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Fractionation of Honey Carbohydrates Using Pressurized Liquid Extraction with Activated Charcoal

A. I. RUIZ-MATUTE, L. RAMOS, I. MARTÍNEZ-CASTRO, AND M. L. SANZ*

Instituto de Química Orgánica General (CSIC) Juan de la Cierva 3, 28006 Madrid, Spain

This article describes the development of a new procedure that combines the use of activated charcoal and pressurized liquid extraction (PLE) to obtain enriched fractions of di- and trisaccharides from honey. Honey was adsorbed onto activated charcoal and packed into a PLE extraction cell. Optimum results were obtained at 10 MPa and 40 °C using two consecutive PLE cycles: first, 1:99 (v/v) ethanol/water for 5 min and second, 50:50 (v/v) ethanol/water for 10 min. Di- and trisaccharide fractions were enriched after PLE treatment, accounting for 73% and 8% of total carbohydrates, respectively. This procedure was also compared with other methodologies reported in the literature for the fractionation of honey carbohydrates (yeast treatment and extraction from activated charcoal). While the removal of monosaccharides was more efficient with yeast treatment, recovery of di- and trisaccharides was higher when either the PLE or the activated charcoal treatment was used. PLE was found to be the faster technique; it also required less solvent volume and minimized handling of the sample.

KEYWORDS: Honey; carbohydrates; PLE; activated charcoal; fractionation

INTRODUCTION

Honey is a natural food highly appreciated by consumers. Since ancient times, many beneficial properties have been attributed to this product, and in the past few years, there has been an increasing number of publications concerning its antioxidant (*1*), antibacterial (*2*), or prebiotic properties (*3, 4*). Moreover, honey is a relatively costly food and may be susceptible to adulterations with cheaper sweeteners (*5, 6*). Recent findings have reported the utility of new indicators (difructose anhydrides; DFAs) to detect honey adulterations with high fructose corn syrups (HFCS) or invert syrups (IS) (*7*). Studies of the oligosaccharide profile of honey have also been useful for determining possible adulteration with corn syrups (CS) (*8*), HFCS, and IS (*9*).

Honey is mainly composed of carbohydrates: high amounts of glucose (22−40%) and fructose (27−44%), which are metabolized in the gastrointestinal tract, and a large number of oligosaccharides with different glycosidic linkages and molecular weights (*10*). The fact that monosaccharides are present in larger amounts may mask the functional properties of other oligossacharides in studies using *in vitro* systems and may also prevent the detection of chemical markers of adulteration. Monosaccharides should therefore be removed to ensure the accurate evaluation of functional properties and the detection of adulterations (*4, 7–9*).

Various different techniques have been suggested to remove glucose and fructose from honey; however, the similarity of the properties of mono- and disaccharides and the high concentrations of the former make this fractionation difficult. Separation of glucose and fructose from honey oligosaccharides has mainly been achieved using charcoal-celite columns (*11, 12*). This procedure is considered quite lengthy and cumbersome (*13*). More recently, Morales et al. (*14*) developed a new methodology based on the adsorption of carbohydrates on activated charcoal and their extraction with different mixtures of ethanol/water by filtering through filter paper. This method resulted in being faster than the one mentioned before and provided better recovery of oligosaccharides with a high degree of polymerization (DP) than the column approach. Nevertheless, the handling of the sample can cause problems of reproducibility. Nanofiltration (*4*) or yeast treatments (*Saccharomyces cerevisiae*) (*4, 7*) have also been used for these purposes. While with nanofiltration relatively large amounts of monosaccharides still remained in the extracts, yeast treatment achieved complete removal of glucose and fructose. However, the latter selectively modifies the oligosaccharide composition of honey and even produces some carbohydrates such as α,α-trehalose by yeast metabolism.

Pressurized liquid extraction (PLE) is in widespread use for the extraction of nonpolar compounds (*15, 16*), although a number of applications for polar compounds, mainly in plant materials, is also available (*17–19*). In both cases, the combined effect of high temperatures and pressures has been found to result in highly efficient extractions, producing a significant

* Corresponding author. Tel: + 34 915622900 (ext. 306). Fax: + 34 915644853. E-mail: mlsanz@iqog.csic.es.

