Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1021 (2003) 145–155
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Application of carbohydrate analysis to verify honey authenticity

J.F. Cotte [a], H. Casabianca [b], S. Chardon [b], J. Lheritier [a], M.F. Grenier-Loustalot [b,*]

[a] *Cooperative France Miel, B.P. 5, Mouchard 39330, France*
[b] *Service Central d'Analyse, USR 059, CNRS, B.P. 22, Vernaison 69390, France*

Received 17 March 2003; received in revised form 25 August 2003; accepted 1 September 2003

Abstract

Gas chromatography and liquid chromatography have been used simultaneously to analyze sugars in honey. After statistical processing by principal components analysis, additions of exogenous sugars could be detected by the appropriate fingerprints of adulteration. Application to acacia, chestnut and lavender honeys enabled the detection of fraud resulting from 5 to 10% addition of sugar syrups. This method may be considered as a replacement of isotopic analysis, that has some limitations.

Keywords: Honey; Principal components analysis; Food analysis; Carbohydrates

1. Introduction

Fraud is an increasingly frequent phenomenon, resulting from the opening of international markets and global competition. The major argument for this practice is extra profit, leading industrialists to condone this illegal act far too often. 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. The beekeeping market is not spared from this phenomenon and control organizations are faced with an increasing number of cases of non-compliance. Honey fraud can be reduced to two situations: it may be the non-compliance of an origin name resulting from mixing (voluntary or not) of honeys from several varieties, or a non-compliance resulting from the deliberate addition of an adulterating syrup. Syrups can be added directly to the honey after harvest or fed to bees during the harvest to improve yield. The upsurge of frauds currently being encountered may ultimately have irremediable economic consequences for the honest sector of the profession that complies with legislation.

A number of control methods exist to combat fraud, one of which is pollen analysis. It can demonstrate the addition of syrup by the microscopic detection of cane sugar annuli or parenchyma, or starch grains [1,2]. This technique provides very good results [3], but there is the problem of possible ultrafiltration of the honey that may considerably reduce the scope of effectiveness of pollen analysis. Under the coverage of obscure health reasons, this determination will soon be officially adopted [publication in the J.O.C.E. (Official Register of the European Community), January 12, 2002, decree not later than August 1, 2003, application not later than August 1, 2004]. It will result in the elimination of natural (pollens) and exogenous tracers (cane sugar annuli, starch grains, etc.) from determinations, that guarantee the relevance of pollen analysis.

Another control method is based on the stable carbon isotope ratio analysis (SCIRA). This method is based on differences in isotopic carbon composition that depend on the origin of the plant. The reactions occurring during photosynthesis lead to the isotopic fractionation of carbon that is specific to the photosynthesis cycle involved. Considerable work has thus shown isotopic differences between products from the Calvin cycle of photosynthesis (C_3) and those from the Hatch and Slack cycle (C_4) [4–6]. Products resulting from the C_3 cycle contain a smaller proportion of carbon 13 [7]. Among the large body of work carried out according to this principle [8,9], the initial studies of honey were based on these differences in isotopic compositions. The creation of databases on the isotopic contents of honeys (most from C_3 plants) has revealed that these contents are relatively uniform [10]. It was thus possible to detect the addition of a corn

* Corresponding author. Tel.: +33-478022262; fax: +33-478024174.
E-mail address: mf.grenier-loutalot@sca.cnrs.fr (M.F. Grenier-Loustalot).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.09.005

or cane sugar syrup (C_4 plants), but starting only at an addition of about 20% [11,12]. This method was subsequently improved by the introduction of an internal standard, honey proteins [13], leading to a refinement of measurements by comparing the isotopic values of these proteins and of the honey in question. This method became the official control method for the addition of high fructose content syrups (HFCS) in honeys [14,15]. The limit of detection was estimated by the authors to be a 7% addition of HFCS [16], a result that was confirmed by many publications on honeys from different countries [17,18].

Another isotopic technique is also applicable for controlling the authenticity of honeys: SNIF-NMR of ethanol deuterium. This method is based on the work of Martin and Martin with wines [19,20], became the official method for the detection of beet sugar syrup addition to fruits [21], and many applications have arisen from its use [22,23]. In the domain of honey, this technique has been used to authenticate certain varieties, e.g. citrus honeys [24]. It is also used to confirm the carbon 13 isotopic method when doubts subsist concerning a sample [25].

There are nevertheless serious limitations when these two isotopic methods are applied. It is in fact very difficult if not impossible to detect the addition of syrup from beets (C_3), that follow the same Calvin cycle of photosynthesis as the honey flower. In addition, anomalies have been reported [26] in the analysis of natural samples declared fraudulent by the official method of White. It is thus indispensable to consider new physicochemical parameters to apply to the control of honey authenticity.

In this sense, sugars can be considered as a family of interest and considerable work has been devoted to these compounds, the majority species in honey. Research was conducted starting in the 1960s to determine the sugar profiles of honeys by first using thin-layer chromatography (TLC) [27], and subsequently by gas chromatography (GC) after the derivation of sugars [28–30]. The latter method enabled honeys to be characterized as a function of their botanical origins [31,32]. The major limitation of GC is the necessity of derivatizing sugars to render them volatile [33,34], a step that may be long and difficult. This explains why liquid chromatography (LC) was considered, since it does not require derivatization. The first application of this technique date from the early 1980s and used a system of an amino-bonded column and a refractometer for detection [35]. The use of this equipment created numerous problems, however, including the formation of Schiff bases [36,37]. A new system involving an anion-exchange column was subsequently developed [38,39].

In the food industry, a large number of applications followed the development of this material [40–42]. Advances in LC techniques led to the characterization of a number of di- and trisaccharides [43], thereby enabling the determination of the floral origin of a honey [44]. Concerning the battle against adulteration, the method is based on the work of Low [45,46] that revealed the presence of HFCS in honey starting at an addition of 5%, using adulteration markers for this purpose [47,48].

This publication describes the method we developed involving the use of LC coupled with pulsed amperometric detection (PAD) to assay fructose and glucose, and GC with flame ionization detection (FID) to measure the entire profile of di- and trisaccharides. The two techniques were combined with a statistical processing of the results by principal components analysis (PCA). This enabled us to demonstrate the addition of syrup to acacia, chestnut and lavender honeys. PCA had previously been used to discriminate authentic and fraudulent raspberry samples [49]. Our work involved creating databases of oligosaccharides using authentic honey samples. In order to determine the limits of fraud detection of our method, we then worked on preparations of a mixture of authentic honey and syrups from three different sugar families. We terminated by examining commercial samples to determine the current status of fraud on the French market.

