GC	Non-polar, for volatile compounds, amines and nitroaromatics compounds
DVB/CAR/PDMS: divinylbenzene/carboxene/polydimethylsiloxane (50/30 μm)	GC	For odours

requires extensive modification of the gas chromatographic injector or the addition of a desorption module (Pawliszyn & Arthur, 1990). Solid-phase microextraction (SPME) eliminates problems associated with dynamic solid phase extraction while retaining the advantages. Solvents are completely eliminated and extraction time can be reduced to a few minutes (Pawliszyn & Arthur, 1990).

SPME has recently been developed as a rapid, inexpensive and solvent-free technique. This technique uses a fine fused silica fibre with a polymeric coating (Table 2) to extract organic compounds from their matrix (Fig. 3) and directly transfer them into the injector of a gas chromatograph for thermal desorption and analysis (Zhang & Pawliszyn, 1995).

The main advantages of SPME are simplicity, high sensitivity, small sample volume, and lower cost per analysis. SPME techniques can be successfully applied for polar and non-polar compounds in gas, liquid and solid samples, and can be easily coupled with various analytical instruments such as GC, GC–MS, HPLC, LC–MS and GC-O (GC-olfactometry) (Kataoka, Lord, & Pawliszyn, 2000; Pawliszyn, 1997; Wardencki, Michulec, & Curylo, 2004). Other significant aspects of SPME techniques are reproducibility, repeatability, fibre stability, and the possibility of quantitative determinations (Pawliszyn, 1997). Since 1992, SPME has been widely used for environmental, food, flavour, fragrance, pheromone, pharmaceutical, clinical, forensic, and reaction monitoring applications (Pawliszyn, 1997).

6.1. Fibre selection

In general, volatile extraction is best achieved when the polarity of the fibre matches the polarity of the analyte (i.e. non-polar fibres for non-polar analytes, and polar fibres for polar analytes) (Fig. 4). This principle also applies in gas chromatography when polar and non-polar analytes require columns of the same polarity for analysis (Mani, 1999).

6.2. Solid-phase microextraction conditions

6.2.1. Optimization of extraction

In SPME, the amount of analyte extracted onto the fibre depends not only on the polarity and thickness of the stationary phase, but also the extraction time and the concentration of analytes in the sample. Extraction of analytes is improved by agitation, addition of salt to the sample, changing the pH, and increasing temperature (Kataoka et al., 2000; Pawliszyn, 1999). Extraction time is mainly determined by the agitation rate and the partition coefficient of the analyte between the fibre coating and

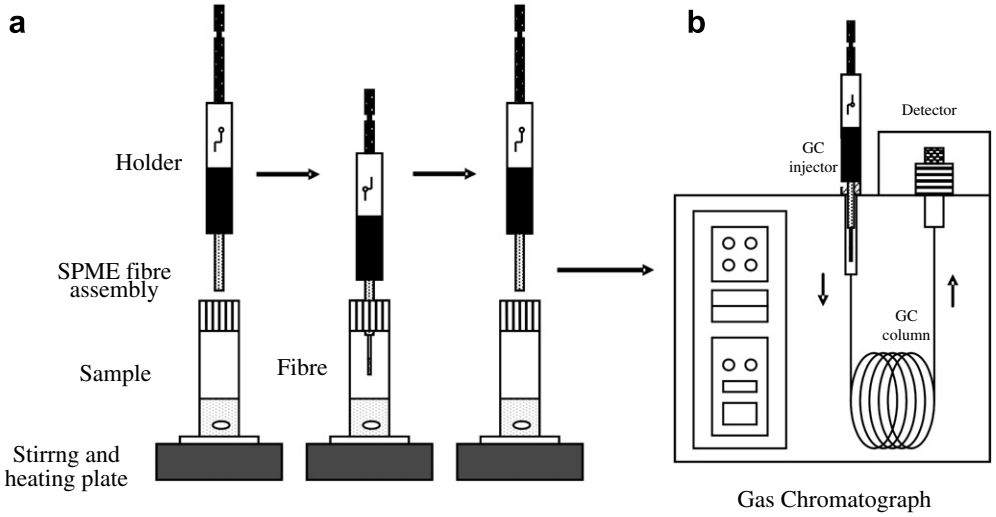

Fig. 3. Extraction process by HS-SPME, and thermal desorption process for GC: (a) HS-SPME process and (b) thermal SPME fibre desorption.

