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Influence of Storage Conditions on Chemical Composition and Sensory Properties of Citrus Honey

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Fresh citrus honey was stored at 10, 20, and 40 °C for 12 months. The effect of storage on the quality of honey was evaluated using physicochemical parameters, volatile compounds, mono-, di-, and trisaccharides, and sensory analysis. Diastase activity and HMF were out of the legal limit in honey stored 12 months at 40 °C. Volatile compounds (especially terpenes and terpene derivatives), monosaccharides, and disaccharides presented important losses during honey storage at any temperature. Honey storage at 10 or 20 °C maintained their floral, fresh, citric, and fresh fruit aroma, while the intensities of these attributes were diminished. Storage at 40 °C during 12 months resulted in the appearance of attributes such as "medicinal, smoked, toasted, cooked vegetable, and ripened fruit", associated with compounds formed during the Maillard reaction or through degradation of sugars such as volatile pyrroles, furanones, pyranones, and pyrazines, which appeared or increased in concentration during honey storage mainly at high temperature.

KEYWORDS: Citrus honey; volatile compounds; sugar compounds; physico-chemical parameters; storage conditions; sensory analysis.

INTRODUCTION

Honey is usually selected by the consumer on the basis of its organoleptic qualities more than for its nutritive properties, flavor and taste being its most significant attributes. The distinctive honey aroma is produced by its volatile compounds which depend on nectar origin, but also on processing and storage conditions; on the other hand, honey taste is mainly caused by honey sugars.

Honey is a highly complex substrate to analyze. It contains many volatile components with different chemical structures, in low concentrations, in a sugar matrix composed mainly of glucose and fructose. The floral origin of honey is usually determined by pollen analysis, physicochemical parameters, and organoleptic properties (*1–4*).

Freshly extracted honey is liquid, but during storage sooner or later honey becomes crystallized. Crystallized honeys are not popular with consumers and can only be marketed liquefied. Gentle heating is mostly used to liquefy crystallized honey. To avoid heat damage of sensitive substances, it is recommended not to heat honey to more than 40–50 °C (*5, 6*).

It is in general admitted that the quality of the honeys decreases with the time of storage; however, under current market conditions, temperatures of up to 40–45 °C can be reached. These temperature changes can have a significant effect on the chemical composition and organoleptic characteristics of honeys.

According to the European Codex Honey Standards (*7*), a well-processed and ready to be consumed honey must contain the following physicochemical quality parameters: maximum moisture content of 20–21 g/100 g, free acidity ≤ 50 mequiv/kg, diastase number (ID) ≥ 8, hydroxymethylfurfural (HMF) content ≤ 40 mg/kg. The last two are very much influenced by heating and time of storage (*8*). Many authors have studied the evolution of HMF and diastase activity in honeys, observing a diminution of ID and an increase of HMF as effect of the storage and heating of honeys (*9–12*). Honey has an acid pH (3.5–4.5) due to its organic acids content, although its sensorial appreciation is masked by the sweet predominant flavor. Maximum free acidity permitted by European legislation is the 50 mequiv/kg. Higher levels involve a possible microbial alteration (*13–16*). Values of free and lactonic acidity increase progressively as a consequence of heating and prolonged storage (*17*).

Variations in levels of volatile components during honey storage are considered to depend largely on the temperature to

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which honey is exposed, although clearly the period of such exposure is also relevant (*18*). Changes in the volatile compounds of honey that has been heated or stored can be attributed to two principal causes: compounds that are heat labile and may be destroyed, and volatile compounds produced by nonenzymatic browning (Maillard reaction), which cause considerable changes in honey flavor (*19*).

A long period of storage or high temperatures are known to produce furan derivatives through sugar degradation, furfural and 5-hydroxymethylfurfural (HMF) concentrations being used as indicators of storage or heating of honeys (*17, 20–23*).

The legal limit for HMF in honeys has been established as 40 ppm (*24*). Other volatile compounds generated by the Maillard reaction that are structurally related to pyrazines and pyrroles, and to a lesser extent furanones, could serve as markers to heating honey (*25*), although there is no information about changes produced during prolonged stored of honeys at low or ambient temperature.

The objectives of this study were to evaluate the changes produced in a citrus honey stored for 12 months at different temperatures, in physicochemical parameters, volatile composition, mono-, di-, and trisaccharides, and sensory properties, in order to identify the presence of chemical and sensorial markers which can be related to the storage and heating of honey.

MATERIALS AND METHODS

A fresh Spanish citrus honey was divided in portions. One fresh portion was analyzed. The other three portions were stored at a constant temperature of 10, 20, and 40 °C for 12 months in 0.5 kg glass jars (70 mm of diameter and 100 mm of height) sealed with a twist-off cap. All treatment procedures were duplicated.

The temperatures of the analyses were selected on the basis of the different situations of storage to which honey can be submitted. 20 °C was selected to study the variations in storage at room temperature. On the other hand, 10 °C was selected to evaluate the evolution of honey in conditions of semirefrigeration, and finally we decided to study the modifications of honey in extreme temperatures of 40 °C that can be in some commercial showcase in the Spanish summer.