2. Experimental

2.1. Database

Pure honeys were obtained from beekeepers of the France Honey Cooperative (Mouchard, France). They were selected according to strict criteria and after the signature of a quality charter and so the natural nature of samples is not questioned. Moreover, these honeys were analyzed by microscopy and organoleptic tests by the cooperative laboratory in order to certify their floral validity. We used 141 French honeys belong to three monofloral origins: 50 acacia, 38 chestnut and 53 lavender.

Thirty-five commercial samples were used, obtained from a broad distribution and from independent beekeepers. There were 23 French honeys (8 acacia, 8 chestnut and 7 lavender), 7 Hungarian honeys (acacia), 3 Chinese honeys (acacia), 1 Spanish honey (lavender) and 1 Moroccan honey (lavender).

Three inexpensive commercial sweeteners were used in this work: Erstein liquid sugar (Erstein, France) (named in our study syrup A): its $\delta^{13}C$ is equal to −22.25‰, and so it is a C_3 sugar syrup; Erstein siroline 728:4-6 consisting of a mixture of invert sugar syrup and glucose syrup (Erstein, France) (named in our study syrup B): its $\delta^{13}C$ is equal to −21.31‰, and so it is probably a mixture of C_3 and C_4 sugar syrups; Cerestar FT 1702 (Hambourdin, France) (named in our study syrup C): its $\delta^{13}C$ is equal to −25.32‰, and so it is a C_3 sugar syrup.

2.2. Analysis by GC-FID

2.2.1. Sample preparation

Samples of pure and adulterated honeys were diluted with ultrapure water (18.2 mΩ) to a final Brix value between 5 and 6. From this sample, 150 μl were transferred to GC autosampler vials that were then freeze-dried for 4 h.

2.2.2. Sample analysis

The freeze-dried sample was derivatized by the addition of 150 μl of trimethylsilylimidazole (98%, Fluka, Saint Quentin Fallavier, France) and 1 ml of pyridine (Fluka). The vial was capped and the solution heated at 80 °C for 1 h. Trimethylsilylated carbohydrates were analyzed with a gas chromatograph (Model 6890 Series, Agilent Technology, Waldbronn, Germany) equipped with an autosampler (Model 7686 Series, Agilent Technology), and piloted by HP GC Chemstation software. Analysis was carried out in split mode with a ratio of 1/30. The carrier gas was UHP helium at a constant pressure of 22 psi at column head (1 psi = 6894.76 Pa). Two microliter samples were injected into the column. The injector temperature was maintained at 280 °C and the capillary column was 30 m × 0.25 m HP5-MS (0.25 μm film thickness, Interchim, Montluçon, France). The program used to separate the carbohydrates was: 150 °C for 5 min, followed by a 3 °C min^{-1} gradient to 325 °C, followed by a 10 min plateau at this temperature. The derivatized carbohydrates were detected with FID at 280 °C.

Identification of carbohydrates was realized with their retention indexes according to the relationship: $I = 100[(t_{ri} - t_{rn})/(t_{rn+1} - t_{rn})] + 100n$ (t_{ri} = retention time of solute, t_{rn} = retention time of alkane n, t_{rn+1} = retention time of alkane $n + 1$, n = number of alkane carbons).

All sugars standard (Fluka) used in this study had been analyzed with the same protocol to measure their retention index. The analyses were repeated many times to certify the correct attribution of peaks on chromatograms.

2.3. Analysis by high-performance anion-exchange chromatography (HPAE)–PAD

2.3.1. Sample preparation

Homogenized samples of natural and adulterated honeys were weighed (between 0.5 and 1 g) and diluted 10 000-fold with water. The solution was filtered (0.2 μm) and transferred to vials.

2.3.2. Sample analysis

Honeys were analyzed on a DX 500 liquid chromatograph (Dionex, Sunnyvale, CA), equipped with a GP 500 gradient pump (Dionex). Carbohydrates (glucose and fructose) were separated on a Carbo Pac PA1 pellicular anion-exchange column (250 mm × 4 mm) (Dionex) at 35 °C. Each sample (25 μl) was injected with an AS 50 autosampler (Dionex). The separation was realized with a gradient of two mobile phases. Phase A was pure water and phase B was 0.5 mM sodium hydroxide (Prolabo, Limonest, France). Flow-rate was 1 ml min^{-1} with 10% B for 15.2 min; at 15.8 min 50% B; at 22.1 min 50% B; at 22.8 min 10% B for 7 min for column reequilibration. Carbohydrates were monitored by a PAD system ED 40 (Dionex) 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: $E_1 = 0.05$ V ($t_1 = 0.40$ s), $E_2 = 0.75$ V ($t_2 = 0.20$ s), $E_3 = -0.15$ V ($t_3 = 0.40$ s). Carbohydrates eluting from the column were plotted with Peak Net version 5.11 (Dionex) software.

2.4. Principal components analysis

This method enabled us to represent objects or variables on a graph, with different objectives: study the proximity of objects in order to classify them and to detect atypical objects; analyze the position of objects in varied representations; assign new objects in a representation characterizing the population; determine the significance of principal components. The PCA does not presuppose a heterogeneity of the objects studied. When each PCA is calculated, we detail the circle of correlations that represent new vectors. These vectors correspond to each variable used in PCA calculations. The study of this circle of correlations and associated vectors provide correlations between vectors and principal axes, and correlations between vectors themselves. The interpretation of variables is based principally on the direction of the vector.

PCA was conducted with Statgraphics US software (STSC, Rockville, MD).

3. Results and discussion

Fig. 1 shows the GC separation obtained with an acacia honey. The monosaccharides zone is between 14 and 20 min, in which two peaks of fructose and two peaks of glucose predominate. There was a broad cluster of peaks between 39 and 46 min, corresponding to disaccharides. Trisaccharides eluted between 54 and 59 min. The most difficult zone for peak identification was that of disaccharides, magnified in Fig. 2. Two peaks are present, corresponding to two anomers of maltose (40.08 and 41.17 min) that surround two peaks of maltulose (40.58 and 40.0 min) and one of turanose (40.92 min). The identification of each compound would have been difficult if not impossible without having calculated retention indices, since only determinations of these indices guarantee correct peak attribution.

