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Polysaccharides as a Marker for Detection of Corn Sugar Syrup Addition in Honey

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Honey is a natural product of high quality. However, because of its limited production and of its relatively high price, some beekeepers or unscrupulous traders do not hesitate to modify and falsify this natural product in order to try to increase its market value. Then, these involved falsification practices, for example intentional addition of cheap sugar syrup to honeys, are sometimes difficult to detect. An effective and simple analytical method is proposed in order to detect adulteration in honey by analysis of polysaccharide profiles. Samples were previously treated with reversed-phase solid phase extraction first to remove monosaccharides and small oligosaccharides and second to concentrate simultaneously traces of polysaccharides. A chromatographic separation using anion exchange stationary phase and pulse amperometric detection was further performed. Polysaccharide fingerprints (degree of polymerization from 11 to 17) were shown to be present in laboratory doped samples, and not detectable or present at very low concentrations in the authentic honey samples. Application to acacia, mountain polyfloral and polyfloral honeys enabled readily the detection of fraud resulting from deliberate addition of 1% of corn syrup.

KEYWORDS: Polysaccharides; honey; sugar syrup; adulteration; solid phase extraction

INTRODUCTION

According to Recommended European Regional Standards (*1*), honey is the sweet substance produced by honey bees from the nectar of blossoms or from secretion of living parts of plants, which they collect, transform and combine with specific substances and store in honeycomb (*2*).

The relatively high price, limited supply and complexity of honey combine to encourage falsification (*3*). Indeed, despite the technological advent of modern analytical instruments, there is still a problem with the adulteration of high-carbohydrate foods, such as honey, with inexpensive syrups. The most common syrups (*4*) used to adulterate honey are corn syrups, inverted syrups and high fructose corn syrup. According to hydrolysis, they contain in variable proportion a mixture of many sugars: glucose, maltose, maltotriose, dextrins, and so on (*5*, *6*). These syrups are inexpensive, and their carbohydrate profiles can be manipulated easily to resemble the carbohydrate profile of honey (*7*, *8*). Honey adulteration has evolved from the basic addition of sugar and water to specially produced syrups from which the chemical composition approximately reproduces the sugar composition and ratios of natural honey.

At the current time, the use of reliable control methods to ensure the compliance of a food product is imperative if we want to limit or eliminate these risks of falsification. Stable carbon isotope ratio analysis has been mainly proposed (*9*−*13*) but NMR (*14*) or differential scanning calorimetry (*15*) or more recently measure of proline content (*16*) can be alternative methods.

Detection of honey adulteration is difficult and depends on the composition of the honey or on the presence of a specific component in the adulterant (*17*). Despite this high variability, attempts have been made to detect honey adulteration from sugar syrup by carbohydrate analysis. Many works concerned the analysis of major sugars in honeys using liquid chromatography coupled to a refractometric detector (*18*−*20*), anion exchange chromatography and pulse amperometric detection (HPAEC−PAD) (*7*, *21*−*27*), gas chromatography coupled to a flame-ionization detector (GC−FID) (*8*, *28*, *29*) or mass spectrometry (*30*−*33*). Some works have noted the presence of oligosaccharides in honeys with degrees of polymerization (DP) between 3 and 6 (*21*, *26*, *27*, *30*). Cotte et al. (*8*) proposed to use oligosaccharide (low DP) analysis in order to control honey authenticity by GC−FID, and deliberate additions of syrup about 5% were detected. More recently, Morales et al. (*26*, *27*) showed that detection of oligosaccharides (mainly DP ≤ 12) in honey is possible when corn syrup or high fructose corn syrup is added. Only, traces of DP13-DP15 were found in the adulterated samples.

To the best of our knowledge, no previous studies were focused on saccharides of higher DP (DP > 12) and none

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highlighted the presence of polysaccharides in honey. Besides, it was shown in some publications (*4*, *26*, *34*−*36*) that oligosaccharides and polysaccharides are present in sugar syrups. The majority of the oligosaccharides in sugar syrup result from the incomplete enzymatic hydrolysis of starch. These oligo-/polysaccharides are composed only of glucose monomer with alpha(1−4) and/or alpha(1−6) linkage (*6*). Consequently, the presence of these compounds in honeys may enable detection of possible doping by this syrup.

Thus, we were interested in the polysaccharides to determine if they could be potentially a good probe for the quality control of honeys. HPAEC−PAD was used to establish the fingerprints of authentic honey samples, which will be compared to adulterated samples prepared in the laboratory. At this time no work was focused on the detection of trace of polysaccharides as a falsification probe. This was the objective of this paper, which presents first results for the detection of corn syrup in honey by the analysis of trace polysaccharides. Because of the complexity of samples and very low concentration of polysaccharides, solid-phase extraction (SPE) was used prior to analysis. This will enable us to detect the addition of syrup in various acacia, mountain polyfloral and polyfloral honeys.