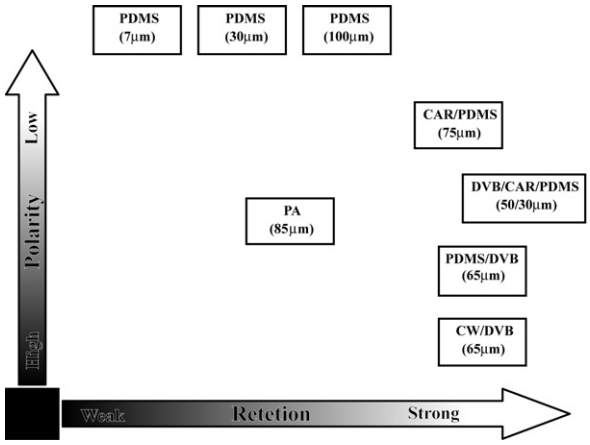

Fig. 4. SPME commercial fibres properties (diagram adapted from Kataoka et al., 2000).

sample matrix. Magnetic stirring is commonly used for agitation. This process accelerates the transfer of analyte from the sample matrix to the coating fibre, however too fast of an agitation can cause a change in equilibrium time and poor measurement precision (Kataoka et al., 2000; Pawliszyn, 1999).

The extraction efficiency is also improved by adding soluble salts such as sodium chloride, sodium hydrogencarbonate, potassium carbonate or ammonium sulphate, to the sample. In principle, supersaturation of the sample with salts is most effective for the extraction of analytes onto the fibre, due to the salting-out effect. The form of the analytes present in the sample mainly depends on the pH of the matrix relative to the analyte and influences the extraction efficiency. In general, the sample is acidified for the extraction of acidic analytes and is made alkaline for the extraction of basic analytes. Non-volatile acid or base is usually used for HS-SPME. In order to increase the concentration of the analytes in the gaseous phase in HS-SPME, the sample is usually heated. An increase in extraction temperature causes an increase in extraction rate, and simultaneously a decrease in the distribution constant. Therefore, an adequate temperature which provides satisfactory sensitivity and extraction rate should be used.

Another critical point is that the vial size and sample volume should remain constant in all samples during analysis by SPME. It was found that equilibrium is reached three times more quickly if 1 mL of liquid is placed in a 5 mL vial than if 10 mL liquid is placed in a 50 mL vial. Usually, the vial is filled to half of its capacity (Kataoka et al., 2000; Pawliszyn, 1999). The analyte extraction is improved when the headspace is minimized; however the minimum headspace volume is limited by the length of the fibre.

6.2.2. Optimization of desorption

Efficient desorption of analytes in a GC injection port is dependent on analyte volatility, the thickness of the fibre coating, injection depth, injection temperature, and exposure time. A narrow bore GC injector insert is required to ensure high linear flow and the fibre needs to be exposed immediately after the needle is introduced into the insert. Split/splitless injectors should be operated in the splitless mode to avoid loss of extracted volatile compounds (Kataoka et al., 2000; Pawliszyn, 1999). Generally, the optimal desorption temperature is approximately equal to the boiling point of the least volatile analyte. To prevent peak bordering, the initial GC column temperature should be kept low, or even cooled (cryofocusing). Thus, concentration of analytes at the head of the column is achieved. The desorption time depends on the injector temperature and the linear flow-rate around the fibre (Kataoka et al., 2000; Pawliszyn, 1999). To achieve reproducibility, constant conditions for analyte extraction and fibre desorption are essential.