We then analyzed authentic honey samples in order to develop a reliable database that could be used to control the natural nature of a sample. The results (Table 1) show a relatively characteristic profile depending on the floral origin in question. It can in fact be seen that the sucrose content of chestnut honeys is lower than that of lavender honeys. The level of this sugar is highly dispersed in this variety, but we were able to distinguish two populations: lavender honeys with high sucrose (almost 10%) that are the case of rapid honey flows, i.e. few enzyme reactions of bees on sugars, and lavender honeys with a lower sucrose content (between 1 and 3%) that are cases of slow honey flows, where enzymes could transform most disaccharides into monosaccharides. This leads to a high variability of the content of this sugar in lavender honeys (principally sucrose). Chestnut honeys are

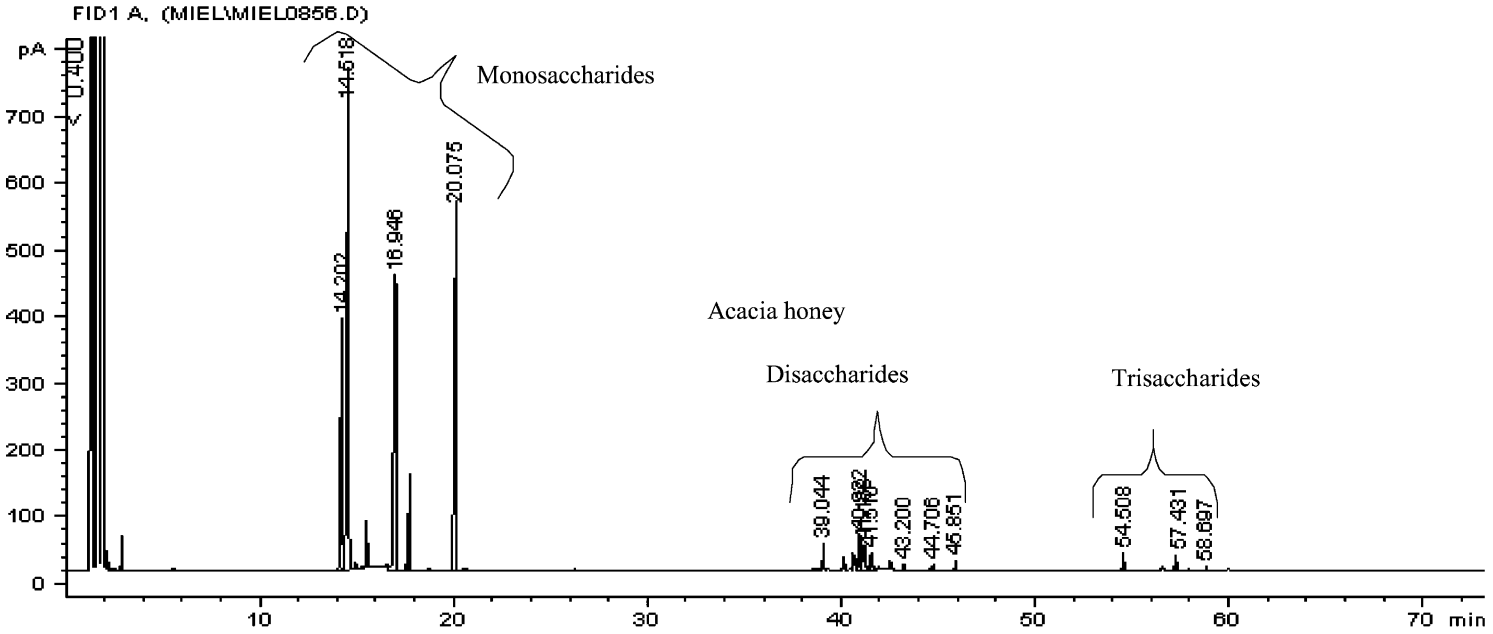

Fig. 1. Gas chromatogram of the sugar of a TMSi pure acacia honey.

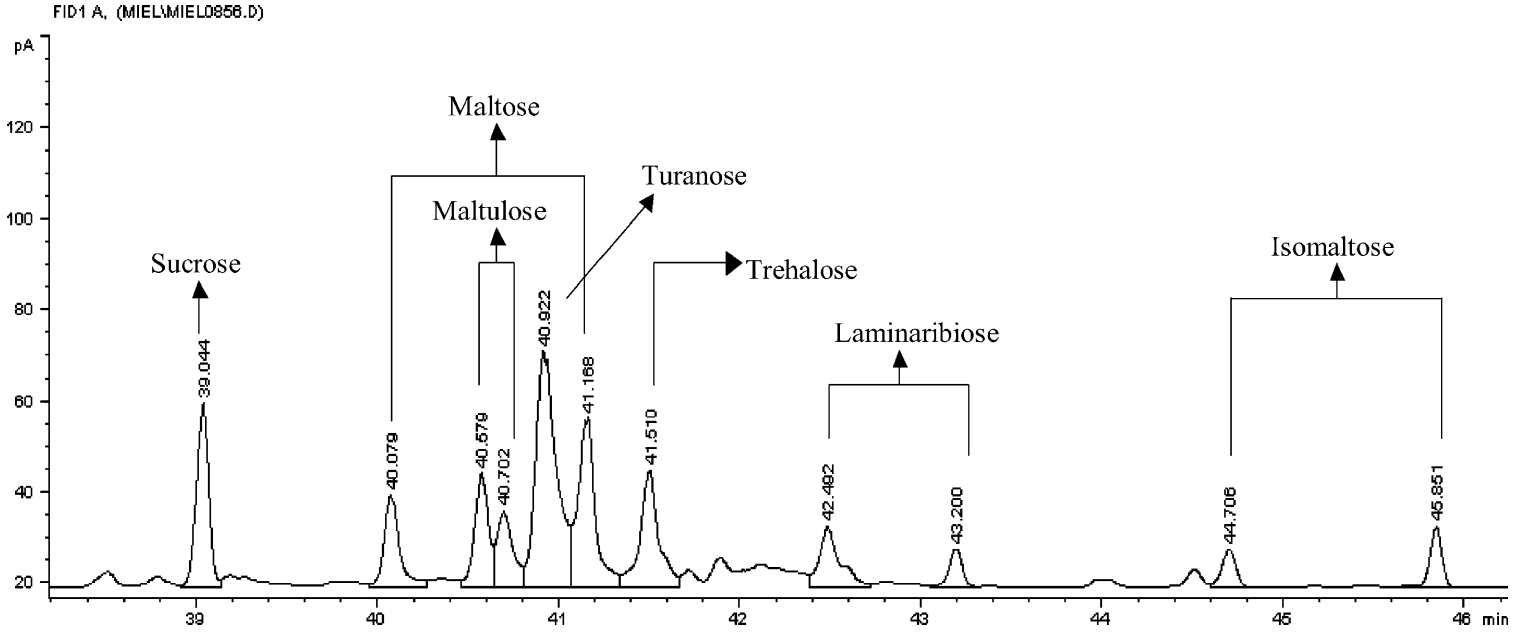

Fig. 2. GC disaccharide profiles of typical pure acacia honey.