MATERIALS AND METHODS

The solutes including glucose, fructose, maltose, maltotriose, maltoheptaose (all used for peak identification) and maltodextrin mixture (DP13−17) were purchased from Sigma-Aldrich (St Quentin Fallavier, France) and used as received. Because pure standard oligosaccharides with DP > 7 were not commercially available, it was not possible to produce accurate quantitation results (*36*). Sodium hydroxide 46/48% solution and sodium acetate were analytical reagent grade (Fisher Scientific, Leicestershire, U.K.). Solvents were of HPLC grade (Baker, Phillipsburg, NJ).

Honeys were obtained from beekeepers of the France Honey Cooperative (France Miel, France): acacia, mountain polyfloral and polyfloral honeys. They were selected according to strict criteria and after the signature of a quality charter to ensure authenticity. Moreover, these honeys were analyzed by microscopy and organoleptic tests by the cooperative laboratory in order to certify their floral validity. All samples were stored at room temperature and preserved sheltered from light until required for analysis. Within a class of honey (acacia, polyfloral), various samples (named A−F) were taken from different honey sources and beekeepers but in the same cooperative.

The commercially inexpensive sweetener employed as adulterant in this study was corn syrup (provided by France Miel, France), previously used in ref *36*, which contains 10% fructose, 45% glucose, 30% maltose, 13% maltotriose and 2% higher oligosaccharides approximately. The syrup was stored at room temperature and preserved sheltered from the light until required for analysis.

Sample Preparation. Adulterated samples were prepared in the laboratory by adding 0.1 or 1% of syrup (w/w) to authentic honey. Such honey doping was carried out mixing well 20 g of honey with 20 or 200 mg (respectively 0.1 and 1%) of corn syrup using a spatula during 10 to 15 min. The samples were then stored two days at ambient temperature and preserved sheltered from the light to equilibrate before analysis. Homogeneity was verified by analyzing two different parts of the same mixture; difference was lower than 10%.

SPE was performed using a 12-port vacuum manifold; flow-rate was approximately 3 $mL \cdot min^{-1}$. The cartridge packed with a polymeric phase, employed in this work, was Envi-ChromP (500 mg/6 mL from Supelco). Solute and saccharide mixtures were dissolved in water as described in **Table 1** and stocked at −20 °C. The sample extraction protocol is presented in **Table 1**. The choice of this SPE method is described in a previous paper (*36*). Among others, this support gave satisfying results in the conditions used.

Prior to chromatographic analysis, samples were filtered on 0.45 μm syringe filters to remove particulate matter, homogenized by mechanical stirring and transferred to vials. It is an important step for honey samples in order to eliminate all remaining wax and bee's fragments and vegetable matter coming from the hive's frames. Samples were analyzed immediately or were stored at −20 °C until analyzed.

Table 1. Protocol for reversed-phase SPE for polysaccharides extraction(sugar syrup, authentic and adulterated honeys) (*36*)

step	solvents
conditioning	5 mL of methanol, then 5 mL of water
sample loading	sugar syrup: 50 mL sample (0.04 $g \cdot L^{-1}$) in water (2 mg loaded)
	honeys: 50 mL sample (40 $g \cdot L^{-1}$) in water (2000 mg loaded)
wash	9 mL of water, allow to dry few minutes, then 5 mL of water
elution	6 mL of methanol−water (30:70)
	evaporation to dryness and dissolution in 0.75 mL of water

HPAEC−PAD Analysis. Samples were analyzed on a Dionex liquid chromatograph equipped with a GP-50 gradient pump and with an ED-50 pulsed amperometric detector PAD (Dionex, Sunnyvale, CA). A 50 μL sample loop was used for the analysis (Rheodyne MX9925). Carbohydrates were separated on a CarboPac 100 (Dionex, Sunnyvale, CA), pellicular anion-exchange column (250 × 4 mm, dp = 8.5 μm). This polyvinylidene/polyvinylbenzene column is suitable for oligo- and polysaccharide analysis. Aqueous sodium hydroxide solution (eluent A: 100 mM NaOH) and sodium hydroxide with sodium acetate solution (eluent B: 100 mM NaOH with 1 M NaOAc) were used as eluents under helium splashing. Oligo- and polysaccharides are separated in their anionic forms, and the use of sodium acetate gradient in sodium hydroxide accelerated and improved their separation. Analyses were performed under elution gradient, and the mobile phase flow rate was fixed at 1 $mL \cdot min^{-1}$. The gradient profile was $t = 0$ min, 5% eluent B; $t = 70$ min, 60% eluent B (concave gradient). Some variations in retention time can be observed from one figure to another; this was due to sodium hydroxide carbonatation of the mobile phase with carbon dioxide of ambient air. In order to attenuate this phenomenon during mobile phase preparation, we subsequently used a smaller storage bottle of sodium hydroxide. However, standard analyses were performed in the same conditions as samples and consequently relative retentions were adequate.