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Table 1. Concentration (n=2) of Mono- (MS), Di- (DS) and Trisaccharides (TS) Expressed in % of Total Carbohydrates Obtained in Each PLE Cycle and in % of Carbohydrates Recovered from the Untreated Original Honeydew Honey for the Different Assayed Conditions in PLE Treatment[a]

treatments					% total carbohydrates in each PLE cycle			% carbohydrates recovered from original honey		
assay no.	T (°C)	PLE cycle no.	ethanol % in aqueous mixture	extraction time (min)	MS	DS	TS	MS	DS	TS
1	40	1	10	30	24	65	11	2	9	9
		2	50	30	0	30	70	0	2	33
		3	50	30	0	26	74	0	0	7
2	40	1	1	30	24	66	10	15	80	68
		2	50	30	14	74	11	1	13	11
		3	50	30	7	80	12	0	3	2
3	40	1	1	5	78	22	0	5	3	0
		2	50	5	14	72	14	1	13	14
		3	50	5	2	81	17	0	19	24
		4	50	5	0	88	12	0	2	2
4	**40**	**1**	**1**	**5**	**85**	**15**	**0**	**11**	**4**	**0**
		2	**50**	**10**	**4**	**83**	**13**	**2**	**78**	**72**
5	40	1	1	5	77	23	0	7	4	0
		2	50	20	4	84	12	2	74	64
6	40	1	1	5	78	22	0	16	8	0
		2	50	30	12	75	14	4	49	52
7	60	1	1	5	88	9	3	34	7	15
		2	50	10	8	80	12	4	79	69

[a] All experiments were carried out at 10 MPa.

reduction in extraction times and solvent volumes as compared to conventional (nonsolvent-enhanced) extraction procedures. PLE has been applied for the first time to the fractionation of carbohydrates by our research group (*20*). In that study, lactulose was separated from lactose using 70:30 (v/v) ethanol/water at 40 °C and 1500 psi.

This article describes the development of a new methodology combining PLE with an in-cell packed adsorbent bed of activated charcoal for the separation of monosaccharides from the oligosaccharide fraction in honey. It also compares this method with extraction using activated charcoal (*14*) and yeast treatment (*7*).

MATERIALS AND METHODS

Standards. Cellobiose, erlose, fructose, gentiobiose, glucose, isomaltose, isomaltotriose, 1-kestose, kojibiose, laminaribiose, maltose, maltotriose, melezitose, nigerose, panose, phenyl-β-glucoside, raffinose, and sucrose were supplied by Sigma Chemical Co. (St. Louis, USA). Leucrose, melibiose, palatinose, α,α-trehalose, α,β-trehalose, and turanose were purchased from Fluka (Madrid, Spain) and maltulose from Aldrich Chem. Co (Milwaukee, WI, US). Trehalulose was kindly gifted by Dr. Wach from Südzucker (Mannheim, Germany) and theanderose by Dr. G.R. Côté (USDA, Peoria, USA).

Samples. One citrus honey and one oak honeydew honey were directly purchased from beekeepers in Madrid (Spain) and used in this study.

Fractionation Techniques. *Yeast Treatment.* Yeast treatment was carried out as indicated by Ruiz-Matute et al. (*7*). A 20% (w/v) solution of honey in Milli-Q water (Milli Q Plus 185, Millipore, Waters, Milford, USA) was treated with 1% (w/v) *Saccharomyces cerevisiae* (Maizena, Unilever) at 30 °C for 52 h. Samples were centrifuged at 7000*g* for 5 min and filtered through 0.22 μm filters (Sartorius, Germany) to remove yeast.

Activated Charcoal Treatment. Oligosaccharides in the honey sample were extracted following the method of Morales et al. (*14*) with some modifications. Briefly, 0.5 g of honey was dissolved in 100 mL of ethanol/water 1:99 (v/v) and stirred with 3 g of Darco G-60 100 mesh activated charcoal (Sigma Chemical Co., St. Louis, MO) for 30 min to remove monosaccharides. This mixture was filtered under vacuum, and the activated charcoal was further washed with 25 mL of 1:99 (v/v) ethanol/water. Oligosaccharides adsorbed onto the activated charcoal were then extracted by stirring for 30 min in 100 mL of 50:50 (v/v) ethanol/water. Activated charcoal was washed with 25 mL of this ethanol/water solution and subsequently eliminated by filtering through paper as previously described. The sample was evaporated under vacuum at 35 °C, reconstituted with 5 mL of water, and filtered through a 0.22 μm filter (Millipore).