6.3. Honey volatile analysis by SPME

6.3.1. Fibre

In recent years, some efforts have been made for analysing honey volatiles using SPME technique (Guidotti & Vitali, 1998; Pérez, Sánchez-Brunete, Calvo, & Tadeo, 2002; Piasenzotto, Gracco, & Conte, 2003; Verzera, Campisi, Zappala, & Bonaccorsi, 2001). Different types of SPME fibres have been evaluated, taking into consideration polarities, fibre coatings and fibre coating film thickness with the purpose of identifying which commercial fibre is most suitable for extracting honey volatiles.

Verzera et al. (2001) found better results with a PDMS/DVB fibre than with a PA fibre when analysing honey derived from eucalyptus, orange, wild flowers, and chestnut. However, Pérez et al. (2002) discovered higher concentrations and a greater number of honey volatiles with CAR/PDMS fibres than with PDMS/DVB fibres. Piasenzotto et al. (2003), in contrast, found more optimal performance with a PA fibre than with a CAR/PDMS fibre.

Soria et al. (2003) suggested using both PA and CAR/PDMS fibres since the PA fibre showed high precision, which can be enhanced by using suitable compound concentration ratios, and is a main requirement for honey characterization when distinguishing among samples of different types. CAR/PDMS fibre test results indicated a better overall sensitivity than PA fibres, which is also necessary for characterization of honey that is poor in volatile compounds. Other researchers (Alissandrakis, Kibaris, et al., 2005; Alissandrakis, Tarantilis, et al., 2005, in press; Ampuero et al., 2004; Zhou, Wintersteen, & Cadwallader, 2002) have found good results when analysing acacia, chestnut, lime, dandelion, buckwheat, cotton, citrus and fir honey volatiles with DVB/CAR/PDMS 50/30 μm composite fibres.

To date, it has not been specified which of the available SPME commercial fibres are best suited for honey volatile analyses. Additionally, it is necessary to take into consideration SPME operation conditions, which have significant

influence on the head space equilibrium and on fibre absorption capacity.

6.3.2. Optimization of extraction and desorption process

Diverse efforts have been made with the purpose of finding optimal SPME conditions for analysing honey aroma. Such efforts have focussed on vial size, salt addition, magnetic stirring, temperature, equilibrium and extraction time, plus GC split/splitless desorption time (Table 3). Verzera et al. (2001), in contrast to Soria et al. (2003), found that the addition of salt improves the extraction of volatiles, since organic hydrophilic compounds are less soluble in aqueous phase (Pawliszyn, 1997; Supelco, 1998). Optimal extraction conditions (Table 3) presently delineated include time and temperature equilibrium between 15 and 30 min at 60–70 °C; time and temperature extraction between 20 and 30 min at 60–70 °C. However, it is still unknown whether artifacts, such as Millard reaction products, can be generated by those temperature and time parameters (Soria et al., 2003; Verzera et al., 2001). Several vial sizes have been utilized, although Verzera et al. (2001), uniquely elaborated criterion for vial selection. The samples ranged from 1 g of undiluted honey placed in 4 mL size vials, to 16 g of diluted honey placed in 40 mL size vials (Table 3). The purpose of varying vial size is to obtain the highest amount of extraction compounds by exposing the SPME fibre to a headspace/sample volume ratio around 1:1 (Pawliszyn, 1997; Supelco, 1998; Verzera et al., 2001). It is also important to indicate that honey diluted with water was used as a means to decrease the matrix density and easily evaporate the analytes of interest retained by the sugars (Verzera et al., 2001). Magnetic stirring improves honey volatiles extraction, however optimum magnetic stirring velocities have not been established (Pérez et al., 2002; Soria et al., 2003; Verzera et al., 2001; Zhou et al., 2002).

Fibre desorption time, using splitless mode, has varied from 2 to 5 min. Soria et al. (2003) found better results employing 2 min of splitless desorption time. Desorption temperatures utilized were those suggested by the fibre manufacturer's manual (Supelco, 1998). Another approach was used by Peña, Barciela, Herrero, and García-Martín (2004). They compared direct immersion (DI)-SPME against HS-SPME for monoterpenes compounds extraction. They reported better recoveries of monoterpenes when applied DI-SPME in mixed floral Galician honeys.