Physicochemical Parameters. Physicochemical analysis of honey samples were carried out by duplicate according to the Spanish Official Methods (*26*).

Electrical conductivity was determined in a solution of 5 g of honey dissolved in 50 mL of deionized water, using a Crison GLP 31 conductimeter previously calibrated at work temperature.

Isolation and Analysis of Volatile Compounds. All honeys were fractionated on styrene–divinylbenzene cartridges (Lichrolut EN, Merck, 0.5 g phase), previously conditioned with 10 mL of dichloromethane, 5 mL of methanol, and 10 mL of ethanol/distilled water (10%) at a flow rate of 2 mL/min.

Ten grams of each honey was dissolved in 50 mL of distilled water, and 25 μL of 2-pentanol (1 g/L) was added as internal standard. These solutions were passed through the cartridges. Then sugars were eluted with 25 mL of distilled water. Volatile compounds of interest were eluted with 30 mL of dichloromethane at a flow rate of 2 mL/min. The organic phase was collected and concentrated in a Vigreux column and analyzed by GC/MS (*27*).

An Agilent 6890 N gas chromatograph, coupled to a 5973 Inert mass selective detector was used. Two microliters (2 μL) of extracts were injected in splitless mode (0.6 min) on a BP-21 capillary column (60 m × 0.25 mm × 0.25 μm of film thickness). The oven temperature program was: 60 °C (3 min) – 2 °C/min–200 °C (30 min). Helium was used as carrier gas at a flow rate of 0.8 mL/min (28 cm/s). Injector and transfer line temperatures were 250 and 280 °C, respectively. Mass detector conditions were as follows: electronic impact (EI) mode at 70 eV and mass acquisition range 40–450 amu. Identification of the volatile components was performed by comparing their GC retention indices and mass spectra with those of authentic standards from Sigma-Aldrich. The tentative identification of compounds for which it was not possible to find reference compounds were carried out by comparison of their mass spectra with spectral data from the Wiley G 1035 A library. Semiquantitative analyses were carried out assuming a response factor equal to 1.

Carbohydrate Analysis. Honey disaccharides and trisaccharides were determined by GC by means of their transformation in trimethylsilyl (TMS) oximes following the method developed by Sanz et al. (*28*). One milliliter of a honey solution (20 mg/mL, 80% ethanol) was mixed with 0.5 mL of the internal standard solution (1 mg/mL of β-phenylglucoside) and evaporated under vacuum until dryness. Sugar oximes were formed using 350 μL of 2.5% hydroxylamine hydrochloride in pyridine and heated to 75 °C for 30 min. After reaction, samples were persilylated adding 350 μL of hexamethyldisilazane (HMDS) and 35 μL of trifluoroacetic acid (TFA) and holding at 45 °C for 30 min. All reactives were purchased from Sigma Chimical (St. Louis, MO). Next, samples were centrifuged at 7000 rpm for 5 min at 5 °C.

Gas chromatographic separation was carried out in an HP 5890 gas chromatograph equipped with a flame ionization detector (FID) (Hewlett-Packard, Palo Alto, CA) using an SPB-1 fused silica capillary column (25 m × 0.25 mm i.d. × 0.25 μm film thickness) from Supelco (Bellefonte, PA). The temperature of the injector and detector was 300 °C. Oven temperature was held at 200 °C for 15 min, then programmed to 270 °C at a heating rate of 15 °C/min and to 290 at 1 °C/min, and finally programmed to 300 at 15 °C/min and held for 30 min. Nitrogen was used as carrier gas, and injections were made in split mode with a split flow of 40 mL/min.

GC-MS analyses were carried out using a Hewlett-Packard 6890 gas chromatograph coupled to a 5973 quadrupole mass detector operating in electronic impact (EI) mode at 70 eV (both from Agilent, Palo Alto, CA, USA). Operating conditions other than carrier gas (He at 1 mL/min) were to those previously described for GC analysis.

Identification of *O*-TMS derivatives of carbohydrates present in honey samples was carried out by comparison of their retention times with those of standard compounds; mass spectral data were used to confirm peak identities. Sixteen disaccharides available as standard compounds were purchased from commercial sources (*28*). Quantitative data for carbohydrates were calculated from FID peak areas using standard solutions of carbohydrates over the expected concentration range in honey, prepared to calculate the response factor (RF) relative to phenyl-β-D-glucoside (internal standard).

Sensory Descriptive Analysis. Citrus honeys were tasted by a group of 10 assessors ranging from 22 to 35 years of age with previous experience in sensory analysis. They were recruited from the staff of University of Castilla-La Mancha. Participation was unremunerated.

At initial three sessions, assessors were trained with references. Solutions of linalool, 2-phenylethanol, γ-decalactone, and eugenol at 25 ppb were used to identify "fresh", "floral", "fresh fruit", and "spices" aromas. Furaneol at 35 ppb was used for "toast" notes. Oak wood chip and plumb were used to describe with "wood" and "ripened fruit" aromas.