Table 1
Mean and standard deviation (S.D.) of sugar contents in acacia, chestnut and lavender honeys

	Acacia ($n = 50$)	Chestnut ($n = 38$)	Lavender ($n = 53$)
Fructose ($g\,l^{-1}$)	438.9 (12.9)	407.2 (22.8)	384.6 (13.0)
Glucose ($g\,l^{-1}$)	263.0 (13.0)	265.2 (20.7)	323.3 (13.1)
F/G ratio[a]	1.67 (0.08)	1.54 (0.11)	1.19 (0.05)
Sucrose (%)	2.02 (1.98)	0.16 (0.22)	4.68 (2.82)
Maltose (%)	2.63 (0.75)	1.48 (0.55)	2.56 (0.58)
Maltulose (%)	1.73 (0.40)	2.63 (0.93)	0.97 (0.29)
Turanose (%)	2.87 (0.60)	2.78 (0.76)	1.77 (0.49)
Trehalose (%)	1.48 (0.39)	2.05 (0.66)	0.79 (0.25)
Palatinose (%)	0.30 (0.12)	0.54 (0.40)	0.16 (0.07)
Laminaribiose (%)	1.16 (0.31)	1.46 (0.51)	0.70 (0.28)
Melibiose (%)	0.14 (0.07)	0.30 (0.26)	0.05 (0.03)
Isomaltose (%)	0.89 (0.28)	1.80 (0.81)	0.47 (0.17)
Gentiobiose (%)	0.03 (0.02)	0.22 (0.47)	0.03 (0.02)
Raffinose (%)	0.03 (0.06)	0.04 (0.11)	0.05 (0.04)
Neo-kestose (%)	0.20 (0.12)	0.19 (0.30)	0.15 (0.05)
1-Kestose (%)	0.06 (0.03)	0.08 (0.14)	0.06 (0.02)
Erlose (%)	1.88 (1.25)	0.24 (0.20)	1.40 (0.59)
Melezitose (%)	0.10 (0.06)	0.22 (0.43)	0.08 (0.07)
Maltotriose (%)	0.39 (0.18)	0.19 (0.13)	0.21 (0.11)
Panose (%)	0.24 (0.12)	0.21 (0.11)	0.12 (0.08)

[a] Fructose/glucose.

also characterized by a low trisaccharide content, in contrast to acacia and lavender honeys that contain, e.g. considerable quantities of erlose (1.88 and 1.40%, respectively).

In the same way, we analyzed three syrups (Table 2), characterized by their fructose and glucose concentrations that are generally lower than in honeys. The fructose/glucose (F/G) ratio, however, is not always different, e.g. the syrup of sugar A (ratio of 1.16) is equivalent to lavender honeys. The sucrose content of the syrup A (30%) was higher than the levels measured in authentic honeys. In addition, the considerable presence of maltose and maltotriose is to be noted in syrups B and C. These sugars than be used as markers since they are not present at these concentrations in honeys.

Table 2
Sugar contents in sugar syrups A, B and C

	Syrup A	Syrup B	Syrup C
Fructose ($g\,l^{-1}$)	287.9	108.3	72.8
Glucose ($g\,l^{-1}$)	248.2	159.2	269.7
F/G ratio	1.16	0.68	0.27
Sucrose (%)	42.1	0.09	0.05
Maltose (%)	0.00	1.61	29.82
Maltulose (%)	0.00	0.35	2.12
Turanose (%)	0.18	0.37	0.00
Trehalose (%)	0.00	0.26	0.00
Palatinose (%)	0.06	0.12	0.07
Laminaribiose (%)	0.00	0.25	0.17
Melibiose (%)	0.00	0.02	0.00
Isomaltose (%)	0.00	0.09	1.6
Gentiobiose (%)	0.00	0.08	0.09
Raffinose (%)	0.09	0.00	1.47
Neo-kestose (%)	0.04	0.00	0.73
1-Kestose (%)	0.03	0.00	0.00
Erlose (%)	0.00	0.05	0.04
Melezitose (%)	0.00	0.04	0.22
Maltotriose (%)	0.00	1.20	6.52
Panose (%)	0.35	0.00	0.90

The ultimate goal of our work was to demonstrate the addition of industrial syrup to a sample. This was done by analyzing preparations containing the three syrups (A, B and C) described above at the concentrations of 5, 10, 20 and 50%. The hypothesis supporting the analysis of carbohydrates as a fraud control method is based on the profile difference between authentic samples and syrups used as adulterants, demonstrated above with the analysis of authentic honeys and sugar syrups. The analysis of database samples revealed certain carbohydrate characteristics for the seven monofloral varieties. Syrups, however, are not a faithful reflection of the sugar composition of honeys, even after feeding bees. It is thus possible to identify markers that can be used to detect the syrup addition. These considerations for the three botanical origins selected enabled PCAs to be established for the control of the natural nature of honeys. This technique enabled the selection of several discriminating parameters: the ratios of sugars calculated from markers identified for each syrup, e.g. sucrose, maltose, maltotriose, etc.

The most discriminating parameters were selected with Student's *t*-test, that compares the mean and variance of paired series of data. This furnishes a theoretical coefficient: the larger the coefficient, the greater the significance of the difference between the two series of data. Four series of data were used in this study: authentic honeys, honeys adulterated with syrup A, honeys adulterated with syrup B and honeys adulterated with syrup C. Student's test was applied in three cases, i.e. between authentic samples and honeys adulterated with each of the three syrups. In the three cases, the parameters used were those with the highest Student's experimental coefficients, i.e. the most discriminating. One parameter may be discriminating for several syrups.