6.3.3. Honey volatile compounds identified by SPME-GC/MS

As previously mentioned, it has been determined that honey volatile compounds are related to their botanical origin, and that many of them are characteristic markers of honeys. By using SPME-GC/MS methods, an important number of organic compounds (Table 1) have been found as components of different types of honeys (Ampuero et al., 2004; Guidotti & Vitali, 1998; Pérez et al., 2002; Piasenzotto et al., 2003; Soria et al., 2003; Verzera et al., 2001; Zhou et al., 2002). Thus, methyl anthranilate was identified as a compound characteristic of citrus honey (Alissandrakis et al., 2005, 2007; Piasenzotto et al., 2003; Soria et al., 2003; Verzera et al., 2001), which was also reported previously by Graddon et al. (1979) and Serra and Coll (1995). Other volatile compounds suggested as markers for citrus honey include lilac aldehyde (Alissandrakis et al., 2005, 2007; Piasenzotto et al., 2003; Soria et al., 2003), limonene diol (Piasenzotto et al., 2003), hotrienol (Guidotti & Vitali, 1998; Verzera et al., 2001) and 1-*p*-menthen-al (Alissandrakis et al., 2005, 2007).

Eucalyptus honey was shown to be distinct by the content of the volatile compounds nonanol, nonanal, nonanoic acid (Guidotti & Vitali, 1998; Piasenzotto et al., 2003; Verzera et al., 2001), and acetoin (Pérez et al., 2002; Piasenzotto et al., 2003; Verzera et al., 2001). Acetoin has been mentioned as a eucalyptus honey marker by Graddon et al. (1979) and Bouseta et al. (1992).

Alternatively, the chestnut honey analyzed by Piasenzotto et al. (2003) and Verzera et al. (2001) produced volatile compounds identified as characteristic markers such as acetophenone, 1-phenylethanol and 2-acetophenone, which have also been mentioned previously by Guyot et al. (1998). Volatile compounds such as 2-methylcyclopentanol and diethylphenol have also been mentioned as characteristic of this unifloral honey because of its detection and abundance (Guidotti & Vitali, 1998).

Heather honey has presented high levels of isophorone (3,5,5-trimethylcyclohexen-2-enone) (Soria et al., 2003), which has been previously mentioned for New Zealand (Tan et al., 1989) and European (Guyot et al., 1999) heather honeys. Nonetheless, this compound has also been found in other unifloral honey such as thyme (Tan, Wilkins, Holland, & McGhie, 1990) and rosemary (Soria et al., 2003).

Other unifloral honey analyzed by SPME-GC/MS have been rosemary honey (Pérez et al., 2002; Soria et al., 2003), lavender honey (Pérez et al., 2002; Soria et al., 2003), Sicilian wild flower honey (Verzera et al., 2001), acacia honey (Guidotti & Vitali, 1998), and thyme honey (Guidotti & Vitali, 1998; Pérez et al., 2002; Piasenzotto et al., 2003). However, further work is needed to reach a consensus on results concerning volatile compounds as characteristic markers of these honeys.

As previously mentioned, some specific aroma compounds have been proposed as markers for characterizing unifloral honeys. However, in many cases the lack of reference samples and the probability of honeys being of mixed origin makes the application of statistical methods to the classification of the honey difficult. A two-step statistical analysis was utilized for classifying four Spanish honeys. The honey aroma compounds were extracted and identified by SPME-MS. The samples most clearly classified by discriminant analysis were selected as ''references samples'' and used in multiple regression analysis to estimate the most representative compounds for each honey. By using this method they were able to classify by origin eucalyptus and citrus honey. However, the other honeys presented difficulties for classification since

Table 3
SPME-GC/MS operation conditions technique used for honey volatile analysis