Pressurized Liquid Extraction (PLE) with Adsorbent Bed of Activated Charcoal. All assays were carried out in a homemade miniaturized PLE system (*21*). The instrument consisted of an oven equipped with temperature control and regulation, in which a stainless steel extraction cell (100 mm × 4.6 mm i.d × 6.6 mm o.d.) was placed. This extraction cell was coupled to an isocratic pump (Hewlett-Packard 1050 series, Palo Alto, USA), which was used to deliver and pressurize the solvent, via a six-port Rheodyne valve (model 7000, Rheodyne L.P., Rohnert Park, CA, USA). Another valve of the same characteristics was connected to the outlet end of the extraction cell. The extraction cell was sealed with 5-μm stainless steel frits (Supelco, Bellefonte, USA) at its lower and upper ends to avoid the access of suspended particles to the outlet stainless-steel tubing, valve, and ultimately to the extraction vial.

Fifty milligrams of honey was dissolved in 2 mL of 1:99 (v/v) ethanol/water and mixed with 300 mg of activated charcoal. The sample was homogenized by stirring for 5 min and packed in the extraction cell with the aid of a vacuum system. Sea sand (Panreac, Barcelona, Spain) was also gradually added during the packing of the sample into the extraction cell to hold the sample and prevent the formation of preferential flow paths.

Carbohydrate fractionation was optimized using successive PLE cycles with different proportions of ethanol/water as solvents at 10 MPa and 40 °C. In a first cycle, the use of 10:90 and 1:99 (v/v) ethanol/water was evaluated, while 50:50 (v/v) ethanol/water was used in a second and a third extractive cycle. Solvent was completely flushed out of the cell after the preselected static period of each cycle, drawing the extract into the collector vial. The effect of static PLE time (5, 10, 20, and 30 min) and the extraction temperature (40 and 60 °C) was also evaluated during the optimization process. Unless otherwise specified, extractions were carried out in duplicate.

Carbohydrate Analysis. *Derivatization Procedure.* Before analysis, carbohydrates were converted into their trimethylsilyl (TMS) oximes following a two-step method described by Sanz et al. (*22*). Briefly, 1.3 mL of the target extracts was mixed with 0.3 mL of a 70% ethanolic solution of phenyl-β-D-glucoside (1 mg/mL), which was employed as an internal standard. After drying the samples under vacuum, 350 μL of 2.5% solution of hydroxylamine chloride (Sigma) in pyridine (Merck, Madrid, Spain) was added. Samples were heated for 30 min at 75 °C, and then, 350 μL of hexamethyldisilazane and 35 μL of trifluoroacetic acid were added, and the mixture was kept at 45 °C for 30 min. After

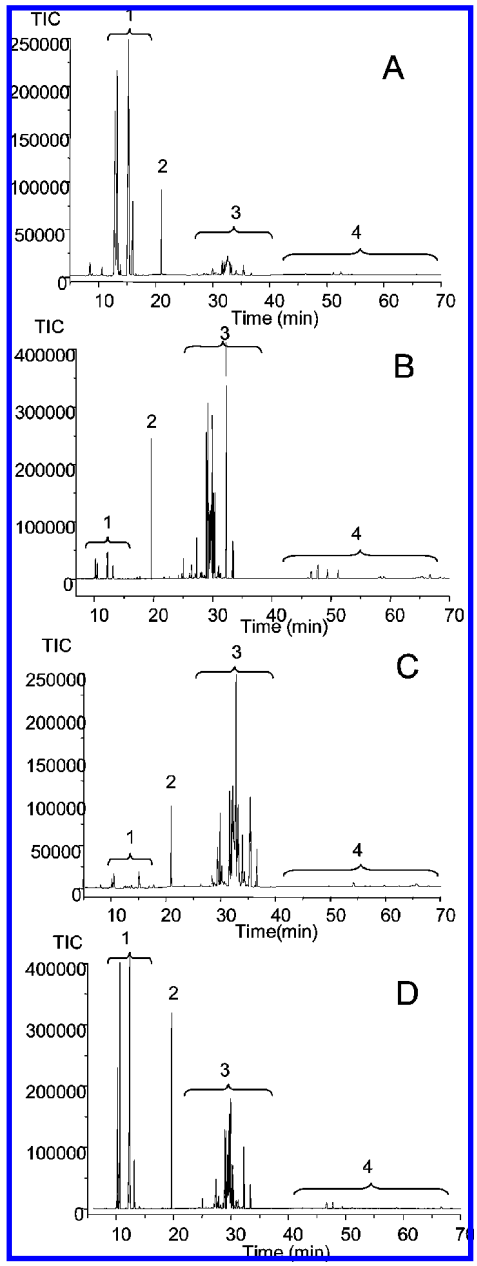

Figure 1. Gas chromatographic profile of (**A**) an untreated citrus honey and the same nectar honey after (**B**) PLE, (**C**) yeast, and (**D**) activated charcoal treatments. (1) Monosaccharides, (2) internal standard, (3) disaccharides, and (4) trisaccharides.