Assessors were trained in descriptive sensory analysis using fresh and storage Spanish citrus honeys during three sessions. Then judges generated sensory terms individually. Over the course of these four sessions, 13 attributes were selected by consensus in order to describe the aroma of fresh and storage citrus honey samples. Another four formal sessions were employed to evaluate the intensity of the13 attributes selected using 10 cm nonstructured scales.

Sessions were performed in a tasting room with 14 individual booths that were separated from the area where the samples were prepared. In the tasting room, the temperature and the humidity were controlled, and the room was isolated for sound. Three different coded samples were presented in random order to each assessor in 40 mL glass vials sealed with a twist-off cap and at room temperature. Mineral water was provided so that assessors could rinse out their mouths between samples. Two replications of all sample assessments were performed.

Statistical Analysis. The Student–Newman–Keuls test was applied to discriminate the means of chemical data. The mean ratings and Fischer's least significant differences (LSD) were calculated for each

Table 1. Mean Values of Physicochemical Parameters and Standard Deviations of Fresh and Storage Citrus Honey

	pH	free acidity (mequiv/kg)	lactonic acidity (mequiv/kg)	diastase index (Gothe)	electrical conductivity (mS/cm)	HMF (mg/kg)	moisture (%)	proline (mg/100 g)
fresh honey	3.8 (0.0)	16.7 (0.3)	8.1 (0.0)	13.0 (0.1)	2.13 (1.1)	10.2 (0.1)	16.4 (0.0)	57.8 (0.8)
honey stored for 12 months (10 °C)	3.8 (0.0)	17.5 (0.0)	9.5 (0.7)	10.7 (0.0)	1.45 (0.1)	23.3 (0.3)	18.0 (0.0)	30.3 (1.5)
honey stored for 12 months (20 °C)	3.9 (0.0)	17.5 (0.0)	10.0 (0.0)	9.7 (0.0)	1.48 (1.1)	30.4 (0.3)	17.4 (0.0)	16.4 (0.2)
honey stored for 12 months (40 °C)	3.8 (0,0)	22.0 (0.4)	13.8 (0.1)	2.2 (0.0)	1.47 (1.6)	284.6 (3.8)	16.6 (0.0)	10.1 (0.2)

sensory descriptor by analysis of variance (ANOVA). Statistical processing was carried out using the SPSS 13.0 for Windows statistical package.

RESULTS AND DISCUSSION

Physicochemical Parameters. Table 1 shows results from physicochemical analysis normally used to verify the quality of honeys. Honey heating and storage significantly increased free and lactonic acids, mainly during storage at 40 °C. Increase of acidity as effect of heating and prolonged storage has been also observed in eucalyptus honeys (*17*).

Diastase activity (ID) and proline decrease after storage for 12 months especially at 40 °C (*6, 29, 30*). Diastase activity decreases 2.3 units in citrus honey samples stored at 10 °C, with respect to fresh samples, during a year. At these temperature conditions only can influence the storage period. Other studies reported a diminution of 4 diastase units at 20 °C after a year (*31*). Sahinler (*32*) concluded that honey diastase number is related to heating temperature and storage time.

Fresh citrus honey presented a low HMF content (10.2 mg/kg). Honey storage during 1 year at 10 and 20 °C generated an increase of this parameter, although the more significant increment took place at 40 °C, amply exceeding the limit allowed by the legislation (40 ppm) (*22, 29*).

Electrical conductivity is a parameter correlated with the mineral content, which increased in stored honeys with respect to fresh honeys, whereas moisture content and pH did not show a clear variation among fresh and stored honeys (*33*). Electrical conductivity does not depend exclusively on the mineral content. This measurement depends on the ash, organic acids, proteins, and some complex sugars (*34*); the higher their content, the higher the resulting conductivity (*35*).

Volatile Compounds. Eighty-nine volatile compounds were quantified in fresh and stored citrus honey samples. Mean values of concentrations (μg/kg) and standard deviations are shown in **Table 2**. Fresh citrus honey's aroma is mainly due to terpenes and derivative compounds, which have been related to the floral and citric attributes characteristic for this kind of honey (*3637*).

Terpene derivatives were some of the compounds that had more influence on the citrus honey aroma. They were some of the compounds that suffered major variability in this work. In some cases, the concentration of these compounds decreased considerably during 12 months of storage. In other cases, they could not be detected in honey samples stored for 1 year. Most of them, like α-terpineol, epoxylinalool, nerolidol, (*Z*)- and (*E*)-*p*-mentha-1(7)-8(10)dien-9-ol, *p*-cymen-8-ol, hotrienol, limonil alcohol, and lilac alcohol isomers, quite representative of these floral sources (*38*), were present in large amount in fresh citrus honey extracts, but they were not detected during 12 months of storage, at any temperature.