Fibre	Sample size	Vial size (ml)	Sample treatment	Equilibrium		Extraction		Desorption (GC)			Ref.
				Temperature (°C)	Time (min)	Temperature (°C)	Time (min)	Time (min)	Temperature (°C)	Split/splitless valve	
PDMS/DVB Supelco	1 g	4	–	70	60	70	30	5	PDMS@ 270 PDMS/ DVB @ 250	Splitless	(Pérez et al., 2002)
PDMS/DVB 65 μm Supelco	16 g	40	Dilution of honey in 7 mL of water + addition of 2 g NaCl	30	30	30	25	3	220	Splitless	Verzera et al. (2001)
PDMS, 100 μm; PA, 80 μm; carboxene, 75 μm. Supelco	3 g	10	Addition of 0.5 g Na_2SO_4 + 0.5 mL of internal standard (ISTD)	70	30	70	20	3	250	Splitless/split valve opening @ 3 min	Piasenzotto et al. (2003)
PDMS 100 μm Supelco	3 g	10	–	70	30	70	20	3	240	Splitless/split valve opening @ 3 min	Guidotti and Vitali (1998)
DVB/CAR/PDMS Supelco	1 g	22	Dilution of honey in 5 mL of a matrix diluent (deodorized aqueous + 1.0 M phosphate–citrate buffer containing saturated NaCl with final pH adjusted to 4.0) + addition of 5 μL of ISTD	60	10	60	5	4	260	Splitless/split valve opening @ 4 min	Zhou et al. (2002)
CAR/PDMS (75 μm); PA (85 μm) Supelco	1.5–2.0 g	5	Addition of 1 mL of milli-Q water	60	15	60	30	2	250	Splitless/split valve opening @ 2 min	Soria et al. (2003)
CAR/PDMS (75 μm)	1.5–2.0 g	5	Addition of 1 mL of milli-Q water	60	15	60	30	2	250	Splitless/split valve opening @ 2 min	de la Fuente et al. (2005)
DVB/CAR/PDMS	7 g	10	Addition of 1 mL of milli-Q water + Addition of 1.05 g NaCl	90	2	90	30	–	190	–	Ampuero et al. (2004)
DVB/CAR/PDMS Stableflex 50/30 Supelco	6 mL (honey solution)	15	Addition of 20 μL of ISTD (benzophenone, 10 μg mL^{-1}) to honey solution (3 g mL^{-1})	60	30	60	60	–	220	–	Alissandrakis et al. (2005, 2007)

they probably were of mixed origin. These results highlight the utility and importance of statistical techniques in constraining possible interpretations.

7. Conclusions

Volatile compound identification with the purpose of assessing the botanical origin of honey has the potential to be an extremely useful strategy. Nonetheless more efforts are still required in order to develop a simple, rapid and objective method. Current methods that use solvents or analyze headspace (static and dynamic) have shown good approximations. However, such methods are complicated and demand additional equipment. Among them, dichloromethane extraction under an inert atmosphere followed by simultaneous steam distillation-dichloromethane extraction appeared to be a useful method for honey aroma characterization. On the other hand, the relatively recent SPME-GC/MS method has proved to be a very reliable technique since high reproducibility and sensibility have been achieved with respect to extraction and identification of honey volatile compounds without the complexity of traditional methods. Moreover, SPME has the advantage of being a flexible, simple and a relatively economic extraction technique. To date, composite SPME fibre coatings, such as CAR/PDMS fibre (75 μm), PDMS/DVB (65 μm) and DVD/CAR/PDMS (50/30 μm), have shown good results with honey volatile extraction. Therefore, the results will be dependent on both the fibre characteristics and extraction conditions used for the analysis. Headspace SPDE is another promising extraction technique with the advantage of the SPME coatings. However, more studies are required before it becomes a more widely accepted technique for honey aroma extraction.

Acknowledgements

The authors thank the Consejo Nacional para la Ciencia y Tecnología (CONACyT) of Mexico for the financial support given and the Consejo del Sistema Nacional para la Educación Tecnológica (CoSNET) for providing Luis Cuevas-Glory doctoral fellowship. Additional support was provided by a National Science Foundation International Research Fellowship to Louis Santiago. We also thank Emily Limm for her review of the article and constructive suggestions.

References

Alissandrakis, E., Daferera, D., Tarantalis, P. A., Polissiou, M., & Harizanis, P. C. (2003). Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey. *Food Chemistry, 82*(4), 575–582.

