JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 7317–7322 7317
DOI:10.1021/jf100758x

Carbohydrate Composition of High-Fructose Corn Syrups (HFCS) Used for Bee Feeding: Effect on Honey Composition

Ana Isabel Ruiz-Matute,[†] Milagra Weiss,[‡] Diana Sammataro,[‡] Jennifer Finely,[‡] and Maria Luz Sanz*,[†]

[†]Instituto de Química Orgánica General (CSIC), 28006 Madrid, Spain, and [‡]USDA-ARS Carl Hayden Honey Bee Research Center, Tucson, Arizona 85719

In this study, the carbohydrate composition of high-fructose corn syrups (HFCS) from commercial manufacturers as well as from beekeepers was characterized by GC-MS. Sucrose syrups (SS) were also included in this work for comparison. Fructosyl-fructoses and some unknown carbohydrates, which could correspond to fructosyl-glucoses, have been detected in HFCS for the first time, whereas SS were mainly characterized by the high contents of sucrose. Hydroxymethylfurfural (HMF) content of samples supplied by beekeepers was much more variable; the mean level of HMF was 64.61 ppm (±16.92 ppm, 95% CI ranging from 26.91 to 102.31 ppm). Syrups were used to feed caged bees and the resulting honeys produced were analyzed in order to determine their influence in carbohydrate composition. Fructosyl-fructoses were mainly detected in honeys from bees fed with HFCS, but not from those honeys coming from free-flying bees or bees fed with SS.

KEYWORDS: High-fructose corn syrup (HFCS); sucrose syrup (SS); HMF; honey; bees; carbohydrates

INTRODUCTION

High-fructose corn syrups (HFCS) are obtained by enzymatic isomerization of corn syrups, which can be produced by both acid and enzymatic hydrolysis of cornstarch; the enzymatic procedure is the most utilized in the manufacturing process. Three different enzymes (α-amylase, glucoamylase, and glucose-isomerase) are needed to transform cornstarch into the simple sugars glucose and fructose. After a complex fractionation and combination process, mixtures with various amounts of fructose can be obtained: HFCS-42 (42% of fructose), HFCS-55 (55% of fructose), or HFCS-90 (90% of fructose) (*1*). These syrups are commonly used as sugar substitutes in processed foods, especially in soft drinks, mainly for economical reasons (*2*).

The sugar profile of certain HFCS has a basic composition similar to that of natural honey: fructose and glucose are the main components, and a high number of oligosaccharides appear in minor amounts (*3*). Because of its low cost, fraudulent additions of HFCS to honey can be carried out. Many studies have been conducted to detect these syrups in honey (*4*−*6*). In addition, their similar compositions encouraged commercial beekeepers to use HFCS as a bee feed (*7*). However, there is some concern that HFCS contains compounds that are detrimental to bees. Moreover, as described by Cordella et al. (*8*), feeding HFCS can affect the quality of the resulting honey, especially if it is done without proper safeguards.

Although beekeepers in many other parts of the world commonly use sucrose syrup (SS) as a supplement (*9*), in the United States, HFCS has become a more readily available carbohydrate source, because corn is widely planted and supported. This made HFCS relatively inexpensive and is now used as supplementary feed by many commercial beekeepers (over 500 colonies) as a source of carbohydrates during periods of dearth (when no nectar is available, such as in winter or early spring), to boost food reserves for overwintering colonies, or to stimulate brood rearing in the spring (*10*). It is easy to transport to the hives, and the ratio of sugars allows the solution to maintain a high concentration of dissolved sugars (71−77%), which is economical in terms of delivering a high number of calories in a small volume. The fructose/glucose ratio is such that crystallization is avoided, and the low pH allows the HFCS to resist fermentation and bacterial contamination. HFCS-55 and HFCS-42 are both commonly used as bee feed; additionally, some suppliers are providing HFCS−sucrose blends (*11*).

Although HFCS is easy to distribute to a large number of colonies, testing its efficacy and safety as a bee feed has been limited (*12*, *13*). In the 1970s, some bee deaths were reported from colonies fed HFCS, and when the syrup was examined, it was found to contain high levels of hydroxymethylfurfural (HMF), which can be a contaminant from the acid-hydrolyzed process used to make HFCS (*14*). These observations of detrimental health effects are likely attributable to HMF contamination and/or the further dehydration products of HMF and levulinic and formic acid, which readily form in acidic fructose solutions. HMF also forms in HFCS under high-heat conditions (a result of improper storage) because of its fructose composition and pH (*1*, *15*).

Beekeepers have reported mixed results from HFCS feeding (*11*). Currently, with the reports of large numbers of colony

*Corresponding author [e-mail mlsanz@iqog.csic.es; telephone + 34 915622900 (ext. 306); fax + 34 915644853].