Special effect in sensorial characteristics of stored honey could have the loss of the α-4-dimethyl-3-cyclohexene-1-acetaldehyde that was quantified as one of the compounds of major concentration found in fresh honey and it has been associated with the "lilac aroma" (*36*). The same effect was observed for the two isomers of sinensal, components of the orange essential oil (*39*) and recently described as floral markers of citrus honeys (*38*).

Lilac aldehyde isomers presented a decrease during the 12 months of storage that was not affected by the storage temperature. In contrast linalool, linalool oxides, and dien-diols derivatives showed an important increase in stored honeys mainly at 40 °C. This increase was also observed by other authors (*40–42*) and could be due to the hydrolysis of glycosilated forms in warm and acid conditions.

Another compound considered as a good floral marker for citrus honey is the methyl anthranilate (*37, 43, 44*). The results showed a marked decrease of its concentration in honeys subjected to temperatures of 40 °C for 12 months.

Compounds resulting from degradation of sugars such as furan derivatives (furfural, 2-acetylfuran, 5-methylfurfural, furfuryl alcohol) were found in fresh citrus honey; they showed a little increase with storage at 10 and 20 °C, but they greatly increased when honey was stored at 40 °C. These compounds have been used as classic indicators of heating (*45*) and could afford "toasted" aromas and tastes when honey is stored at temperature of 40 °C for a long time.

Other compounds were clearly formed during honey storage, since they were absent in fresh citrus honeys. Some of them were formed only during honey storage at 40 °C, but not at 10 or 20 °C, such as (4*H*)pyran-4-one, 3,4-dihydro-(2*H*)pyranone, furaneol, 2-propyl-4-methylfuran, maltol, and maltol derivatives. These compounds could be good indicators for honey storage at high temperatures, specially the furaneol (2,5-dimethyl-4-hydroxy-3[2*H*]-furanone) which can be responsible for the sugar toasty flavor in heating honeys and other toasted products like coffee, cooked meats, malt, hazelnuts, toasted almonds, and popcorn (*46*). Its olfactory threshold is 31 ppb (*47*) that have been only exceeded by honeys heated at 40 °C.

The formation of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one (DDMP) has been described as the result of the decomposition of Amadori compounds formed by Maillard reaction. This compound together with the 2-methoxy-6-methylpyrazine and methyl furoate appeared in honeys stored at 10 and 20 °C, significantly increasing their concentration when honey was stored at 40 °C. Thermal degradation of DDMP can lead to the formation of compounds such as maltol and hydroxymaltol (*46, 48*).

γ-Butyrolactone and pantolactone present similar changes. They are not found in fresh citrus honey extracts, and their concentrations progressively increased in honey stored at 10, 20, and 40 °C. Some organic acids, ketones, and benzene compounds such as 1-hydroxy-2-propanone, butanoic acid, benzyl alcohol, or 2-phenylethanol, presented in fresh honey showed a progressive increase with storage temperature.

Table 2. Mean Concentrations of Volatile Compounds of Citrus Honeys (μg/kg) and Standard Deviations ($n = 2$)[a]