this reaction time, samples were centrifuged at 7000*g* for 5 min at 5 °C, and 1 μL of the supernatant was injected onto the GC column.

Qualitative Analysis. Qualitative analysis of carbohydrates was carried out by gas chromatography mass spectrometry (GC-MS) using a Hewlett-Packard 6890 gas chromatograph coupled to a 5971 quadrupole mass detector operating in electronic impact (EI) mode at 70 eV (both from Hewlett-Packard, Palo Alto, CA, USA). A 25 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, USA) was used. The 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 and interface temperatures were 300 and 270 °C, respectively. Injections were made in the split mode, with a split ratio of 1:40 and the carrier gas was helium at 1 mL min^{-1}. Acquisition was done using a HPChem Station software (Hewlett-Packard, Palo Alto, CA, USA).

Quantitative Analysis. Quantitative analysis of samples derivatized as their TMS oximes was carried out by GC with a FID detector (HP 5890, Palo Alto, CA, USA). Operating conditions other than the carrier gas (nitrogen) were identical to those previously described for GC-MS analysis. Carbohydrate quantitative data were obtained from FID peak areas using the method described by de la Fuente et al. (*23*). Standard solutions of carbohydrates over the expected concentration range in honey (from 1 to 500 mg of monosaccharides/g honey and from 0.1 to 30 mg of di- and trisaccharides/g honey) were prepared to calculate the response factor (RF) relative to phenyl-β-D-glucoside (internal standard). Response was linear in the studied range, and the relative standard deviation of the GC method was in all instances lower

Table 2. Comparison of Carbohydrate Amounts (n = 3) Obtained from Citrus Honey after PLE with Adsorbent Bed of Activated Charcoal, Charcoal Extraction, and Yeast Treatment[a]

	mg of carbohydrate/g honey (% of individual carbohydrate with respect to the total carbohydrate group)			
carbohydrates	original	PLE	charcoal	yeast
fructose	331.2(55.9)	14.1(44.3)	49.3(48.2)	2.9(58.0)
glucose	261.8(44.1)	17.7(55.7)	53.0(51.8)	2.1(42.0)
total monosaccharides	**593.0**	**31.8**	**102.3**	**5.0**
sucrose	1.8(1.1)	0.6(0.5)	0.6(0.6)	0.0
α,α-trehalose	0.5(0.3)	0.9(0.7)	0.6(0.6)	2.3(3.3)
α,β-trehalose	7.1(4.3)	4.5(3.7)	4.2(4.0)	4.1(5.9)
cellobiose	2.2(1.3)	1.2(1.0)	1.4(1.3)	0.2(0.3)
laminaribiose	3.3(2.0)	3.1(2.6)	2.1(2.0)	2.1(3.0)
maltulose	23.6(14.4)	22.2(18.4)	19.4(18.4)	9.9(14.2)
nigerose	18(11.0)	13.7(11.4)	10.7(10.1)	8.6(12.3)
turanose	28.7(17.5)	21.5(17.8)	21(19.9)	6.5(9.3)
maltose	19.5(11.9)	10.4(8.6)	7.9(7.5)	2.4(3.4)
kojibiose	27.8(17.0)	16.8(13.9)	13.6(12.9)	14.3(20.5)
trehalulose	11.3(6.9)	9.4(7.8)	8.9(8.4)	6.5(9.3)
palatinose	3.3(2.0)	1.9(1.6)	2.3(2.2)	2.0(2.9)
gentiobiose	0.2(0.1)	1.3(1.1)	0.6(0.6)	0.3(0.4)
isomaltose	16.2(9.9)	13(10.8)	12.1(11.5)	10.6(15.2)
melibiose	0.2(0.1)	0.0	0.2(0.2)	0.1(0.1)
total disaccharides	**163.7**	**120.5**	**105.6**	**69.9**
raffinose	0.1(0.6)	0.0	0.0	0.0
1-kestose	4.1(24.4)	3.0(22.7)	1.8(17.6)	0.0
erlose	5.0(29.8)	2.7(20.5)	2(19.6)	0.0
melezitose	0.9(5.4)	0.8(6.1)	0.6(5.9)	1.0(13.3)
theanderose	0.6(3.6)	0.6(4.5)	0.4(3.9)	0.2(2.7)
unknown trisaccharide	0.6(3.6)	0.6(4.5)	0.6(5.9)	0.5(6.7)
maltotriose	1.5(8.9)	1.2(9.1)	1.3(12.7)	0.9(12.0)
unknown trisaccharide	1.5(8.9)	1.7(12.9)	1.5(14.7)	1.8(24.0)
panose	2.5(14.9)	2.6(19.7)	2(19.6)	3.1(41.3)
total trisaccharides	**16.8**	**13.2**	**10.2**	**7.5**
total carbohydrates	**773.5**	**165.5**	**218.1**	**82.4**