Published on Web 05/21/2010

deaths, called colony collapse disorder (CCD), the authors examined HFCS more closely, taking into account that a detailed study of the minor compounds of these syrups has not yet been done and the identity of most of their oligosaccharides is still unknown. Sucrose syrups (SS) were also included in this work for comparison.

A bee-feeding experiment was performed in Tucson, AZ, to evaluate the effect of supplying hives with SS and HFCS. Bees were fed in an enclosed flight arena and in an apiary, and the carbohydrate compositions of both the bee feed and the honeys produced were evaluated and compared to those made by free-flying bees outside.

MATERIALS AND METHODS

Standards. Cellobiose, fructose, gentiobiose, glucose, isomaltose, kojibiose, laminaribiose, maltose, nigerose, panose, phenyl-β-D-glucoside, and sucrose were obtained from Sigma Chemical Co. (St. Louis, MO). Maltulose was purchased from Aldrich Chemical Co (Milwaukee, WI), and leucrose, palatinose, α,α-trehalose, α,β-trehalose, and turanose were purchased from Fluka (Madrid, Spain). Trehalulose was provided by Dr. Wach from Südzucker (Mannheim, Germany). Beneo P95 containing inulobiose was provided by Orafti (Tienen, Belgium).

Samples. HFCS samples were procured directly from the manufacturers, Roquette, Archer Daniels Midland, and Tate & Lyle, and from a bee supply house, Mann Lake Ltd. (Hackensack, MN). These consisted of three samples of HFCS-42 (71% dissolved solids; M1−M3) and four samples of HFCS-55 formulation (77% dissolved solids; M4−M7).

Other syrup samples were supplied voluntarily by beekeepers from various regions and of different sized beekeeping operations. The samples were variable in terms of formulation and manufacturer: HFCS-75 ($n = 2$; B1, B2), HFCS-55 ($n = 5$; B3−B7), HFCS-42 ($n = 1$; B8), and mixtures HFCS + SS ($n = 4$; B9−B12) and SS ($n = 3$; B13−B15). A survey was taken to ascertain the storage conditions and feeding requirements of the syrups; for example, samples B1, B4, B5, and B6 had been stored outside in metal tanks for up to 1 year. Beekeepers mailed the samples to the Carl Hayden Bee Research Center (CHBRC) in Tucson, AZ, using cold-storage shipping packages to avoid a high-heat transit period during which HMF might form.

Nine honeys (five from HFCS and four from SS) produced by bees inside a flight arena and four honeys produced by bees in an apiary (fed on HFCS or not fed but allowed to collect natural nectar) were obtained as indicated below. Ten control honeys produced by free-flying bees were included in this study for comparison.

Colony Feeding in Greenhouse. Ten nucleus (nuc) colonies (5 frames each, about 10000 workers) were placed in a closed foraging arena (modified Quonset style greenhouse) at the University of Arizona Agricultural Research Center, Tucson, AZ; another 10 nuc colonies were placed in the CHBRC apiary. All colonies were fitted with a nuc-top feeder in which the randomly assigned treatment of either commercially purchased HFCS or sucrose syrup was continually supplied (both solutions, 67% solids w/v). All colonies were supplied with MegaBee (Castle Dome Solutions) protein supplement *ad libitum*; this material contained no natural pollen. Syrup fed to the nuc colonies and "honey" produced from these feedings were collected.

Hydroxymethylfurfural Analysis. HMF content was determined colorimetrically using a method adapted by LeBlanc et al. (*1*) after Winkler (*16*). For each sample, 5 g of syrup was diluted in 5 mL of deoxygenated deionized water. Five hundred microliters of this dilution was incubated with *p*-toluidine and barbituric acid and absorbance measured at 550 nm using a 96-well plate reader (Synergy HT). Absorbances were interpreted against a polynomial curve generated using serial dilutions of an HMF standard (Sigma-Aldrich).

Carbohydrate Analysis. Carbohydrates of syrups were converted to their trimethylsilyl oximes (TMSO) after reaction with 350 μL of 2.5% hydroxylamine chloride in pyridine (30 min at 75 °C), 350 μL of hexamethyldisilazane, and 35 μL of trifluoroacetic acid (45 °C for 30 min). Derivatized samples were centrifuged, and 1 μL of supernatant was injected into the injection port of the gas chromatograph (*4*).