			12 months of storage					
	fresh		10 °C		20 °C		40 °C	
compd	x̄	SD	x̄	SD	x̄	SD	x̄	SD
Terpenes and Derivatives								
(*Z*)-linalool oxide	220.3 a	10.3	58.7 b	2.3	71.1 b	3.8	328.8 c	21.5
(*E*)-linalool oxide	387.4 a	66.1	55.2 b	10.4	69.9 b	0.6	280.0 c	16.4
lilac aldehyde (isomer I)	421.7 a	26.2	76.5 b	7.3	78.2 b	2.9	57.2 b	15.1
linalool	317.4 a	7.7	343.3 a	9.4	343.9 a	1.6	850.1 b	117.3
lilac aldehyde (isomer II)	534.5 a	49.7	132.7 b	16.3	140.9 b	5.9	112.1 b	3.7
lilac aldehyde (isomer III)	396.8 a	3.5	75.4 b	4.8	72.5 b	1.0	60.2 b	6.6
lilac aldehyde (isomer IV)	533.0 a	52.0	102.8 b	6.3	109.3 b	12.1	88.9 b	3.1
hotrienol	263.5	35.7	n.d.		n.d.		n.d.	
α-4-dimethyl-3-cyclohexene-1-acetaldehyde (isomer I)	3096.8	42.5	Tr		Tr		Tr	
α-4-dimethyl-3-cyclohexene-1-acetaldehyde (isomer II)	306.2 a	39.4	37.1 b	0.9	48.3 b	1.1	34.7 b	1.3
α-terpineol	76.6	14.6	n.d.		n.d.		n.d.	
lilac alcohol (isomer I)	22.0	5.0	n.d.		n.d.		n.d.	
2-caren-4-one	42.1	7.1	n.d.		n.d.		n.d.	
lilac alcohol (isomer II)	84.0	2.2	n.d.		n.d.		n.d.	
epoxylinalool	12.8	1.4	n.d.		n.d.		n.d.	
lilac alcohol (isomer III)	66.0	4.7	n.d.		n.d.		n.d.	
α-4-dimethyl-3-cyclohexene-1-acetaldehyde (isomer III)	102.7	3.2	Tr		Tr		Tr	
lilac alcohol (isomer IV)	92.6	5.8	n.d.		n.d.		n.d.	
p-cymen-8-ol	140.2	18.3	n.d.		n.d.		n.d.	
sinensal (isomer I)	16.2	1.3	n.d.		n.d.		n.d.	
sinensal (isomer II)	215.4	31.2	n.d.		n.d.		n.d.	
limonil alcohol	Tr		n.d.		n.d.		n.d.	
(*Z*)-*p*-mentha (7),8(10)dien-9-ol	229.7	27.8	n.d.		n.d.		n.d.	
(*E*)-*p*-mentha (7),8(10)dien-9-ol	170.9	20.8	n.d.		n.d.		n.d.	
2,6-dimethyl-1,7-octadien-3-ol	211.0 a	1.5	738.9 b	42.9	636.8 b	86.0	227.9 a	2.1
(*E*)-3,7-dimethyl-1,3,6-octatriene	639.7	8.3	n.d.		n.d.		n.d.	
nerolidol	73.1	21.2	n.d.		n.d.		n.d.	
β-maliene	98.0		n.d.		n.d.		n.d.	
(*Z*)-2,6-dimethyl-2,7-octadien-1,6-diol	n.d.	3.3	710.8 a	91.0	746.7 a	29.1	417.5 b	12.1
(*E*)-3,7,11-trimethyl-6,10-dodecadien-1-ol,	52.6	19.9	n.d.		n.d.		n.d.	
(*E*)-2,6-dimethyl-2,7-octadien-1,6-diol	70.2 a	10.3	8456.3 b	321.3	9014.3 b	405.6	3125.2 c	184.4
Furan and Pyran Compounds and Maillard Derivatives								
5-methyl-2(3*H*)furanone	33.3	7.3	27.8	5.1	24.8	5.0	23.2	2.8
furfural	144.5 a	1.1	244.7 b	23.1	399.0 c	8,3	4326.5 d	24.2
2-acetylfuran	63.0 a	7.1	43.1 a	8.7	49.7 a	0,5	216.7 b	1.8
6-methyl-3,5-dihydroxy-2,3-dihydro(4*H*)pyranone	n.d.		341.6 a	11.3	360.1 a	12,7	822.8 b	75.0
5-methylfurfural	149.2 a	2.7	147.1 a	12.6	144.4 a	1,3	913.4 b	118.7
(4*H*)pyran-4-one	n.d.		n.d.		n.d.		94.1	20.8
5-methyldihydro-2-(3*H*)furanone	n.d.		47.7	1.0	50.9	2,2	50.5	9.3
furfuryl alcohol	30.7 a	1.6	64.9 a	9.5	55.7 a	0,6	213.8 b	80.2
5-methyl-5-ethenyldihydro-2(3*H*)furanone	81.4 a	4.4	43.5 b	0.0	70.3 c	0,6	133.5 d	2.8
3,4-dihydro-(2*H*)-pyranone	n.d.		n.d.		7.8 a	2,9	34.5 b	7.3
3,5-dimethyl-5-ethyl-4,5-dihydropyrazol	n.d.		n.d.		26.6 a	1,7	178.8 b	24.9
furanol	n.d.		n.d.		n.d.		87.0	29.3
2-propyl-4-methylfuran	n.d.		n.d.		n.d.		44.0	28.7
maltol	n.d.		n.d.		15.9 a	0,7	423.1 b	243.7
2-methoxy-6-methyl pyrazine	n.d.		1637.6 a	60.6	6543.9 b	359,9	16640.4 c	682.3
methyl furoate	n.d.		1243.5 a	106.9	2203.6 a	57,3	13831.5 b	829.9
cyclotene	n.d.		Tr		Tr		Tr	
maltol derivative (isomer I)(126/79/109/154)	n.d.		n.d.		n.d.		511.5	11.8
maltol derivative (isomer II)	n.d.		n.d.		n.d.		280.9	34.0
2,3-dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one	n.d.		853.8 a	42.7	871.3 a	21,8	10193.2 b	1141.6
6-methoxy-(6*H*)-dibenzopyrane	n.d.		n.d.		1184.2 a	82,9	1846.6 b	171.7
Ketones								
3-hydroxy-2-butanone	59.7 a	9.7	60.7 a	12.7	65.6 a	0.9	91.1 b	3.3
1-hydroxy-2-propanone	35.0 a	4.8	39.4 a	2.9	43.4 a	0.4	359.5 b	3.6
1-hydroxy-2-butanone	Tr		n.d.		n.d.		77.4	17.9
Aldehydes								
nonanal	231.1 a	18.3	100.7 b	5.0	85.9 b	2.2	193.1 c	1.5
decanal	129.7	36.7	70.6	0.7	63.5	0.1	124.9	1.0
Alcohols								
2-hexanol	68.5 a	5.4	42.1 a	1.0	34.1 a	3.0	37.6 b	24.8
2-methyl-2-buten-1-ol	42.7 a	3.5	20.4 b	2.5	19.0 b	1.6	12.0 c	2.5
2-ethyl-1-hexanol	Tr		49.4 a	4.3	53.5 a	1.2	79.2 b	1.4
Acids								
acetic acid	n.d.		260.5 a	6.5	260.7 a	12.6	443.9 b	59.2
butanoic acid	14.1 a	1.1	167.2 b	1.6	144.1 c	5.0	156.9 d	4.2
3-methylbutanoic acid	25.2 a	4.8	42.5 b	0.2	43.1 b	1.8	55.8 c	2.8