Alissandrakis, E., Kibaris, A. C., Tarantilis, P. A., Harizanis, P. C., & Polissiou, M. (2005). Flavour compounds of Greek cotton honey. *Journal of the Science of Food and Agriculture, 85*, 1444–1452.

Alissandrakis, E., Tarantilis, P. A., Harizanis, P. C., & Polissiou, M. (2005). Evaluation of four isolation techniques for honey aroma compounds. *Journal of the Science of Food and Agriculture, 85*, 91–97.

Alissandrakis, E., Tarantalis, P. A., Harizanis, P. C., & Polissiou, M. (2007). Aroma investigation of unifloral Greek citrus honey using solid-phase microextraction coupled to gas chromatographic–mass spectrometric analysis. *Food Chemistry*, *100*(1), 396–404.

Ampuero, S., Bodganov, S., & Bosset, J. O. (2004). Classification of unifloral honeys with an MS-based electronic nose using different sampling modes SHS SPME and INDEX. *European Food Research Technology, 218*, 194–207.

Ampuero, S., & Bosset, J. O. (2003). The electronic nose applied to dairy products: a review. *Sensors and Actuators B, 94*, 1–12.

Anklam, E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry, 63*(4), 549–562.

Anklam, E., & Radovic, B. S. (2001). Suitable analytical methods for determining the origin of European honey. *American Laboratory*, 60–64.

Barcarolo, R., & Casson, P. (1997). Modified capillary GC/MS system enabling dynamic headspace sampling with on-line cryofucusing and cold on-column injection of liquid samples. *Journal of High Resolution Chromatography, 20*(1), 24–28.

Berna, A. Z., Lammertyn, J., Buysens, S., Di Natale, C., & Nicola, B. M. (2005). Mapping consumer liking of tomatoes with fast aroma profiling techniques. *Postharvest Biology and Technology, 38*, 115–127.

Bianchi, F., Careri, M., & Musci, M. (2005). Volatile norisoprenoids as markers of botanical origin of Sardinian strawberry-tree (*Arbutus unedo* L.) honey: characterisation of aroma compounds by dynamic headspace extraction and gas chromatography–mass spectrometry. *Food Chemistry, 89*, 527–535.

Bicchi, C., Belliardo, F., & Fratinni, C. (1983). Identification of the volatile components of some piedmontese honeys. *Journal of Apicultural Research, 22*(2), 130–136.

Bicchi, C., Cordero, C., Liberto, E., Rubiolo, P., & Sgorbini, B. (2004). Automated headspace solid-phase dynamic extraction to analyse the volatile fraction of food matrices. *Journal of Chromatography A, 1024*, 217–226.

Bouseta, A., & Collin, S. (1995). Optimized Likens–Nickerson methodology for quantifying honey flavors. *Journal of Agricultural and Food Chemistry, 43*(7), 1890–1897.

Bouseta, A., Collin, S., & Doufour, J. (1992). Characteristic aroma profiles of unifloral honeys obtained with a dynamic headspace GC–MS system. *Journal of Apicultural Research, 32*(2), 96–109.

Careri, M., Mangia, A., Barbieri, G., Bolzoni, L., Virgili, R., Parolari, G., et al. (1994). Sensory property relationship to chemical data italian-type dry-cured ham. *Journal of Food Science, 27*(5), 491–495.

Cocito, C., Gaetano, G., & Delfini, C. (1995). Rapid extraction of aroma compounds in must and wine by means of ultrasound. *Food Chemistry, 52*, 311–320.

D'Arcy, B. R., Rintoul, G. B., Rowland, C. Y., & Blackman, A. J. (1997). Composition of Australian honeys extractives. 1 Noroisoprenoids, monoterpenes, and others natural volatiles from. *Journal of Agricultural and Food Chemistry, 45*(5), 1834–1843.

de la Fuente, E., Martínez-Castro, I., & Sanz, J. (2005). Characterization of Spanish unifloral honeys by solid phase microextraction and gas chromatography–mass spectrometry. *Journal of Separation Science, 28*, 1093–1100.

Graddon, A. D., Morrison, J. D., & Smith, J. S. (1979). Volatile constituents of some unifloral Australian honeys. *Journal of Agricultural and Food Chemistry, 27*(4), 832–837.