GC-MS analyses were carried out using a Hewlett-Packard 7890 gas chromatograph coupled to a 5975 quadrupole mass detector (both from Agilent, Palo Alto, CA), using helium as carrier gas (average linear velocity of ~20 cm s^{-1}). A 30 m × 0.25 mm i.d. × 0.25 μm film thickness fused silica column coated with SPB-1 (cross-linked methyl silicone) from Supelco (Bellefonte, PA) was used. Oven temperature was held at 200 °C for 20 min, then programmed to 270 °C at a heating rate of 15 °C min^{-1}, then programmed to 290 °C at 1 °C min^{-1}, and finally programmed to 300 °C at 15 °C min^{-1} and held for 40 min. The injector was kept at 300 °C, and injections were made in split mode with a split ratio 1:40. Mass spectrometer was operated in electronic impact (EI) mode at 70 eV, scanning the m/z 35−700 range. Interface and source temperatures were 280 and 230 °C, respectively. Acquisition was done using HPChem Station software (Hewlett-Packard, Palo Alto, CA).

Identification of TMSO derivatives of carbohydrates present in syrups was carried out by comparison of their retention times with those of standard compounds; mass spectral data were used to confirm peak assignation and to tentatively identify those peaks that were not available as commercial standards.

Quantitative data for disaccharides of control honeys were calculated from FID peak areas according to the method proposed by de la Fuente et al. (*17*). However, taking into account the appearance of unknown carbohydrates coeluting with identified disaccharides in honeys from HFCS and SS, results could not be independently given.

GC analyses were carried out in a gas chromatograph equipped with a flame ionization detector (FID) (HP 5890, Palo Alto, CA). Experimental conditions other than the carrier gas (nitrogen; average linear velocity of ~12 cm s^{-1}) and detector temperature (300 °C) were the same as those previously described for GC-MS analysis. Chromatographic peaks were measured using a Chrom-Card 1.20 acquisition system (CE Instruments, Milan, Italy). Standard solutions of carbohydrates covering the expected concentration range in honey were prepared to determine the response factor (RF) relative to phenyl-β-D-glucoside (internal standard). Due to the lack of standards, concentration of difructose anhydrides (DFAs) was estimated assuming a response factor equal to 1. All analyses were carried out in duplicate.

RESULTS AND DISCUSSION

Syrups. *Carbohydrates*. **Figure 1** shows the gas chromatographic profiles obtained for a HFCS (**Figure 1A**) and a SS (**Figure 1B**). Glucose, fructose, and sucrose were detected in both kinds of syrups, whereas several peaks eluting between 22 and 29 min, characterized as disaccharides (**Figure 2**), were observed in only HFCS. It is noteworthy that the disaccharide profile was very similar in all examined HFCS samples. Maltose (peaks 19 and 22), which coeluted with other unknown carbohydrates, and isomaltose (peaks 26 and 27), composed by glucose units with α linkages in 1−4 or 1−6, respectively, were identified in these syrups by comparison of their MS and retention data with their corresponding standards. These disaccharides are characteristic of starch and undoubtedly are from the incomplete hydrolysis of cornstarch during HFCS production. The high intensity of the m/z 307 ion observed in MS of peaks 10, 11, 15−18, and 23 is characteristic of disaccharides with a reducing ketose substituted in C1 or C3. In addition, the presence of the m/z 437 ion (characteristic of ketohexoses at both pyranose and furanose rings (*18*)) in their mass spectra means that these peaks could be attributed to fructosyl-fructoses. Peak 11 was assigned to inulobiose by comparison with retention time and mass spectra of that from Beneo P95 (*4*); however, linkages of the other remaining fructosyl-fructoses cannot be determined due to the absence of standards.

Some unknown disaccharides (peaks 8, 9, 12−14, 20, 21, 24, and 25) showed the presence of m/z 437, 451, and 538 (characteristic of oximes) ions in their mass spectra. These peaks could be attributed to fructosyl-glucoses, although the identities of these disaccharides cannot be assessed.

Peaks eluting from 20 to 23 min (peaks 1−5 and 7) were identified as DFAs from their mass spectra (m/z 217 and 509 ions). These compounds were first detected in HFCS by Ruiz-Matute et al. (*4*) and are called pseudodisaccharides, consisting of two

fructose residues with a 1,4-dioxane intersaccharide ring and different linkages (*19*).