Table 2. Continued

			12 months of storage					
	fresh		10 °C		20 °C		40 °C	
compd	x̄	SD	x̄	SD	x̄	SD	x̄	SD
pentanoic acid	2.4 a	0.3	108.3 b	5.1	88.8 c	2.0	62.7 d	5.1
hexanoic acid	108.6 a	4.9	239.5 b	12.7	229.6 b	1.6	218.7 b	2.8
2-ethylhexanoic acid	37.4 a	10.7	454.6 b	34.1	434.6 b	3.9	575.9 c	32.8
heptanoic acid	41.4 a	7.4	89.2 b	0.5	75.0 c	0.2	32.7 a	1.9
octanoic acid	213.9 a	50.1	474.5 b	2.8	463.7 b	6.5	484.7 b	5.8
nonanoic acid	249.3 a	17.9	264.3 a	18.5	269.7 a	8.9	528.5 b	11.6
benzoic acid	142.3 a	42.1	1494.6 b	43.3	1606.6 b	9.6	2143.2 c	195.0
benceneacetic acid	n.d.		834.6 a	102.7	782.0 a	10.2	1102.9 b	22.1
tetradecanoic acid	606.4 a	88.5	1338.3 b	8.0	697.4 a	21.6	1259.9 b	122.2
			Benzene Compounds					
benzaldehyde	376.5 a	59.5	58.5 b	9.6	47.1 b	5.8	61.1 b	0.6
phenylacetaldehyde	742.4 a	40.0	271.0 a	3.8	315.0 a	4.1	294.9 b	8.0
2-methyl hydroxybenzoate	22.1	9.3	n.d.		n.d.		n.d.	
guaiacol	18.2	0.6	21.0	3.7	17.5	2.3	13.3	0.0
benzyl alcohol	74.6 a	9.2	192.7 b	2.5	193.9 b	4.8	164.7 c	7.9
2-phenylethanol	271.8 a	6.0	494.8 b	13.4	537.0 b	33.3	513.6 b	81.1
2,3,5,6-tetramethylphenol	59.0 a	15.5	261.5 b	6.5	271.9 b	13.3	180.8 c	13.0
3,4,5-trimethylphenol	79.9	8.1	n.d.		n.d.		n.d.	
2,4-bis-(1,1)-dimethylethylphenol	n.d.		n.d.		n.d.		2387.3	114.6
3,5-dimethylbenzoate	n.d.		83.7 a	6.1	68.4 b	3.8	118.2 c	2.0
benzeneacetamide	n.d.		274.3 a	18.9	296.1 a	4.1	235.8 a	17.2
			Lactones					
γ-butyrolactone	n.d.		65.1 a	11.1	107.6 a	7.3	221.6 b	56.0
pantolactone	n.d.		423.7 a	15.7	703.4 b	38.0	940.8 c	64.9
			Miscellaneous					
dimethyl trisulfide	18.5 a	1.6	n.d.		n.d.		74.3 b	11.6
ketoisophorone	20.5 a	0.1	43.6 b	3.2	34.7 c	0.8	41.6 b	1.5
methyl anthranilate	1524.1 a	355.1	330.6 b	0.7	330.4 b	1.0	37.8 b	1.0

[a] Tr = traces. a,b,c,d: In each case, according to the result of the Student–Newman–Keuls, values that do not share a common letter are significantly different at $p < 0.05$. n.d. = not detected.