3.1. Acacia honeys

Student's test enabled the extraction of six discriminating variables for acacia honey: the fructose and glucose contents, and the sucrose/maltose, fructose/glucose, maltotriose/trisaccharides and maltotriose/turanose ratios. The experimental coefficients (Table 3) show that the parameters fructose, glucose and sucrose/maltose, discriminate

Table 3
Experimental Student's coefficients for acacia honeys

	Syrup A	Syrup B	Syrup C
Fructose	11.1	9.4	5.0
Glucose	6.2	1.6	1.9
F/G ratio	2.5	4.5	1.5
Sucrose/maltose	6.0	1.9	2.3
Maltotriose/trisaccharides	0.7	2.6	10
Maltotriose/turanose	0.7	4.1	7.6

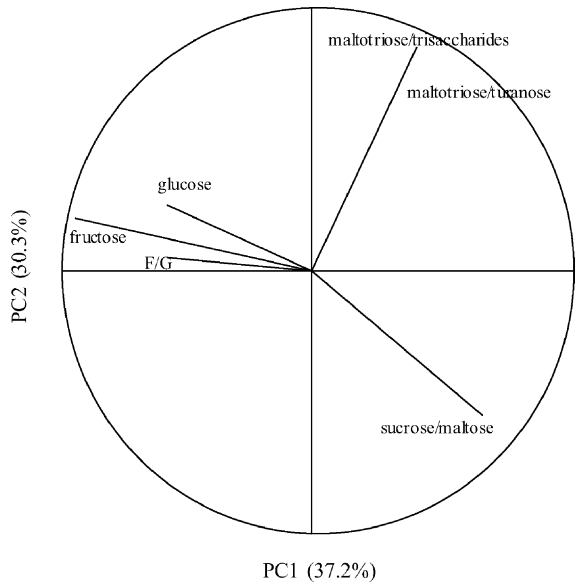

Fig. 3. Circle of correlation and associated vectors of PCA calculated with six variables: fructose, glucose, F/G, sucrose/maltose, maltotriose/trisaccharides and maltotriose/turanose.

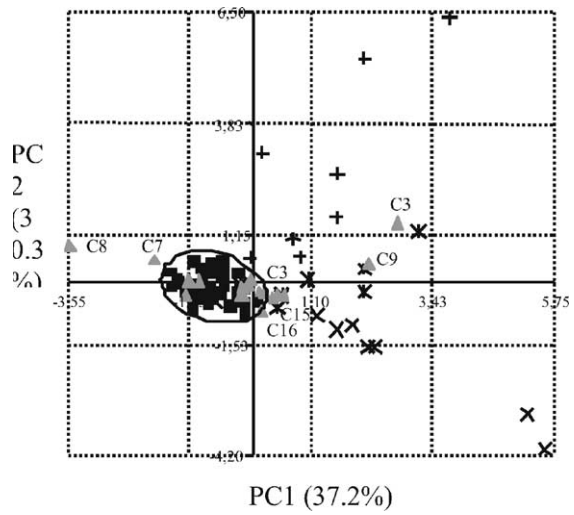

Fig. 4. PCA with pure acacia honeys (■), honeys adulterated with sugar syrup A (×), honeys adulterated with sugar syrup B (✳), honeys adulterated with sugar syrup C (+) and commercial honeys (▲).

preparations with syrup A; fructose, F/G and maltotriose/trisaccharides ratios distinguish preparations with syrup B and fructose, maltotriose/trisaccharides and maltotriose/turanose discriminate those with syrup C. The calculation of the PCA with these variables generated a circle of correlations (Fig. 3) accounting for 67.5% of total variance, with 37.2% for PC1 and 30.3% for PC2. The majority of PC1 was constructed from fructose contents and to a lesser extent from glucose, F/G and sucrose/maltose. PC2 arose from maltotriose/trisaccharides and maltotriose/turanose. Since the latter two parameters were perfectly correlated, we thought that one of them could be eliminated to improve variance, but this led to a loss of discrimination. The choice of these two ratios was thus imposed. The parameters fructose and glucose were significantly correlated with the F/G ratio, logical since the calculation of this ratio involves these two variables.

The graphic representation of individual samples on the plane defined by the first two principal components (Fig. 4) shows the homogeneity of the group of authentic acacia honeys in the center of the figure. All these honey samples were inside the tolerance ellipse. Preparations with syrup A are on the right side of the figure and are distinguished from authentic samples by positively following the sucrose/maltose ratio. Only one point of a 5% addition was not discriminated. Sucrose is thus a good marker, since its concentration in syrup A is higher than in acacia honeys. Preparations with syrup C are situated in the upper right quadrant of the PCA. All their points are outside the natural zone defined by the ellipse. This total separation is ensured by the maltotriose/trisaccharides and maltotriose/turanose ratios. The points from preparations with syrup B are along PC1, negatively correlated with fructose, glucose and F/G. These three variables lead to a practically total distinction, except for one point at 5% addition, represented for authentic samples.

When the representations of commercial samples were examined, seven anomalies were found (C3, C5, C7, C8, C9, C15 and C16), i.e. almost half the commercial acacia samples analyzed (18 in all). Nevertheless, the interpretation of these seven anomalies was not the same. Commercial samples C7 and C8 are to the left of the figure, i.e. diametrically opposite the preparations from the three syrups. They were thus either samples that were adulterated with a type of syrup not tested in our study, or samples not belonging to the acacia floral variety. If a doubt remains concerning honey C7 because of its representation relatively close to the ellipse, then sample C8 must be downgraded to fraudulent. The points of samples C3, C5, C9, C15 and C16 are at the right of the figure, i.e. with the preparations. Samples C3 and C9 were very probably adulterated with the same type of syrup as syrup B, since these commercial samples were differentiated according to the variables fructose, glucose and F/G. It was more difficult to identify the type of syrup in the case of samples C15 and C16 because they were situated in a zone of coincidence of preparations from the three syrups. Sample C5, as sample C7, is doubtful but it was difficult to certify fraud because of the position of the sample in proximity to the ellipse.