Guidotti, M., & Vitali, M. (1998). Identification of volatile organic compounds present in different honeys through SPME and GC/MS. *Industrie Alimentari, XXXVII*, 351–356.

Guyot, C., Bouseta, A., Scheirman, V., & Collin, S. (1998). Floral origin markers of chestnut and lime tree honeys. *Journal of Agricultural and Food Chemistry, 46*(2), 625–633.

Guyot-Declerck, C., Chevance, F., Lermusieau, G., & Collin, S. (2000). Optimized extraction procedure for quantifying norisoprenoids in

honey and honey food products. *Journal of Agricultural and Food Chemistry, 48*(12), 5850–5855.

Guyot, C., Scheirman, V., & Collin, S. (1999). Floral origin markers of heather honeys: *Calluna vulgaris* and *Erica arborea*. *Food Chemistry, 64*(1), 3–11.

Iglesias, M. T., De Lorenzo, C., Polo, M., Martín-Alvarez, P. J., & Pueyo, E. (2004). Usefulness of amino acid composition to discriminate between honeydew and floral honeys. Application to honeys from a small geographic area. *Journal of Agricultural and Food Chemistry, 52*(1), 84–89.

Kataoka, H., Lord, H. L., & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of Chromatography, 880*, 35–62.

Lammertyn, J., Veraverbeke, E. A., & Irudayaraj, J. (2004). zNose™ technology for the classification of honey based on rapid aroma profiling. *Sensors and Actuators B, 98*, 54–62.

Linder, R., & Pöppl, S. J. (2003). A new neural network approach classifies olfactory signals with high accuracy. *Food Quality and Preference, 14*, 435–440.

Maignial, L., Pibarot, P., Bonetti, G., Chaintreau, A., & Marion, J. P. (1992). Simultaneous distillation-extraction under static vacuum: isolation of volatile compounds at room temperature. *Journal of Chromatography, 606*, 87–94.

Mani, V. (1999). Properties of commercial SPME coatings. In J. Pawliszyn (Ed.), *Application of solid phase microextraction* (pp. 57–72). Bodmin, Cornwall, UK: Royal Society of Chemistry.

Moreira, R. F. A., Trugo, L. C., Pietroluongo, M., & De Maria, C. A. B. (2002). Flavor composition of cashew (*Anacardium occidentale*) and Marmeleiro (*Croton Species*) honeys. *Journal of Agricultural and Food Chemistry, 50*, 7616–7621.

Nickerson, G. B., & Likens, S. T. (1966). Gas chromatographic evidence for the occurrence oil components in beer. *Journal of Chromatography, 21*, 1–5.

Overton, S. V., & Manura, J. J. (1994). Flavor and aroma in natural bee honey. *American Laboratory*.

Pawliszyn, J. (1997). *Solid-phase microextraction. Theory and practice*. New York: Wiley–VCH.

Pawliszyn, J. (1999). *Application of solid phase microextraction*. Ontario, Canada: Royal Society of Chemistry.

Pawliszyn, J., & Arthur, C. L. (1990). Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry, 62*, 2145–2146.

Peña, R. M., Barciela, J., Herrero, C., & García-Martín, S. (2004). Solid-phase microextraction gas chromatography mass spectrometry determination of monoterpenes in honey. *Journal of Separation Science, 27*, 1540–1544.

Pérez, R. A., Sánchez-Brunete, C., Calvo, R. M., & Tadeo, J. L. (2002). Analysis of volatiles from spanish honeys by solid-phase microextraction and gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry, 50*(9), 2633–2637.

Piasenzotto, L., Gracco, L., & Conte, L. (2003). Solid phase microextraction (SPME) applied to honey quality control. *Journal of Science of Food and Agriculture, 83*, 1037–1044.

Radovic, B. S., Careri, M., Mangia, A., Musci, M., Gerboles, M., Anklam, E., et al. (2001). Contribution of dynamic headspace GC–MS analysis of aroma compounds to authenticity testing of honey. *Food Chemistry, 72*(4), 511–520.