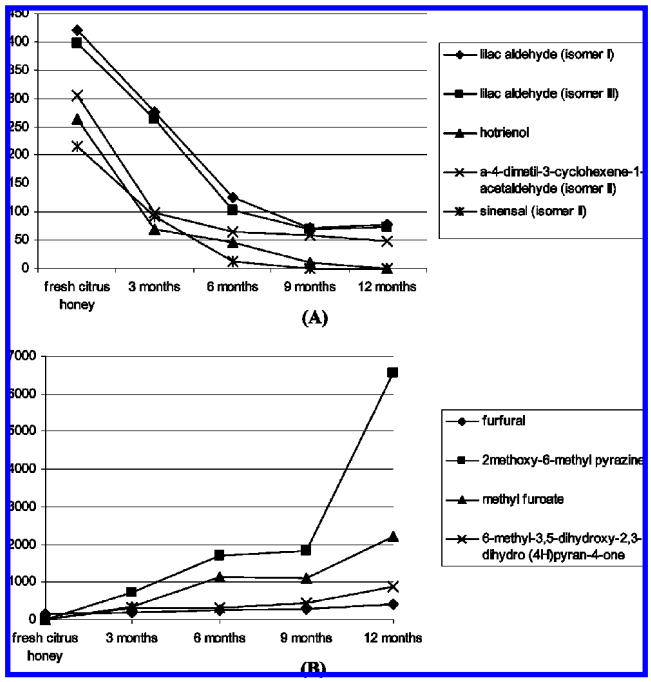

Figure 1. Changes in some terpene derivatives and furan and pyran compounds found in citrus honey during the storage at room temperature.

We also have analyzed the samples stored at 20 °C during 3, 6, and 9 months. **Figure 1A** shows the evolution of some terpene compounds of citrus honey at room temperature (20 °C). In all cases, an aggressive decrease of concentrations was produced until 6 months. From 6 to 12 months of storage, terpene derivatives continued declining their concentrations but in a more moderate way. Furan and pyran compounds were another interesting group of compounds, because their concentrations increased as consequence of the storage conditions. **Figure 1B** shows the evolution of some of the mentioned compounds present in citrus honey stored at room temperature (20 °C) during 3, 6, 9, and 12 months. The majority of them were not present in fresh honey whereas their concentrations increased until 6 months of storage. There had not been many variations between 6 and 9 months and during 12 months of storage were when the most significant increases of concentrations were produced.

Carbohydrate Composition. Table 3 presents the concentrations (mg/g) of carbohydrates in the analyzed samples. Monosaccharides presented very important losses during storage which amount to a 13.5, 25, and 25.2% for 10, 20, and 40 °C, respectively. RSD values for the determination of carbohydrates as their *O*-TMS oximes using this method were below 10% for different compounds both in standard mixtures and in samples (*49*).

Disaccharide concentrations showed a general increasing trend during storage, the changes being more marked at 40 °C. This behavior is common to the main disaccharides (nigerose, turanose, maltulose, isomaltose, and kojibiose). The most important change during storage corresponded to maltose, present initially at 2.5 mg/g and becoming one of the major disaccharides in the sample (23.2 mg/g) after 1 year at 40 °C, while α,β-trehalose decreased at this temperature. The trend for the other disaccharides was less clear.

Changes in trisaccharide composition during storage appeared to depend on the individual component being considered. Since

Table 3. Mean Concentrations of Monosaccharides, Disaccharides, and Trisaccharides of Fresh Citrus Honey and Honey in Storage for 12 months[a]

		12 months of storage		
	fresh	10 °C	20 °C	40 °C
	Monosaccharide (g/100 g)			
fructose	43.37	36.84	32.28	32.5
glucose	28.34	25.17	21.53	21.11
	Disaccharide (g/100 g)			
sacarose	0.05	0.26	0.11	0.17
α-trehalose	n.d.	n.d.	n.d.	n.d.
β-trehalose	0.51	0.53	0.54	0.40
celobiose	0.12	0.18	0.17	0.58
laminaribiose	0.20	0.21	0.21	0.74
maltulose	1.63	1.71	1.89	1.96
nigerose	1.25	1.39	1.33	1.69
turanose	2.39	2.52	2.47	2.74
leucrose	n.d.	n.d.	n.d.	n.d.
maltose	0.25	1.49	1.31	2.32
kojibiose	1.69	2.09	2.05	2.62
trehalulose	0.80	0.87	0.96	1.05
palatinose	0.19	0.25	0.27	0.25
gentiobiose	0.01	0.01	0.01	0.18
isomaltose	1.09	1.24	1.34	1.42
melibiose	n.d.	n.d.	n.d.	n.d.
	Trisaccharide (g/100 g)			
unknown	n.d.	0.16	0.04	0.03
raffinose	n.d.	0.34	0.29	0.08
1-kestose	0.27	0.39	0.38	0.20
erlose	0.04	0.05	0.06	0.04
melezitose	0.07	0.04	0.04	0.04
unknown	n.d.	0.05	0.04	0.07
unknown	0.03	0.09	0.06	0.08
maltotriose	n.d.	n.d.	n.d.	0.03
panose	n.d.	n.d.	n.d.	n.d

[a] n.d. = not detected.

their concentration was low, these changes did not appear to be related to sensorial properties.

Monosaccharide losses are important to sensorial properties since they can partially correspond to chemical degradations which have as end products furyl derivatives and acids, or compounds associated with the Maillard reaction, with strong organoleptic properties.