Carbohydrate elution was monitored by a PAD with a dual gold electrode at a sensitivity of 50 μA. The working electrode was maintained at the following potentials and durations during the operation: $E_1 = 0.1$ V ($t_1 = 400$ ms), $E_2 = -2$ V ($t_2 = 20$ ms), $E_3 = 0.1$ V ($t_3 = 10$ ms), $E_4 = -2$ V ($t_4 = 60$ ms). The detection signal of carbohydrates was plotted with Chromeleon software (6.60 version, Dionex). All experiments were conducted at room temperature.

RESULTS AND DISCUSSION

The detection of honey adulteration is difficult because of the high complexity of the matrix. As far as the minor carbohydrate analysis is concerned, the high level of major monosaccharides is another difficulty. Efficient purification is required with high enrichment factor for minor components of interest.

We recently introduced two methods to isolate and concentrate selectively the carbohydrates using SPE (*36*). The choice of the SPE system is highly dependent on the degree of polymerization of the analytes (oligosaccharides, $2 \leq DP \leq 12$; polysaccharides, DP > 12). The method using apolar stationary phase for extraction was revealed to be well adapted for trace analysis of polysaccharides. Recovery was approximately 91%. This SPE system will be applied to sugar syrup and authentic or adulterated honey prior analysis using ion exchange chromatography. Besides purification of samples, since the sample volume was 50 mL and the final volume was 0.75 mL, a concentration factor of 67 is obtained. This was essential for polysaccharides analysis.

Sugar Syrup Analysis. Figure 1 reports a chromatogram showing the carbohydrate profiles from the sugar syrup with or without SPE extraction. SPE protocol is reported in **Table 1**.