[a] Percentages of the individual compounds once normalized against the total mono-, di-, and trisaccharides are shown within parentheses.

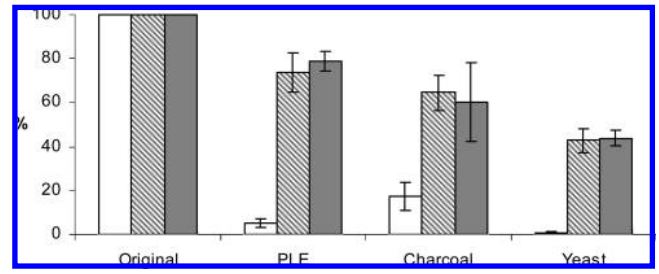

Figure 2. Recovery (% of the original content) of mono- (white), di- (hatched), and trisaccharides (solid) from citrus honey after PLE with adsorbent bed of activated charcoal, charcoal extraction, and yeast treatment (n = 3).

than 5%. Detection limits (LOD) were 0.03 mg/g honey for di- and trisaccharides.

RESULTS AND DISCUSSION

In order to optimize the PLE fractionation procedure, different assays using honeydew honey were performed varying (i) the composition of the solvent (ethanol/water proportions), (ii) the number of static extraction cycles, (iii) the duration of each cycle, and (iv) the extraction temperature. Recovered concentrations of mono-, di-, and trisaccharides in each PLE assay are summarized in **Table 1** and compared with those values found by GC-FID analysis for the same untreated honey.

The first aspect considered was the influence of the nature of the extraction solvents on PLE efficiency. An initial experiment was carried out using ethanol/water 10:90 (v/v) in the first PLE cycle and ethanol/water 50:50 (v/v) in the second and third cycles. Solvent mixtures were selected on the basis of the existing data on carbohydrate solubilities (*20, 24*) and also on the behavior adsorption of carbohydrates on charcoal (*11–14*). As the table shows, the fraction obtained in the second cycle of assay 1 was enriched in di- and trisaccharides, the latter representing 70% of the total carbohydrates obtained by PLE of the sample. However, the real amounts recovered from original honey were very low, accounting for only 2% of disaccharides and 33% of trisaccharides. These ethanol/water proportions were proposed by Morales et al. (*14*) to obtain fractions enriched on oligosaccharides with a degree of polymerization higher than 3 and remove most of the disaccharides from the sample during extraction. The composition of the first cycle solvent was therefore modified in the present study. By using a 1:99 (v/v) mixture in the first cycle followed by the 50:50 (v:v) mixture in the second cycle (assay 2), it was possible to obtain a fraction rich in disaccharides, which represented the 74% of total carbohydrates. However, in this assay most of the carbohydrates were obtained in the first PLE cycle, and the separation was not effective.

Second, in order to evaluate the number of static cycles, a third cycle was performed also using 50:50 (v/v) ethanol/water, in which only very small amounts of oligosaccharides were recovered (assay 2).

Third, different extraction times were assayed to reduce the amounts of di- and trisaccharides extracted in the first PLE cycle (data not shown), 5 min being the time eventually selected for this purpose. The rest of the experimental conditions were the same as those in assay 2. As can be observed in assay 3, 5 min of static extraction with ethanol/water 50:50 (v/v) in the second cycle was not enough to recover all of the oligosaccharides in the original sample, and two more PLE cycles were necessary to achieve this aim (recovered percentages of di- and trisaccharides in the third and fourth PLE cycles of 19 and 24%, and 2 and 2%, respectively). Therefore, the extraction time in the second cycle was also optimized (assays 4−6); the optimum static extraction time selected for the second PLE cycle was 10 min.