Roberts, T. A., Aureli, P., Flamini, C., & Yndestad, M. (2002). Honey and microbioogical hazards. In *Proceedings of the scientific committee on veterinary measures relating to public health*.

Rowland, C. Y., Blackman, A. J., D'Arcy, B. R., & Rintoul, G. B. (1995). Comparison of organic extractives found in leatherwoos (*Eucryphia lucida*) honey and leatherwood flowers and leaves. *Journal of Agricultural and Food Chemistry, 43*(3), 753–763.

Saevels, S., Lammertyn, J., Berna, A. Z., Veraverbeke, E. A., Di Natale, C., Nicolaï, B. M., et al. (2003). Electronic nose as a non-destructive tool to evaluate the optimal harvest date of apples. *Postharvest Biology and Technology, 30*, 3–14.

Serra, B. J., & Coll, V. (1995). Characterization of citrus honey (Citrus Spp.) produced in Spain. *Journal of Agricultural and Food Chemistry, 43*(8), 2053–2057.

Shimoda, M., Wu, Y., & Osajima, Y. (1996). Aroma compounds from aqueous solution of haze (*Rhus sucedánea*) honey determined by absorptive column chromatography. *Journal of Agricultural and Food Chemistry, 44*(1), 3913–3918.

Soria, A. C., Martínez-Castro, I., & Sanz, J. (2003). Analysis of volatile composition of honey by solid phase microextraction and gas chromatography–mass spectrometry. *Journal of Separation Science, 26*, 793–801.

Supelco. (1998). Solid phase microextraction: theory and optimization of conditions. Sigma–Aldrich Co. (Bulletin 923).

Supelco. (2001). Solid phase microextraction (SPME). Chromatography products for analysis and purification. Sigma-Aldrich Co. (Catalog), 349–371.

Tan, S. T., Holland, P. T., Wilkins, A. L., & Molan, C. P. (1988). Extractives from New Zealand honeys. 1. White clover, manuka, and kanuka unifloral honeys. *Journal of Agricultural and Food Chemistry, 36*, 453–460.

Tan, S. T., Wilkins, A. L., Holland, P. T., & McGhie, T. K. (1989). Extractives from New Zealand honeys. 2, degraded carotenoids and other substances. *Journal of Agricultural and Food Chemistry, 37*(5), 1217–1221.

Tan, S. T., Wilkins, A. L., Holland, P. T., & McGhie, T. K. (1990). Extractives from New Zealand honeys. 3. Unifloral thyme and willow honeys constituents. *Journal of Agricultural and Food Chemistry, 38*(9), 1833–1838.

Verzera, A., Campisi, S., Zappala, M., & Bonaccorsi, I. (2001). SPME-GC–MS analysis of honey volatile components for the characterization of different floral origin. *American Laboratory*, 18–21.

Wardencki, W., Michulec, M., & Curylo, J. (2004). A review of theoretical and practical aspects of solid-phase microextraction in food analysis. *International Journal of Food Science and Technology, 39*, 703–717.

Wilkins, A. L., Lu, Y., & Tan, S. T. (1993). Extractives from New Zealand honeys. 4. Linalool derivatives and others components from nodding thistle (*Cardans nutans*) honey. *Journal of Agricultural and Food Chemistry, 41*(6), 873–878.

Wintoch, H., Morales, A., Duke, C., & Schreier, P. (1993). (*R*)-(−)-(*E*)-2,6-dimethyI-3,7-octadiene-2,6-di6ol-0-8-D-glucopyranoside: natural precursor of hotrienol from lulo fruit (*Solanum vestissimum* D.) peelings. *Journal of Agricultural and Food Chemistry, 41*(8), 1311–1314.

Zhang, Z., & Pawliszyn, J. (1995). Quantitative extraction using an internal cooled solid phase microextraction device. *Analytical Chemistry, 67*, 34–43.

Zhou, Q., Wintersteen, C. L., & Cadwallader, K. R. (2002). Identification and quantification of aroma-active components that contribute to the distinct malty flavor of buckwheat honey. *Journal of Agricultural and Food Chemistry, 50*(7), 2016–2021.