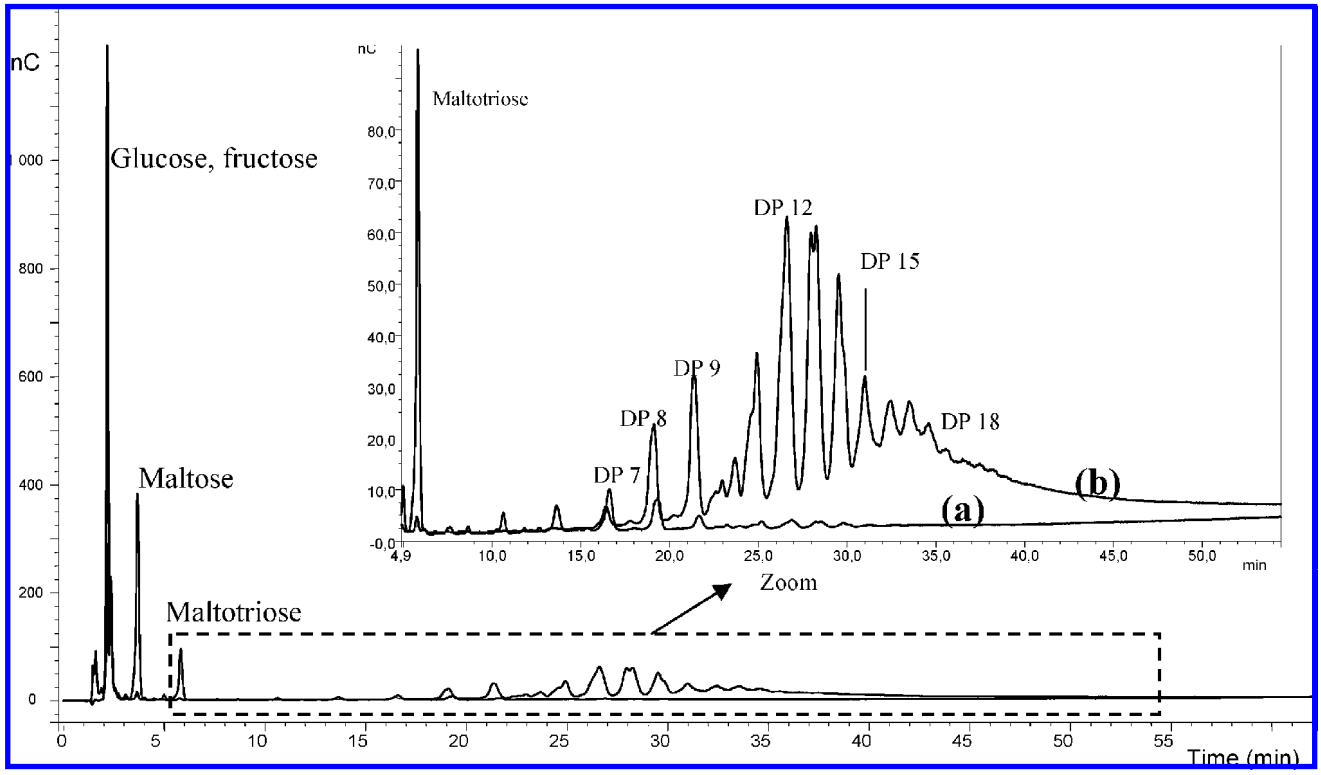

Figure 1. HPAEC−PAD analysis of corn syrup (0.04 g·L^{-1}): raw sample (**a**) and SPE extract (**b**). Conditions: see **Table 1** and Materials and Methods.

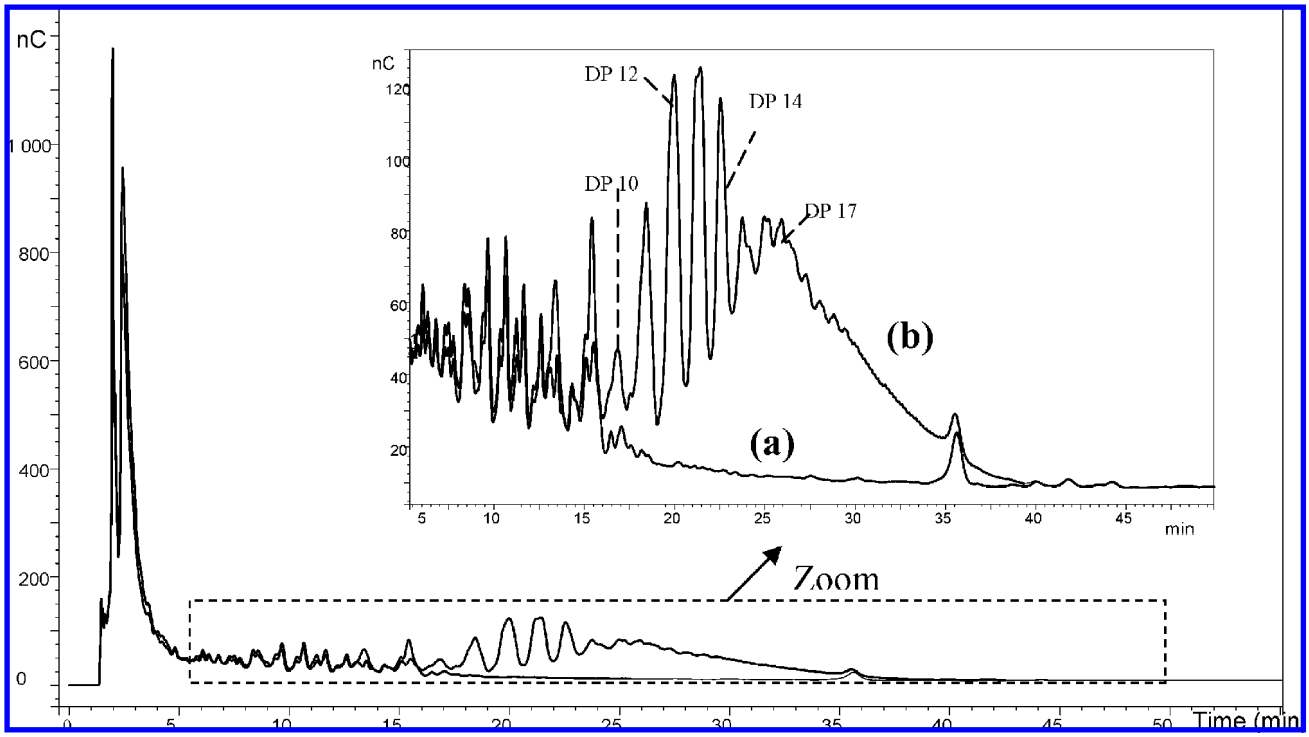

Figure 2. HPAEC−PAD analysis of SPE extract from authentic (**a**) and adulterated (**b**) mountain polyfloral honey (40 g·L^{-1}) with 1% corn syrup. Conditions: see **Table 1** and Materials and Methods.