Finally, the influence of the extraction temperature (40 and 60 °C) on PLE efficiency was also evaluated under these experimental conditions (assays 4 and 6). The results at 40 and 60 °C were fairly similar; therefore, it was concluded that, in the investigated range, temperature did not affect carbohydrate extraction, and the lower temperature was used in further experiments.

The experimental conditions corresponding to assay 4, first cycle 5 min static PLE with ethanol/water 1:99 (v/v), followed by a second cycle 10 min static PLE with ethanol/water 50:50 (v/v), both at 40 °C and 10 MPa, were chosen for subsequent experiments.

To evaluate the reproducibility of the PLE method with in-cell fractionation as developed, the honeydew honey was subjected to five separate PLE (assay 4) plus GC-MS analyses. Relative standard deviations (RSDs) were around 10% for the studied di- and trisaccharides ($n = 5$), which is similar to those reported for the fractionation of honey carbohydrates using yeast (*7*).

To confirm the effectiveness of the enrichment procedure using the combination of PLE and activated charcoal, this methodology was also applied to the analysis of a citrus honey. This type of sample was selected because different authors have reported (*25, 26*) that nectar honey normally contains relatively less di- and trisaccharides than honeydew honey.

Figure 1 shows the chromatographic profiles obtained by GC-MS of the TMS oxime carbohydrates of citrus honey before (**A**) and after (**B**) PLE treatment. Monosaccharide concentration was noticeably reduced after the PLE-fractionation procedure, and the resulting fraction was clearly richer in di- and trisaccharides. **Table 2** shows the changes of mono-, di-, and trisaccharide concentrations after PLE treatment for citrus honey. Although absolute concentrations of di- and trisaccharides after the fractionation process decreased, their relative proportions were higher than those calculated for the untreated honey. Monosaccharides were reduced from 77% to 19% of total carbohydrates. Disaccharides were enriched up to 73% of total carbohydrates, whereas the trisaccharide fraction accounted for 8% of the total carbohydrates.

A comparison with other fractionation procedures (yeast treatment (*7*) and activated charcoal extraction (*14*)) was also performed (**Figure 1C** and **D**, respectively). As indicated in the Materials and Methods section, the percentages of ethanol/water used in the extraction of carbohydrates from activated charcoal were modified from those used by Morales et al. (*14*) for the purpose of comparison between these results and those obtained with the PLE-fractionation procedure proposed here.

Table 2 shows the concentrations (mg/g) of mono-, di-, and trisaccharides calculated for the citrus honey with the three extraction procedures assayed and their proportions with respect to the total carbohydrate group in each sample. As can be observed, all three techniques (PLE, yeast, and activated charcoal) were useful for selective removal of monosaccharides from honey, yeast treatment being the most effective (from 593 mg/g to 5 mg/g). However, as **Figure 2** shows, the recovery of di- and trisaccharides was higher when the PLE procedure was used (74% and 79%, respectively). Intermediate results were obtained with the activated charcoal fractionation (65% and 60%, respectively).

After yeast treatment, sucrose and its derivatives kestose and erlose were completely removed, and there was a selective reduction of many disaccharides and trisaccharides, while the relative proportions of carbohydrates such as α,α-trehalose, isomaltose, and panose increased (**Table 2**). In contrast to yeast treatment, the PLE and activated charcoal methods produced only slight modifications in the relative proportions of di- and trisaccharides of honey, with the highest total values being obtained with the PLE procedure. As expected, the behavior of carbohydrates during PLE and activated charcoal treatments was quite similar. However, PLE allows for more speed and automation of processes, minimizes the handling of the sample, and involves smaller volumes of solvents.

In conclusion, the proposed method using PLE with in-cell adsorbent bed of charcoal for carbohydrate fractionation of honey is feasible, rapid, and efficient. Compared with other methods (i.e., yeast treatment and activated charcoal extraction),

it presents several advantages, such as reduced extraction time, smaller volumes of solvent, and higher recovery of di- and trisaccharides while preserving their profile. Further experiments would be helpful to evaluate the utility of this procedure for the fractionation of carbohydrates from other sources.