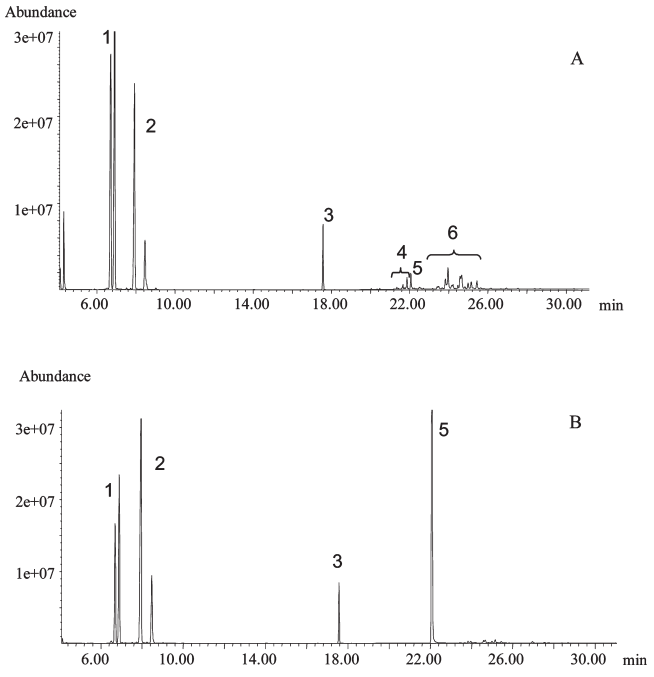

Figure 1. Gas chromatographic profiles of trimethylsilyl oxime derivatives of carbohydrates in a HFCS (**A**) and a SS (**B**). Peaks: 1, fructose; 2, glucose; 3, phenyl-β-D-glucoside (intenal standard); 4, difructose anhydrides (DFAs); 5, sucrose; 6, other disaccharides.

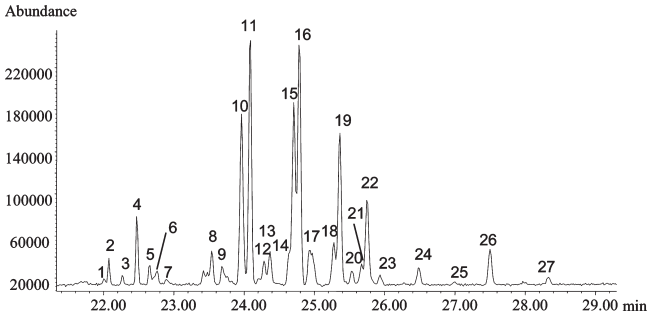

Figure 2. Gas chromatographic profile of trimethylsilyl oxime derivatives of disaccharide region in HFCS. Peaks: 1−5, 7, difructose anhydrides (DFAs); 6, sucrose; 8, 9, unknowns; 10, fructosyl-fructose; 11, inulobiose; 12−14, unknowns; 15−18, 23, fructosyl-fructoses; 19, maltose *E* + unknown; 20−21, unknowns; 22, maltose *Z* + unknown; 24, 25, unknowns; 26, 27, isomaltose *E* and *Z*..

Considering that these syrups have been obtained after enzymatic hydrolysis, we could assume that all of the detected disaccharides keep the α-configuration of starch. However, in those cases when chemical hydrolysis is performed, linkage transglycosylation can occur and the appearance of both α- and β- linkages could be detected (*20*).

Table 1 shows mean values of carbohydrates (mg g^{-1} syrup) detected in HFCS samples, SS samples, and mixtures of both syrups. As has been previously indicated, HFCS can be classified depending on their isomerization degree. In this work, we have analyzed three different types of HFCS (42, 55, and 75% isomerized), and their carbohydrate composition is independently shown in **Table 1**.

As expected, glucose and fructose were the main carbohydrates detected in HFCS, whereas sucrose was the most abundant for SS. Fructosyl-fructoses were the main disaccharides of HFCS. Among the different types of syrups, HFCS-75 showed the highest content of fructose and fructosyl-fructoses, these values decreasing for HFCS-55 and HFCS-42. However, no variations were observed in maltose and isomaltose concentrations in these syrups. Moreover, no differences were found between those syrups obtained from beekeepers and from manufacturers.

Hydroxymethylfurfural. HMF contents of HFCS samples from the manufacturers were on average below the level deemed to be acceptable in honey by the international Codex Alimentarius Commission [40 ppm (*21*)]. The mean level of HMF found in HFCS supplied directly by the manufacturers was 23.48 ppm (± 4.92 ppm, 95% CI 11.09−33.8 ppm) (**Figure 3A**). HMF contents of samples supplied by beekeepers were much more variable; the mean level of HMF was 64.61 ppm (± 16.92 ppm, 95% CI from 26.91 to 102.31 ppm) (**Figure 3B**). SS and blends of HFCS and SS did not show or showed very low values of HMF.

In general, mean values of HMF were lower for HFCS samples supplied by manufacturers, indicating that they were maintained at more controlled conditions than those provided by beekeepers. Several of the beekeepers' samples were well above the internationally accepted level of HMF for honey, whereas others were quite low or nonexistent. As indicated before, syrups B1, B4, B5, and B6 were stored outside in metal tanks in full sun for up to 12 months, which could explain the high HMF values of these samples.

Honeys. Figure 4 shows the GC profiles of honeys obtained after feeding bees HFCS (**Figure 4A**) and SS (**Figure 4B**) in the flight arena. **Table 2** shows the mean values of mono- and disaccharides (mg g^{-1} of honey) of the analyzed honeys.