Syrup profile showed a high number of oligosaccharides. Peaks with retention times from 2 to 17 min were identified as glucose, fructose, maltose, maltotriose and maltoheptaose (DP7) by comparison with commercial standards. Since retention is a function of the DP, the retention times of maltotetraose, maltopentaose, maltohexaose can be easily deduced from the chromatogram. Indeed, the qualitative composition of glucose syrups is well-known and they contain oligo- and polysaccharides whose monomer is mainly α(1−4) D-glucose. Thus, peaks with retention times longer than 17 min could be assigned to gluco-oligosaccharides and gluco-polysaccharides from DP8 to DP18. These oligo- and polysaccharides are not hydrolyzed by the enzymes used during the conversion of corn starch to corn syrup (*37*, *38*).

The reversed-phase SPE method allows the easy detection of the polysaccharide fraction from sugar syrup (SPE extract

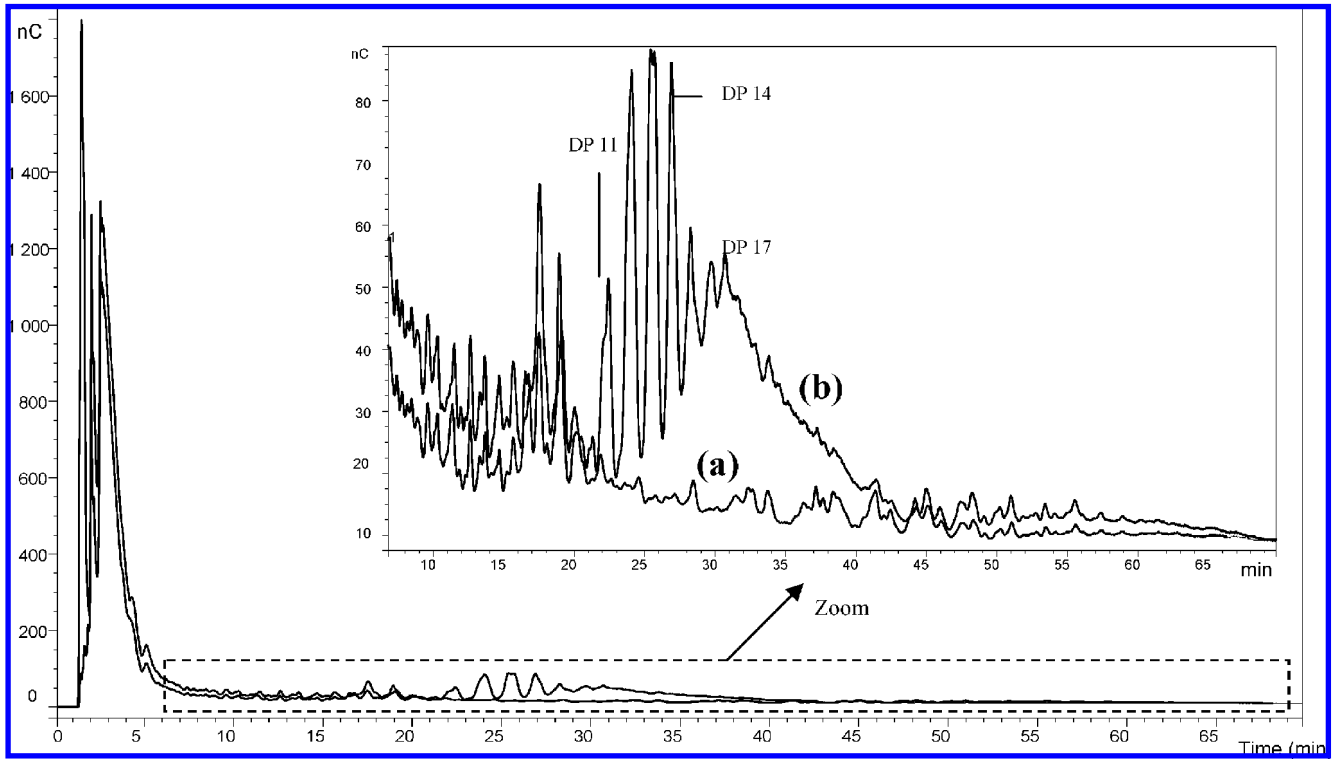

Figure 3. HPAEC−PAD analysis of authentic (**a**) and adulterated (**b**) polyfloral honey SPE extract (40 g·L^{-1}) with 1% corn syrup. Conditions: see **Table 1** and Materials and Methods. Polyfloral honey A.