Honey monosaccharide losses can also be ascribed in part to oligosaccharide formation during storage. Decreases of more than 9% per year have been observed in honey stored at 26 °C (*50*), while the concentration of reducing disaccharides increased 69% (*51*). Disaccharide formation has been attributed to two different processes: enzyme activity can produce transglycosylation through the action of glucosidases present in honeys, while chemical transformation of monosaccharides to disaccharides and higher sugar has been found to occur in concentrated solution of monosaccharides at low pH values.

Sensory Analysis of Honey. All honeys were assessed by skilled tasters, using attributes previously agreed upon as the best for describing sensorial characteristics of honeys. **Figure 2** shows a spiderweb diagram illustrating the average scores for intensities of honey aroma attributes.

The most intense attributes detected by assessors in the aroma profile of fresh citrus honey were described as "floral", "citric", "fresh fruit", and "caramel/sweet" odor. Some of these attributes, floral and caramel odor, related with compounds such as lilac aldehydes, lilac alcohols, and terpineol principally. "Fresh", "fresh fruit", and "citric" attributes which were of moderate intensity can be correlated with the presence of the sinensal isomers, compounds described as components of citric fruits with odor perception thresholds of 0.05 ppb (*52*).

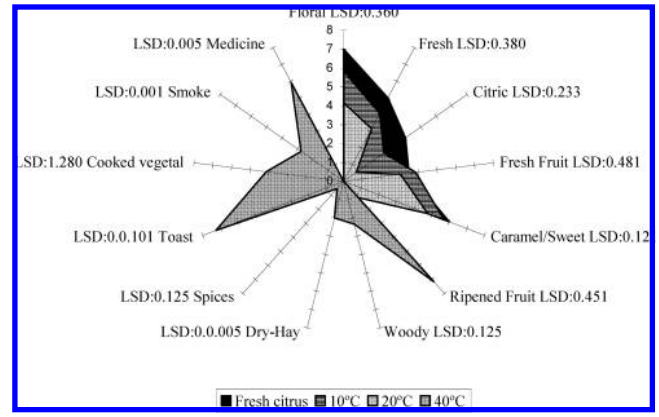

Figure 2. Sensory descriptive analysis of citrus honeys. Fresh and storage for 12 months. Mean scores of 10 judges (two replicates) and least significant differences (LSD $p < 0.05$) are shown.

Citrus honeys stored at 10 °C for 12 months conserved their organoleptic characteristics practically intact; some freshness was lost, but they retained much of the intensity of their original attributes. In honeys stored at 20 °C, more pronounced decreases in intensity were observed, although they were still accepted bythe assessors. That correlated with the loss of certain volatile compounds characteristic of fresh honeys, such as hotrienol, lilac alcohol isomers, or sinensal isomers, maintaining other floral compounds such as linalool, linalool oxides, and lilac aldehydes. On the other hand, compounds causing off-flavors were not found or were found at low concentration in these samples.

Honeys stored at 40 °C for a year lost all the freshness attributes, acquiring other attributes related to heating like "toast", "ripened fruit", "smoke", and "medicine". Storage conditions at 40 °C induced the production of sugar degradation compounds and the formation of Maillard products in high concentrations that probably exceed their odor detection threshold. Both maltol and furaneol (2-hydroxy-3-methyl-2-cyclopenten-1-one) can be responsible for the "burnt sugar" and "toasted caramel" aromas detected by tasters in honeys heated to 40 °C (*46*), whereas pyran and furan compounds could be related with smoke, spices, and medicine aromas.

Honeys stored at 40 °C were considered organoleptically altered and had a totally different sensorial profile compared to the fresh honey, being rejected by the assessors. Another negative sensorial aspect of stored honeys is the increase in acidity detected by tasters, higher for samples kept at 40 °C, which can be explained from the increase in the concentration of organic acids (specially acetic acid), noticeable at 40 °C.

CONCLUSIONS

Changes produced in volatile and sugar composition of honey stored for 1 year at 10 and 20 °C were less evident than those produced at 40 °C, although some compounds suffered loss or increased at low temperature which suggests chemical changes could be produced during storage at low or ambient temperature.

Physicochemical parameters can be useful as indicator of heating or honey storage at high temperature, specially diastase activity and HMF, but variation of these parameters during storage at low temperature is not very evident.

On the other hand, volatile compounds allow distinguishing between fresh honey and honey stored for long time, since most of terpenes and derivatives present in fresh citrus honey, some of them described as floral marker, disappear during storage independently of the temperature. In contrast, furan and pyran

compounds are formed during honey storage mainly at 40 °C. In the same way, low levels of monosaccharides or high levels of maltose could be used as indicator of prolonged storage of honey.

Sensory analysis of honey is useful to detect storage at high temperature but assessors did not detect aroma defects in citrus honey stored 12 months at 10 or 20 °C.

From these results, honey should be stored at low temperatures, preferably 20 °C or below. In these conditions, honey conserves many of its compounds with aromatic properties, at least during 1 year. Nevertheless, the observed decrease in their concentration makes it advisable to reduce the storage time in order to maintain the flavor characteristics of fresh honey.

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