It is worth noting the different profiles obtained for honeys from HFCS and from SS. SS honeys were clearly characterized by the high amounts of sucrose present, whereas HFCS honeys showed several carbohydrates with two fructose units from the correspondent syrup. DFAs were detected in small amounts in

Table 1. Carbohydrate Contents of High-Fructose Corn Syrups (HFCS) from Manufacturers and from Beekeepers, Sucrose Syrups (SS), and Mixtures of HFCS and SS

			carbohydrates (mg g^{-1} of syrup)							
sample			fructose	glucose	DFAs[a]	sucrose	fructosyl-fructoses	maltose + unknown DS[b]	isomaltose	unknown DS
manufacturers	HFCS-42 (n = 3)	M1−M3	304.8 (31.9)[c]	402.2 (36.8)	1.0 (0.3)	0.4 (0.1)	9.5 (0.9)	9.0 (2.7)	6.2 (1.0)	7.9 (3.0)
	HFCS-55 (n = 4)	M4−M7	413.9 (25.2)	346.9 (24.9)	0.7 (0.4)	0.8 (1.0)	15.3 (5.7)	9.1 (1.7)	6.2 (2.4)	6.5 (3.0)
beekeepers	HFCS-75 (n = 2)	B1, B2	543.2 (31.7)	234.4 (27.1)	2.8 (3.4)	1.9 (0.3)	29.8 (22.8)	8.0 (2.0)	5.0 (3.1)	10.2 (8.2)
	HFCS-55 (n = 5)	B3−B7	405.7 (26.8)	331.8 (24.9)	0.9 (0.5)	0.4 (0.6)	17.3 (3.8)	9.2 (2.1)	6.1 (1.3)	5.7 (1.7)
	HFCS-42 (n = 1)	B8	315.6	342.2	1.2	0.2	13.5	11.5	7.0	7.0
	HFCS + SS (n = 4)	B9−B12	165.9 (55.6)	212.6 (75.9)	0.1 (0.1)	356.7 (87.4)	8.4 (1.4)	3.7 (2.7)	1.9 (1.6)	2.1 (0.5)
	SS (n = 3)	B13−B15	12.7 (10.6)	25.1 (19.8)	0.0	725.2 (68.2)	0.0	0.0	0.0	0.0

[a] DFAs, difructose anhydrides. [b] DS, disaccharides. [c] Standard deviation is given in parentheses.

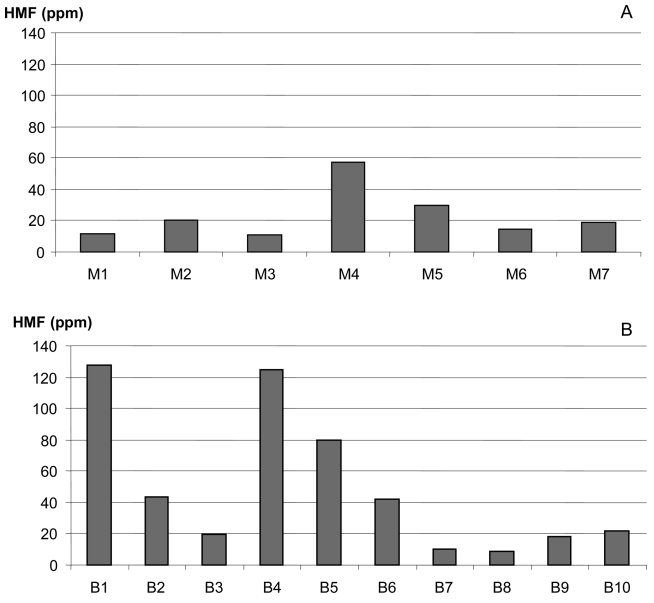

Figure 3. Hydroxymethylfurfural (HMF) concentration of high-fructose corn syrup (HFCS) samples supplied by manufacturers (M1−M3 = HFCS-42, M4−M7 = HFCS-55) (**A**) and by beekeepers (B1 and B2 = HFCS-75, B3−B7 = HFCS-55, B8 = HFCS-42, B9 and B10 = HFCS + SS) (**B**). HMF values of samples B11−B15 were zero and are not shown. The concentration of HMF (ppm) was measured colorimetrically by visible spectroscopy.

HFCS honeys (0.4−0.8 mg g^{-1} of honey), and only traces of these compounds were observed in SS honeys. These compounds had been previously described by Ruiz-Matute et al. (*4*) as indicative of honey adulterations with HFCS.