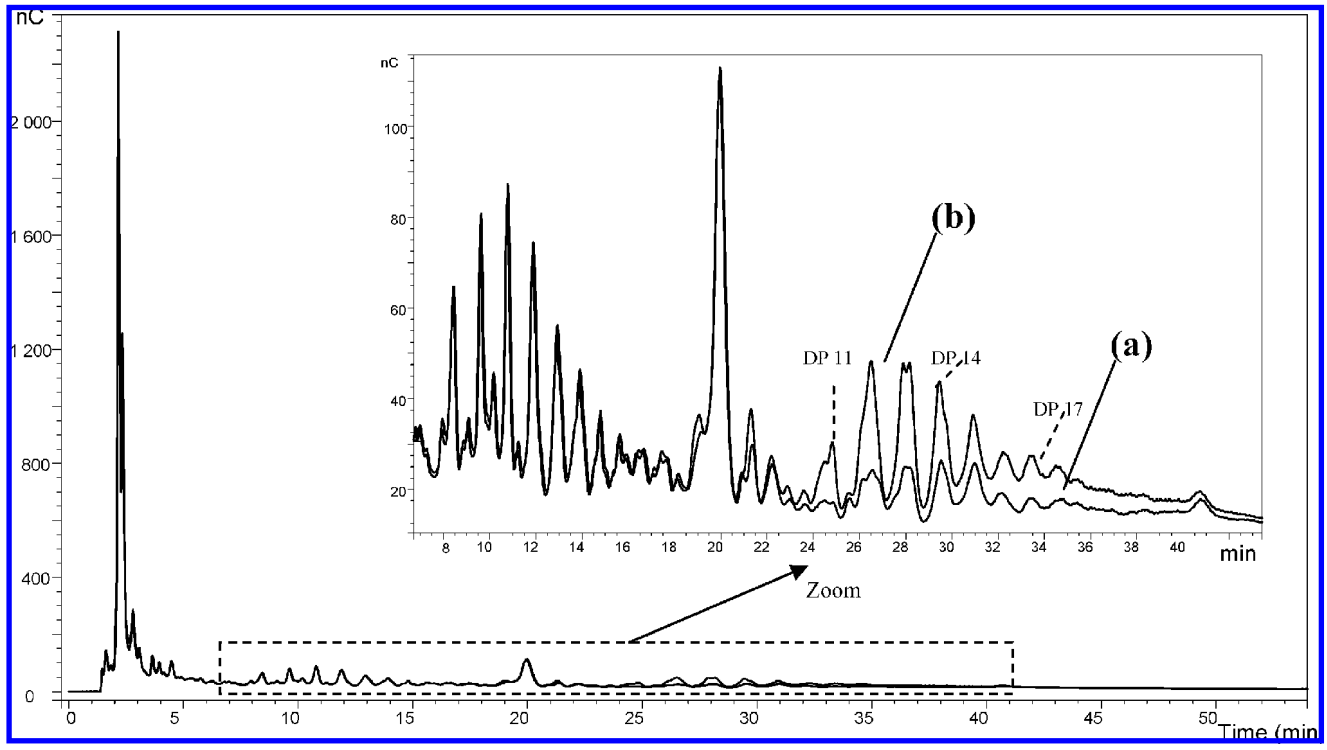

Figure 4. HPAEC−PAD analysis of authentic (**a**) and adulterated (**b**) acacia honey SPE extract (40 g·L^{-1}) with 0.1% corn syrup. Conditions: see **Table 1** and Materials and Methods. Acacia honey A.

analysis in **Figure 1b**). Oligosaccharides having DP lower than 8 were not retained in the SPE stationary phase, whereas those having 8 ≥ DP ≥ 10 and polysaccharides were efficiently retained and concentrated. The presence of small amounts of fructose and glucose after SPE is due to high initial content of monosaccharides in these syrups. The use of HPAEC−PAD allows, in this study, better sensitivity and specificity than liquid chromatography with evaporative light scattering detector used in our previous work (*6*, *36*).

Authentic Honey Analysis. Authentic honey concentrated solutions (40 g·L^{-1}) were extracted using reversed-phase SPE (**Table 1**). Because of low concentration of analytes of