3.2. Chestnut honeys

Four parameters were used to calculate the PCA for chestnut honeys: the sucrose/turanose, sucrose/maltose, maltotriose/melezitose and maltotriose/trisaccharides ratios. The ratios containing sucrose (Table 4) discriminated preparations with syrup A. The ratios involving the maltotriose content differentiated authentic samples from preparations with both syrups B and C. Calculation using these four

Table 4
Experimental Student's coefficients for chestnut honeys

	Syrup A	Syrup B	Syrup C
Sucrose/turanose	7.2	0.9	1.5
Sucrose/maltose	12.0	1.1	2.7
Maltotriose/trisaccharides	0.9	6.9	14.5
Maltotriose/melezitose	0.1	7.1	5.8

variables provided a PCA whose variance on PC1 was 54.5% and was 36.3% on PC2, i.e. a total variance of 90.8%. The vectors representing the sucrose/maltose and sucrose/turanose ratios have the same direction on the circle of correlations (Fig. 5), signifying that they are highly correlated. In spite of this characteristic, the elimination of one of these two parameters led to reduced discrimination, requiring them to be retained in the calculation of the PCA. These two parameters were included in the construction of PC1 and were negatively correlated with it. PC2 is generated by the variables maltotriose/melezitose and maltotriose/trisaccharides that are also mutually correlated. The points representing authentic chestnut honeys on the PCA (Fig. 6) are clustered in the lower half of the figure. The authentic samples are slightly dispersed along the direction of the maltotriose/melezitose and maltotriose/trisaccharides vectors. On the other hand, the separation of points was very low along the vectors of the sucrose/maltose and sucrose/turanose ratios, indicating a very good homogeneity of these two carbohydrate characteristics. Authentic sample 355, however, is outside the natural zone, to its left, since its sucrose content (1.28%) was higher than the mean of its original group. Preparations with syrup A are dispersed in the upper left of the figure. The two variables enabling this differentiation are the sucrose/maltose and sucrose/turanose

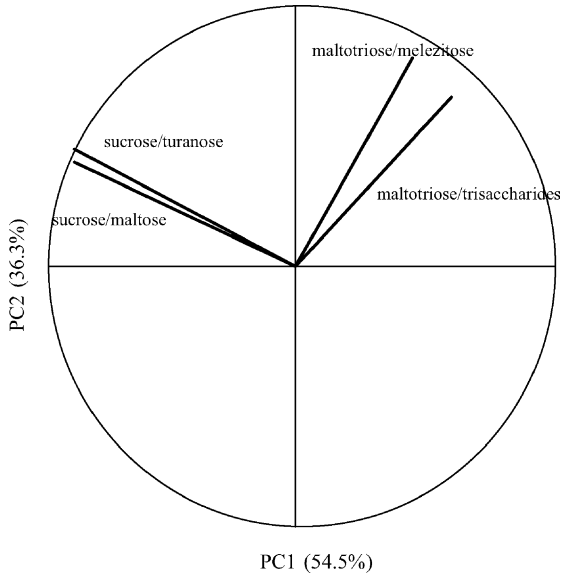

Fig. 5. Circle of correlation and associated vectors of PCA calculated with four variables: sucrose/maltose, sucrose/turanose, maltotriose/trisaccharides and maltotriose/melezitose.

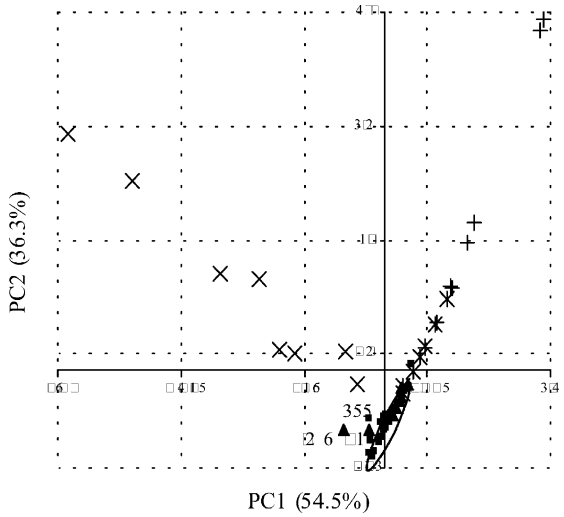

Fig. 6. PCA with pure chestnut honeys (■), honeys adulterated with sugar syrup A (×), honeys adulterated with sugar syrup B (✳), honeys adulterated with sugar syrup C (+) and commercial honeys (▲).

ratios. The addition of this syrup is detectable starting at 5%, since all points representing the preparations are outside the natural zone. The variables maltotriose/melezitose and maltotriose/trisaccharides discriminated preparations with both syrups B and C, a logical outcome of our interpretation of Student's coefficients (Table 4). The points of these preparations positively follow the direction of the vectors of these two ratios. The limit of detection was 5% for syrup C, but 10% for syrup B since the two points of the smallest additions (5%) are positioned inside the ellipse. This better limit of fraud detection is due to a higher maltotriose content in syrup C (10%) compared to syrup B (1.2%). The detection of this trisaccharide and thus of the addition, was facilitated in syrup C compared to syrup B. Examination of commercial samples shows that two of them (C1 and C26) appeared doubtful. Concerning the localization of these points in the same direction as preparations with syrup A, it is consistent to think that these two honeys were adulterated with a syrup of this family, i.e. a syrup containing some invert sugar. Sample C1 is very close to the natural zone, situated alongside sample 355 that is outside the ellipse. In this case, it is thus difficult to certify non-compliance of the sample. Sample C26, on the other hand, was clearly distinguished from authentic samples, leaving no doubt on the required downgrading of this sample.