Honey samples obtained from bees naturally foraging were also included in this study for comparative purposes (**Figure 4C**). Natural honeys contain complex mixtures of glucosyl-glucoses and glucosyl-fructoses, although some fructosyl-fructoses such as inulobiose have been also detected (*4*). SS honeys only differed from natural honeys in the high levels of sucrose (131.7 ± 17.1 mg g^{-1} of SS honey). These values are extremely high compared with those found in control honeys (5.0 ± 7.1 mg g^{-1} of honey). This may be due in part to the confinement of the nucleus colonies as they were under stress and had lower than normal bee populations, which could lead to improperly cured honey. Nevertheless, although the control honey samples included in this study did not show high amounts of sucrose, its concentration is commonly variable and can achieve values even higher than those found in SS honey [up to 15% for honeydew honeys (*22*)]. For this reason, the use of this carbohydrate as indicative of bee-feeding with these syrups cannot be used if its value does not exceed the legal limits.

On the other hand, whereas differences in fructose and glucose amounts between HFCS honeys and control honeys were not appreciable, noticeable changes were observed in the disaccharide profile of both types of honeys. As mentioned above, disaccharides identified in the original syrup (most of them fructosyl-fructoses) could be easily detected together with those characteristic of honeys (glucosyl-glucoses and glucosyl-fructoses). However, as described by Cordella et al. (*8*), a dilution effect on these last compounds was observed. This composition can be explained considering the action of secreted enzymes by bees: It has been described that honey invertase is an α-glucosidase which is responsible for sucrose hydrolysis into glucose and fructose, but it also transfers the glucosyl moiety of sucrose to other acceptors, giving rise to the oligosaccharides present in honey (*23*). However,

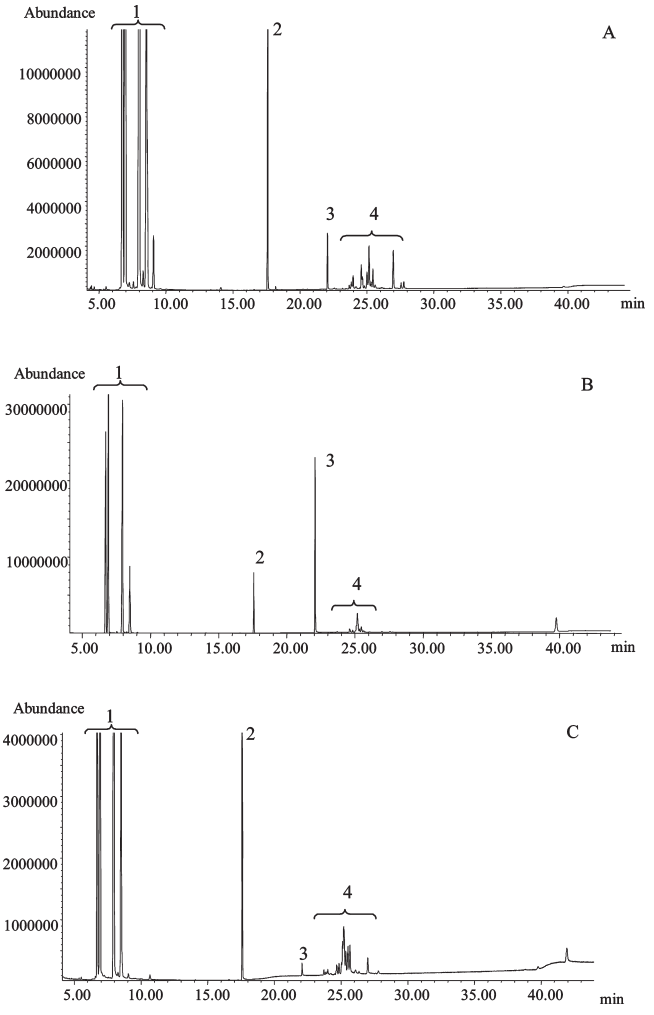

Figure 4. Gas chromatographic profile of trimethylsilyl oxime derivatives of disaccharide region in honeys obtained from caged bees fed HFCS (**A**) and SS (**B**) and from unfed foraging bees (**C**). Peaks: 1, monosaccharides; 2, phenyl-β-D-glucoside (intenal standard); 3, sucrose; 4, other disaccharides.

no fructosidase activity, apart from that from pollen (*24*), has been reported in honey. As caged bees produce honey without pollen, the hydrolysis of fructosyl-fructose disaccharides from the syrup is not possible and could explain the characteristic carbohydrate profile observed in these samples.

However, when the GC carbohydrate content of honeys produced by the bees confined in the flight arena was compared with that of those from the apiary, only a lower value of sucrose for these last was observed (see **Table 2**). These results may indicate that the low activity of pollen fructosidases is not sufficient to remove the content of fructosyl-fructoses from HFCS.