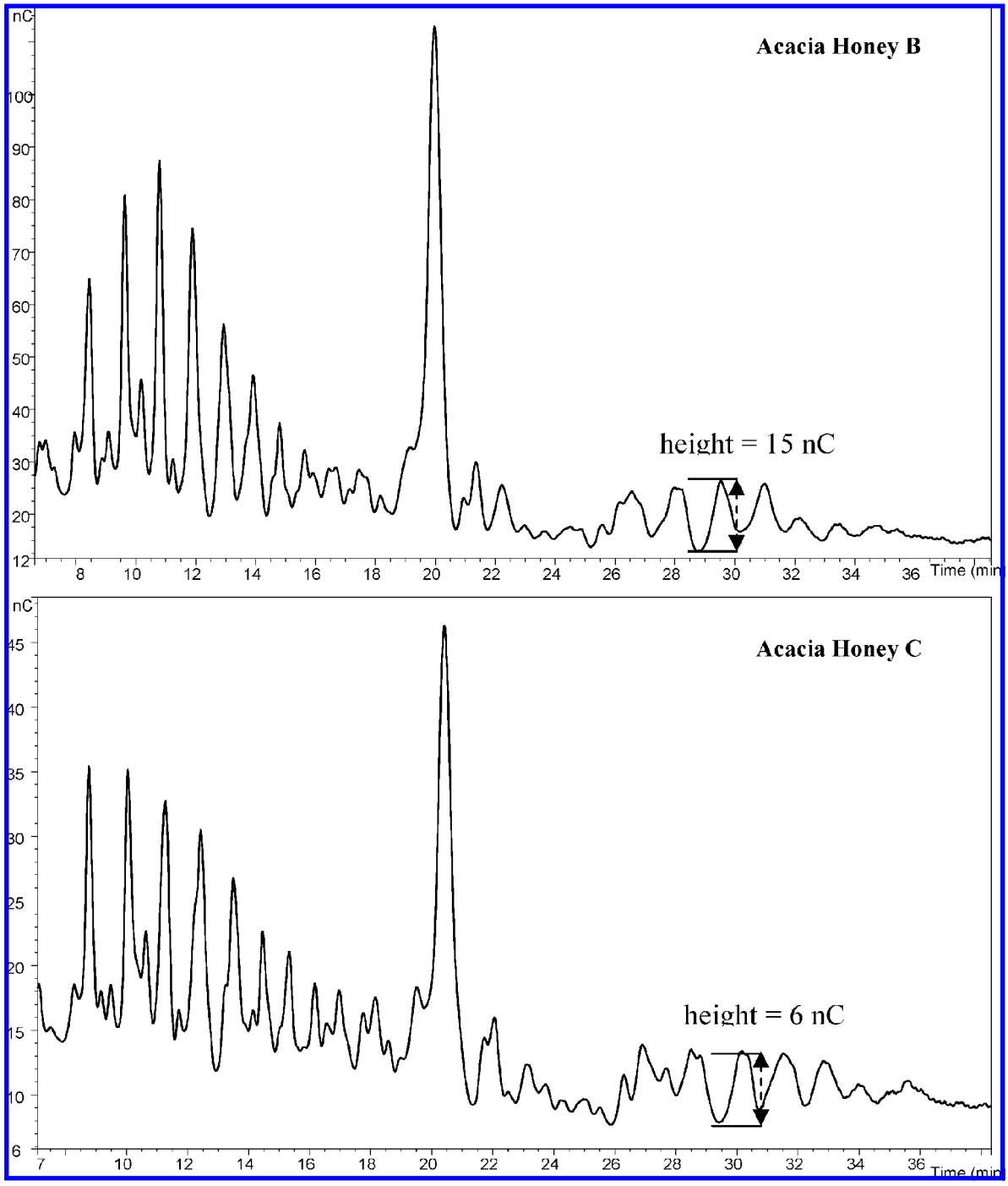

Figure 5. Zoom of chromatograms of SPE extracts from two different authentic acacia honeys B and C in the same conditions. Conditions: see **Table 1** and Materials and Methods.

interest, sufficient quantities of honey (2 g) must be loaded. Monosaccharides (more than 85% of the dry matter) are not retained on the SPE cartridge. HPAEC−PAD was then easily used to determine the oligo- and polysaccharide profile of honey samples after the SPE. Chromatograms which are representative of the authentic honey samples (mountain polyfloral, polyfloral and acacia honeys) analyzed in this study are shown in **Figures 2a**, **3a** and **4a**. As previously observed, because of the important quantity of sample loaded on the SPE cartridge, low levels of monosaccharides still remain in the extract. Clearly, with an enrichment factor of 67, the presence of high DP oligosaccharides and polysaccharides (DP > 10) can be detected in the samples. This was confirmed by mass spectrometry analysis with a MALDI-TOF system of the honey SPE extract (*36*). In fact, the mass spectrum highlights peak differences of 162 mass units which are characteristic of the saccharidic sequence (Na^+ adduct).

Each of the three classes of honey (acacia, mountain polyfloral and polyfloral) analyzed in this study presents specific chromatographic profiles. Major differences are in polysaccharide fingerprints in the 20−45 min range, magnified in **Figures 2a**, **3a** and **4a**.