3.3. Lavender honeys

Four parameters were selected using the calculation of Student's coefficients: fructose, glucose, and the maltotriose/trisaccharides and maltotriose/maltose ratios. The fructose content enabled the addition of the three syrups to be detected (Table 5). The variable glucose also participates concomitantly in the differentiation of syrups A and B. The experimental coefficients of the maltotriose/trisaccharides

Table 5
Experimental Student's coefficients for lavender honeys

	Syrup A	Syrup B	Syrup C
Fructose	10.6	7.0	8.0
Glucose	12.0	6.1	1.1
Maltotriose/trisaccharides	1.7	7.2	14.2
Maltotriose/maltose	0.5	5.0	11.0

and maltotriose/maltose ratios indicate that these two parameters demonstrate the presence of syrups B and C. PC1 calculated from these parameters is constructed primarily with the variables fructose, maltotriose/maltose and maltotriose/trisaccharides (Fig. 7). The latter two parameters were highly correlated but the elimination of one of them was not possible without losing discrimination. PC2 was established with the variable glucose since the vector representing this parameter was negatively correlated with this component. Total information contained in the PCA is 90.7% with PC1 possessing 59.8% of the information and PC2 30.9%. The population of authentic lavender samples is relatively clustered on the right side of the PCA (Fig. 8). There is a certain degree of dispersion observed according to PC2 and thus with reference to the glucose concentration. Authentic sample 257 is outside the ellipse symbolizing the natural zone. This sample is characterized by a maltotriose content (0.7%) much higher than the mean of this trisaccharide in lavender samples (0.2%), explaining its position in the direction of vectors involving maltotriose. This honey sample exhibited the dispersion that could exist in authentic samples. The glucose content enabled the differentiation of preparations with syrup A. One sample was nevertheless localized at the limit of the ellipse characterizing the natural zone. The limit of detection is thus 10%. Additions of

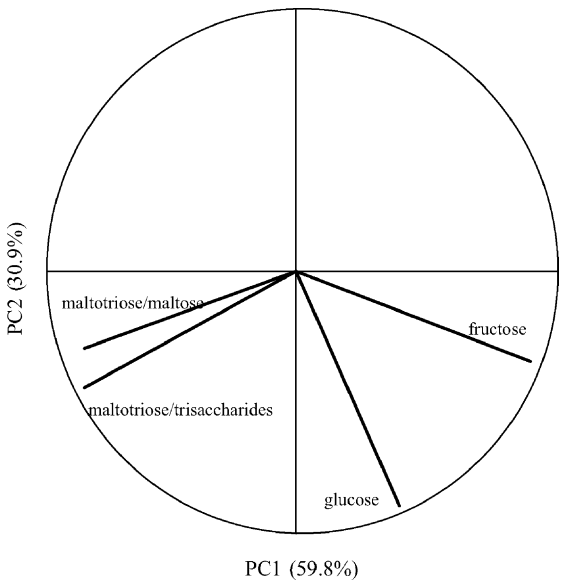

Fig. 7. Circle of correlation and associated vectors of PCA calculated with four variables: fructose, glucose, maltotriose/trisaccharides and maltotriose/maltose.

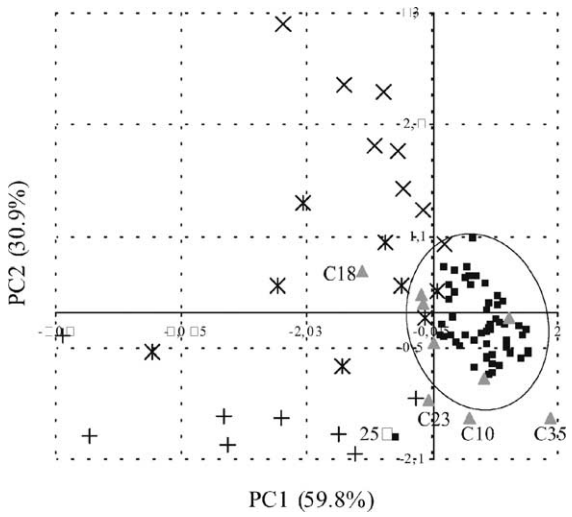

Fig. 8. PCA with pure lavender honeys (■), honeys adulterated with sugar syrup A (×), honeys adulterated with sugar syrup B (✳), honeys adulterated with sugar syrup C (+) and commercial honeys (▲).

syrups B and C were characterized by a shift of the points representing them into the negative part of PC1. The variables leading to this distinction are the maltotriose/maltose and maltotriose/trisaccharides ratios, as well as the fructose content, since component 1 is correlated with these three parameters. Two points corresponding to a 5% addition of syrup B are inside the ellipse and so this syrup can be detected starting only at 10%. All preparations with syrup C were discriminated from the natural zone with a limit of detection of 5%. The low fructose and glucose contents in the three syrups (Table 2) compared to the levels of these sugars in natural lavender samples enabled their presence in the preparations to be demonstrated. Lavender, on the other hand, was characterized by a high sucrose content (mean of 4.68%) and also by the values encountered in certain cases of rapid honey flows that could reach almost 10% of this disaccharide. The addition of syrup A, also characterized by a high sucrose content, could thus not be detected by the variables involving this sugar. The analysis of commercial samples showed that four (C10, C18, C23 and C35) were outside the ellipse. Samples C10 and C35 are not represented on the side of the preparations from our three syrups. They were thus either adulterated with another type of syrup or were samples lacking the carbohydrate characteristics of lavender honey as a result of mixing honey types. Inversely, sample C18 was differentiated from the natural zone in the direction of preparations from syrup B and sample C23 in the direction of syrup C. It is thus possible that this type of syrup was used for these two samples. In light of the distance of point C18, it is clear that this sample is non-compliant. For samples C10 and C23, on the other hand, a conclusion of fraud cannot be reached unambiguously.

Finally, Table 6 represents the comparison of conclusions on the authenticity of commercial honeys, according to the official ISCIRA method [15] with the protocol we previously

Table 6
Comparison between results obtained with the ISCIRA method [26] and sugar analysis

Variety of sample	Reference of sample	ISCIRA index[a]	Sugars analysis
Acacia	C2		
	C3	NC[b]	NC
	C5		D
	C7	D[c]	NC
	C8	D	NC
	C9	NC	NC
	C14		
	C15		NC
	C16		NC
	C17		
	C19		
	C25		
	C32		
	C34		
	C37		
	C38		
	C45		
	C46		
Chestnut	C1		
	C11		
	C13		
	C24		
	C26		NC
	C30		
	C33		
	C44		
Lavender	C6		
	C10	NC	D
	C12	D	
	C18		NC
	C23		D
	C35		NC
	C39		
	C40		

[a] Results obtained with AOAC official method [15,26].
[b] Non-compliance.
[c] Doubtful.

detailed [26], and according to the analysis of sugars followed by the statistical processing of data by PCA. It is seen that three commercial honeys were non-compliant and three were doubtful according to the ISCIRA index, whereas nine honeys were non-compliant and three doubtful according to the analysis of sugars. Except for lavender honey C12, all the samples declared non-compliant by the official method were also downgraded by the analysis of sugars. This method also detected seven additional cases of non-compliance, arguing for its greater versatility. The analysis of sugars followed by statistical processing enables all syrup families to be detected, in contrast to the official AOAC method [15] that could detect only the addition of a C_3 syrup.