In conclusion, the disaccharide fraction of HFCS has been studied in great detail, and the presence of fructosyl-fructoses in these products has been detected for the first time. Mean values of HMF were lower for samples supplied by manufacturers than for those provided by beekeepers, which may have been stored in temperature ranges that would favor HMF formation. Carbohydrate composition of honeys produced by bees fed HFCS was characterized by the presence of fructosyl-fructoses from the syrups; this composition was notably different from those from free-foraging bees and from bees fed SS. Honey from bees fed HFCS can be easily detected using GC-MS, although more studies with a higher number of honeys from confined and free-flying bees would be necessary to confirm these results. Further studies are

Table 2. Mono- and Disaccharide (Milligrams per Gram of Honey) Composition of Honeys Produced by Bees Fed High-Fructose Corn Syrups (HFCS) or Sucrose Syrups (SS) and by Naturally Fed Bees (Control)

	honeys from			
	HFCS		SS	
carbohydrate	flight arena (n = 5)	apiary (n = 4)	flight arena (n = 4)	control (n = 10)
fructose	403.3 (18.1)[a]	435.7 (5.7)[a]	323.3 (45.6)	364.2 (41.7)
glucose	298.5 (14.3)	323.3 (6.7)	254.7 (48.7)	301.1 (57.6)
DFAs[c]	0.4 (0.1)	0.8 (0.5)	tr[b]	0.00
sucrose	10.9 (4.4)	1.7 (0.6)	131.7 (17.1)	5.0 (7.1)
α,α trehalose	0.6 (0.1)	1.1 (0.5)	0.4 (0.2)	0.4 (0.3)
α, β trehalose	1.2 (0.1)	1.7 (0.2)	2.4 (0.7)	3.9 (1.9)
fructosyl-fructose	2.3 (0.2)	3.8 (1.6)	1.0 (0.3)	2.5 (1.6)
inulobiose	3.6 (0.3)	5.5 (1.8)	1.1 (0.3)	3.3 (2.0)
unknown DS[d]	1.3 (0.1)	4.2 (3.2)	1.0 (0.6)	2.7 (1.8)
fructosyl-fructose	0.6 (0.1)	0.9 (0.3)		
cellobiose				1.7 (0.8)
laminaribiose + unknown DS			9.1 (3.0)	
laminaribiose				1.9 (1.1)
fructosyl-fructose + unknown DS + maltulose 1	9.0 (1.0)	9.3 (1.8)		
fructosyl-fructose + maltulose 2	0.9 (0.2)	1.0 (0.2)		
maltulose			8.4 (2.6)	15.7 (11.8)
fructosyl-fructose + nigerose *E*	4.1 (0.2)	5.2 (0.9)		
nigerose			4.7 (2.3)	9.2 (5.7)
maltose *E* + turanose	10.7 (2.6)	11.3 (1.5)		19.5 (6.7)
maltose *E* + turanose + unknown DS			55.4 (21.0)	
maltose				16.8 (6.1)
kojibiose	2.4 (0.2)	2.9 (0.3)	5.2 (4.0)	15.0 (9.3)
maltose *Z* + trehalulose 1 + unknown DS	4.6 (0.5)	5.2 (0.3)		
nigerose *Z* + trehalulose 2	1.2 (0.1)	1.5 (0.2)		
trehalulose			4.4 (0.8)	11.0 (7.3)
unknown DS	0.3 (0.0)	0.4 (0.1)		
palatinose	0.7 (0.2)	0.7 (0.2)	1.2 (0.3)	2.4 (1.9)
gentiobiose			0.1 (0.1)	0.1 (0.1)
melibiose	1.4 (2.4)	0.3 (0.1)		0.1 (0.1)
isomaltose	10.7 (1.7)	12.9 (0.3)	4.6 (1.3)	13.4 (11.8)

[a] Standard deviation is given in parentheses. [b] tr, traces. [c] DFAs, difructose anhydrides. [d] DS, disaccharides.

being done to determine the effects of these carbohydrates on bees.

ACKNOWLEDGMENT

We are grateful to Dr. I. Martínez-Castro for critical reading of the manuscript.

LITERATURE CITED

(1) LeBlanc, B. W.; Eggleston, G.; Sammataro, D.; Cornett, C.; Dufault, R.; Deeby, T.; St. Cyr, E. Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee (*Apis mellifera*). *J. Agric. Food Chem.* **2009**, *57*, 7369–7376.

(2) Long, J. E. High fructose corn syrup. *Food Sci. Technol.* **1991**, *48*, 247–258.

(3) Bogdanov, S.; Jurendic, T.; Sieber, R.; Gallmann, P. Honey for nutrition and health: a review. *J. Am. Coll. Nutr.* **2008**, *27*, 677–689.

(4) Ruiz-Matute, A. I.; Soria, A. C.; Martínez-Castro, I.; Sanz, M. L. A new methodology based on GC-MS to detect honey. Adulteration with commercial syrups. *J. Agric. Food Chem.* **2007**, *55*, 7264–7269.