First, these chromatograms show the complex mixture of carbohydrates in this food, in particular the oligosaccharide zone, from 6 to 16 min, with difficult peak identification because of high varieties of monomer and linkage in sugars. Second, **Figures 2a**−**4a** show the wide natural range in oligo- and polysaccharide content of authentic honey from various origins.

Table 2. Maximum Peak Height in the Retention Window Corresponding to Polysaccharides (DP12−DP17) for the Authentic and Some Adulterated Honeys[a]

class	sample	max peak height (nC)
mountain polyfloral	authentic	<1 (not detected)
	doped 1%	75
polyfloral		
A	authentic	5
	doped 1%	70
B	authentic	30
C	authentic	15
D	authentic	27
E	authentic	20
acacia		
A	authentic	12
	doped 0.1%	38
B	authentic	15
C	authentic	6
D	authentic	2
E	authentic	10
F	authentic	15

[a] Examples of chromatographic profiles are shown in **Figure 5** where the retention window is 25−35 min.

Besides, the oligo- and polysaccharide profiles of extracts from two different acacia honeys were similar (**Figure 5**). Only some minor variations in the peak intensities of oligo- and polysaccharides can be observed. Explanations for these variations in quantities include variation in pH, presence of minerals and other compounds that could inhibit or favor the enzymatic formation of oligo- and polysaccharides, and other parameters such as storage time of the honey, storage temperature, mono- and oligosaccharide concentrations in the original nectar (*39*−*41*) or polysaccharide concentrations in the pollen (*42*). Relative intensities of peaks were very close within a class of honey: five different polyfloral honeys provide intensities from 0 to 30 nC, and six different acacia honeys provide intensities from 2 to 15 nC; values are reported in **Table 2**. Obviously, more studies are required to better understanding these variations in various classes of honey.

Adulterated Honey Analysis. Honey samples (acacia, mountain polyfloral and polyfloral) were intentionally adulterated with 0.1 or 1% glucose syrup (see Materials and Methods) in order to determine the capability of the method to detect the addition of sugar syrup. Upon removal of the majority of monosaccharides by reversed-phase SPE, both initial and deliberately adulterated honeys were analyzed and compared. The polysaccharide patterns of authentic and adulterated honeys can be readily discriminated by HPAEC−PAD.

Each adulterated honey chromatogram (**Figures 2b**, **3b** and **4b**) is very different from the one obtained for initial samples (**Figures 2a**, **3a** and **4a**). The presence of the polysaccharide peaks that elute approximately in the 20 to 34 min range of the chromatogram clearly indicates whether a honey has been adulterated. These peaks correspond to polysaccharides DP12−DP17 from added sugar syrup as stated previously. Consequently, these polysaccharides can be used as markers since they are not detected or are present in very low concentrations in mountain polyfloral, polyfloral and acacia authentic honeys (**Table 2**).

Oligo- and polysaccharide fingerprints resulting from honey samples show that adulteration levels of 1% are readily detected. Adulteration at the 0.1% level in acacia honey produced a significant increase of the peak height: from 12 nC for authentic acacia honeys to 38 nC for the doped acacia honey at 0.1% (**Table 2**). Detection at the 0.1% level seems to be difficult to detect in an unknown sample because of the natural variability from one sample to another. However, this level of fraud is not of concern because of the low economical difference between natural and doped honey.

As with fruit juices or wines, the upsurge of fraud in the honey industry may ultimately have irremediable economic consequences. Honey and inexpensive sweeteners/syrups each contain a complex mixture of oligo- and polysaccharides. These carbohydrates are either present in the raw material or arise during production/processing. Many more studies and chemometric methods are required to interpret the presence and the behavior of polysaccharides in natural honey.

By comparing intentionally adulterated samples to authentic honey samples, reversed-phase SPE and HPAEC−PAD allowed revelation of the presence of polysaccharides (DP11−DP17) coming from corn syrup that can be used as a marker for its deliberate addition. Under these conditions, the developed analytical method allowed detection of the addition of corn syrup at the 1% level in all three floral types of honey considered. Therefore, the analysis of polysaccharides will be a simple and efficient tool for honey quality control and detecting product adulteration.

Various varieties of honey from different geographical areas and other syrup types such as high fructose corn syrup must be analyzed and characterized. Then, this method might be a simple and universal method which can be considered as an alternative to official isotopic analysis. In addition, other food products (natural fruit juices, maple syrup, etc.) could be analyzed in order to control their quality and determine their possible adulteration with the proposed analytical method.

ABBREVIATIONS USED

DP, degree of polymerization; GC-FID, gas chromatography−flame ionization detection; HPAEC−PAD, high performance anion exchange chromatography−pulsed amperometric detection; NMR, nuclear magnetic resonance; SPE, solid phase extraction.

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