4. Conclusion

The application of honey sugar analysis with two chromatographic techniques (GC and LC), combined with a relevant statistical processing of the results by PCA, has enabled us to detect the addition of industrial syrups to honey. The limits of detection are very good (between 5 and 10%) for acacia, chestnut and lavender honeys. This method has the advantage of being universal with respect to a number of syrup types (C_3 and C_4) and thus provides better possibilities than carbon 13 isotopic analysis. The analysis of commercial samples revealed fraud in a number of cases and in some cases the type of syrups with which the adulteration was realized. This method is certainly applicable to other honey varieties and so we propose that it replace the current isotopic method.

References

[1] J. Louveau, A. Maurizio, G. Vorwohl, Bee Wildl. 59 (1978) 139.
[2] J. Kerkvliet, M. Shrestha, K. Tuladar, H. Manandhar, Apidologie 26 (1995) 131.
[3] J. Kerkvliet, H.A.J. Meijer, Apidologie 31 (2000) 717.
[4] M.M. Bender, Radiocarbon 10 (1968) 468.
[5] B.N. Smith, S. Epstein, Plant Physiol. 47 (1971) 380.
[6] M.M. Bender, Phytochemistry 10 (1971) 1239.
[7] W.H. Krueger, R.H. Reesman, Mass Spectrom. Rev. 1 (1982) 205.
[8] H. Casabianca, J.B. Graff, S. Guillaumet, Rivista Italiana EPPOS 7 (1996) 244.
[9] H. Casabianca, C. Perruchietti, P. Jame, C. Grenier, J.F. Cotte, M.C. Bertrand, D. Kustner, Ann. Fals. Exp. Chim. 51 (2000) 179.
[10] L.W. Doner, J.W. White, Science 197 (1977) 891.
[11] J.W. White, Am. Bee J. 117 (1977) 440.
[12] J.W. White, L.W. Doner, J. Assoc. Off. Anal. Chem. 61 (1978) 746.
[13] J.W. White, K. Winters, J. Assoc. Off. Anal. Chem. 72 (1989) 907.
[14] J.W. White, J. AOAC Int. 75 (1992) 543.
[15] Official Methods of Analysis, 5th Revision, AOAC International, Gaithersburg, MD, 16th ed., 1999, Method 998.12.
[16] J.W. White, K. Winters, P. Martin, A. Rossman, J. AOAC Int. 81 (1998) 610.
[17] J. Serra Bonvehi, F. Ventura Call, Food Sci. Technol. Int. 1 (1995) 25.
[18] E. Cienfuegos, I. Casar, P. Morales, J. Apic. Res. 36 (1997) 169.
[19] G.J. Martin, M.L. Martin, Tetrahedron Lett. 22 (1981) 225.
[20] G.J. Martin, M.L. Martin, in: H.F. Linkens, J.F. Jackson (Eds.), Modern Methods of Plant Analysis, vol. 6, Springer, Berlin 1988, p. 258.
[21] Official Methods of Analysis, 5th Revision, AOAC International, Gaithersburg, MD, 16th ed., 1999, Method 995.17.
[22] R.A. Culp, J.E. Noakes, J. Agric. Food Chem. 38 (1992) 1249.
[23] G.S. Remaud, Y.L. Martin, G.J. Martin, G.G. Martin, J. Agric. Food Chem. 45 (1997) 859.
[24] P. Lindner, E. Bermann, B. Gamarnik, J. Agric. Food Chem. 44 (1996) 139.
[25] S. Giraudon, M. Danzart, M.H. Merle, J. AOAC Int. 83 (2000) 1401.
[26] J.F. Cotte, H. Casabianca, C. Perruchietti, C. Sanglar, H. Waton, M.F. Grenier-Loustalot, J. Agric. Food Chem., in press.
[27] J. Pourtallier, Bull. Apic. (1964) 197.
[28] J. Pourtallier, Bull. Apic. 2 (1967) 209.
[29] I.R. Siddiqui, B. Furgala, J. Apic. Res. 6 (1967) 139.
[30] I.R. Siddiqui, B. Furgala, J. Apic. Res. 7 (1968) 51.
[31] M. Bernardini Battaglini, G. Bosi, Apiacta 7 (1972) 5.
[32] L.W. Doner, J. Sci. Food Agric. 28 (1977) 443.
[33] A.G. Mac Innes, D.H. Ball, F.P. Cooper, J. Bishop, J. Chromatogr. 1 (1958) 556.
[34] R. Mateo, F. Bosch, A. Pastor, M. Jimenez, J. Chromatogr. 410 (1987) 319.

[35] H. Binder, J. Chromatogr. 189 (1980) 414.
[36] E. Brons, J. Olieman, J. Chromatogr. 159 (1983) 79.
[37] B. Porsch, J. Chromatogr. 253 (1982) 49.
[38] A.G.J. Voragen, H.A. Scholl, J.A. Devries, W. Pilnik, J. Chromatogr. 244 (1982) 327.
[39] P. Nebinger, M. Koel, A. Franz, E. Werries, J. Chromatogr. 265 (1983) 19.
[40] M. Callul, E. Loppez, R.M. Marce, J.C. Olucha, F. Borrul, J. Chromatogr. 500 (1992) 453.
[41] A. Klockow, A. Paulus, V. Figueirido, R. Amado, H.M. Widmer, J. Chromatogr. A 680 (1994) 187.
[42] E. Verette, F. Qian, F. Mangani, J. Chromatogr. A 705 (1995) 195.
[43] K.W. Swallow, N.H. Low, J. Agric. Food Chem. 38 (1990) 1828.
[44] I. Goodall, M.J. Dennis, I. Parker, M. Sharman, J. Chromatogr. A 706 (1995) 353.
[45] N.H. Low, P. Sporn, J. Food Sci. 53 (1988) 558.
[46] N.H. Low, J. AOAC Int. 79 (1996) 724.
[47] N.H. Low, J. AOAC Int. 78 (1995) 1210.
[48] N.H. Low, Book of Abstracts 212th ACS National Meeting, Orlando, 1996, p. 1.
[49] H. Casabianca, J.B. Graff, J. Chromatogr. A 684 (1994) 360.