(5) Morales, V.; Corzo, N.; Sanz, M. L. HPAEC-PAD oligosaccharide analysis to detect adulterations of honey with sugar syrups. *Food Chem.* **2008**, *107*, 922–928.

(6) Doner, L. W.; White, J. W.; Phillips, J. G. Gas−liquid chromatographic test for honey adulteration by high fructose corn syrup. *J. Assoc. Off. Anal. Chem.* **1979**, *69*, 186–189.

(7) Nabors, R. A. Using mixtures of different sugars to feed bees. *Am. Bee J.* **1996**, *136*, 785–786.

(8) Cordella, C.; Militao, J. S. L. T.; Clement, M. C.; Drajnudel, P.; Cabrol-Bass, D. Detection and quantification of honey adulteration via direct incorporation of sugar syrups or bee-feeding: preliminary study using high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and chemometrics. *Anal. Chim. Acta* **2005**, *531*, 239–248.

(9) Somerville, D. *Fat Bees Skinny Bees − a Manual on Honey Bee Nutrition for Beekeepers*; Rural Industries Research and Development Corporation Australian Government: Barton, Australia, 2005.

(10) Standifer, L. N.; Moeller, F. E.; Kauffeld, N. M.; Herbert, E. W., Jr.; Shimanuki, H. *Supplemental Feeding of Honey Bee Colonies*; USDA Information Bulletin 413; U.S. GPO: WAshington, DC, **1977**; 8 pp, illus.

(11) Oliver, R. Fat bees − part III. *Am. Bee J.* **2007**, *147*, 965–969.

(12) Barker, R. J.; Lehner, Y. Laboratory comparison of high fructose corn syrup, grape syrup, honey, and sucrose syrup as maintenance food for caged honey bees. *Apidologie* **1978**, *9*, 111–116.

(13) Severson, D. W.; Erickson, E. H., Jr. Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates. *J. Econ. Entomol.* **1984**, *77*, 1473–1478.

(14) Jachimowicz, T.; El Sheribiny, G. Problematic Der Verwenung Von Invertzucker Fur Die Bienenfutterung. *Apidologie* **1975**, *6*, 121–143.

(15) Kim, B.-S.; Nahmgoong, B.; Shin, D.-B.; Jeong, M.-C.; Kim, Q.-W. Quality change of high fructose corn syrups during storage. *Han'guk Nonghwa Hakhoechi* **1995**, *38*, 232–238.

(16) Winkler, O. Beitrag zum nachweis und zur bestimmung von oxymethylfurfurol in honig und kunsthonig. *Z. Lebensm. Forsch. A* **1955**, *102*, 161–167.

(17) De la Fuente, E.; Sanz, M. L.; Martínez-Castro, I.; Sanz, J. Development of a robust method for the quantitative determination of disaccharides in honey by gas chromatography. *J. Chromatogr., A* **2006**, *1135*, 212–218.

(18) Kamerling, J. P.; Vliegenthart, J. F. G.; Vink, J.; de Ridder, J. J. Mass spectrometry of pertrimethylsilyl oligosaccharides containing fructose units. *Tetrahedron* **1972**, *28*, 4375–4387.
(19) Ratsimba, V.; García Fernández, J. M.; Defaye, J.; Nigey, H.; Voilley, A. Qualitative and quantitative evaluation of mono- and disaccharides in D-fructose, D-glucose and sucrose caramels by gas−liquid chromatography−mass spectrometry. Di-D-fructose anhydrides as tracers of caramel authenticity. *J. Chromatogr., A*. **1999**, *844*, 283–293.
(20) Thompson, A.; Anno, K.; Wolfrom, M. L.; Inatome, M. Acid reversion products from D-glucose. *J. Am. Chem. Soc*. **1954**, *76*, 1309–1311.
(21) Codex Alimentarius Commission. *Codex standard for honey, CODEX STAN 12-1981*; Food and Agriculture Organization of the United Nations and the World Health Organization.: Rome, Italy, 2001.
(22) Council Directive 2001/110/EC of 20 Dec 2001 relating to honey. The Council of the European Union.
(23) White, J. W., Jr.; Maher, J. Transglucosidation by honey invertase. *Arch. Biochem. Biophys*. **1953**, *42*, 360–367.
(24) Siddiqui, I. R. The sugars of honey. *Adv. Carbohydr. Chem. Biochem*. **1970**, *7*, 51–59.

Received for review February 25, 2010. Revised manuscript received April 30, 2010. Accepted May 7, 2010. This work has been funded by Projects CTQ2006-14993/BQU (financed by CICYT) and ANALISYC2 (financed by Comunidad de Madrid). The Project *Apis mellifera* (Pam) and The Society for the Preservation of Honey Bees funded the work in Arizona.