LITERATURE CITED

(1) Blasa, M.; Candiracci, M.; Accorsi, A.; Placentini, M. P.; Piatti, E. Honey flavonoids as protection agents against oxidative damage to human red blood cells. *Food Chem*. **2007**, *104*, 1635–1640.

(2) Basualdo, C.; Sgroy, V.; Finola, M. S.; Marioli, J. M. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet. Microb*. **2007**, *124*, 375–381.

(3) Shin, H. S.; Ustunol, Z. Carbohydrate composition and content of honey from different floral sources and their influence on growth of intestinal bacteria: An in vitro comparison. *Food Res. Int*. **2005**, *38*, 721–728.

(4) Sanz, M. L.; Polemis, N.; Morales, V.; Corzo, N.; Drakoularakou, A.; Gibson, G.; Rastall, A. An in vitro investigation into the potential prebiotic activity of honey oligosaccharides. *J. Agric. Food Chem*. **2005**, *53*, 2914–2921.

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

(6) Cotte, J. F.; Casabianca, H.; Chardon, S.; Lheritier, J.; Grenier-Loustalot, M. F. Application of carbohydrate analysis to verify honey authenticity. *J. Chromatogr., A* **2003**, *1021*, 145–155.

(7) 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.

(8) 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.

(9) Swallow, K. W.; Low, N. H. Determination of honey authenticity by anion-exchange liquid chromatography. *J. AOAC Int*. **1994**, *77*, 695–702.

(10) White, J. W., Jr. Composition of Honey. In Honey: A Comprehensive Survey; Crane, E., Ed.; Heinemann: London, 1975; pp157−206.

(11) Whistle, R. L.; Durso, D. F. Chromatographic separation of sugars on charcoal. *J. Am. Chem. Soc*. **1950**, *72*, 677–679.

(12) Swallow, K. W.; Low, N. H. Analysis and quantitation of the carbohydrates in honey using high-performance liquid-chromatography. *J. Agric. Food Chem*. **1990**, *38*, 1828–1832.

(13) Low, N. H.; South, W. Determination of honey authenticity by capillary gas chromatography. *J. AOAC Int*. **1995**, *78*, 1210–1218.

(14) Morales, V.; Sanz, M. L.; Olano, A.; Corzo, N. Rapid separation on activated charcoal of high oligosaccharides in honey. *Chromatographia* **2006**, *64*, 233–238.

(15) Korta, E.; Bakkali, A.; Berrueta, L. A.; Gallo, B.; Vicente, F. Study of an accelerated solvent extraction procedure for the determination of acaricide residues in honey by high-performance liquid chromatography-diode array detector. *J. Food Protect*. **2002**, *65*, 161–166.

(16) Ramos, J. J.; Dietz, C.; González, M. J.; Ramos, L. Miniaturised selective pressurised liquid extraction of polychlorinated biphenyls from foodstuffs. *J. Chromatogr., A* **2007**, *1152*, 254–261.

(17) Herrero, M.; Simó, C.; Ibáñez, E.; Cifuentes, A. Capillary electrophoresis-mass spectrometry of *Spirulina platensis* proteins obtained by pressurized liquid extraction. *Electrophoresis* **2005**, *26*, 4215–4242.

(18) Herrero, M.; Cifuentes, A.; Ibañez, E. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae. A review. *Food Chem*. **2006**, *98*, 136–148.

(19) Basile, A.; Jiménez-Carmona, M. M.; Clifford, A. A. Extraction of rosemary by superheated water. *J. Agric. Food Chem*. **1998**, *46*, 5205–5209.

(20) Ruiz-Matute, A. I.; Sanz, M. L.; Corzo, N.; Martín-Alvarez, P. J.; Ibañez, E.; Martínez-Castro, I.; Olano, A. Purification of lactulose from mixtures with lactose using pressurized liquid extraction with ethanol-water at different temperatures. *J. Agric. Food Chem*. **2007**, *55*, 3346–3350.

(21) Ramos, J. J.; Dietz, C. H.; Camara, C.; Ramos, L. Dispositivo miniaturizado de extraction con líquidos a presión. Universidad Complutense de Madrid-CSIC. Patent PTC-ES 2006/000311, 2006.

(22) Sanz, M. L.; Sanz, J.; Martínez-Castro, I. Gas chromatographic-mass spectrometric method for the qualitative and quantitative determination of disaccharides and trisaccharides in honey. *J. Chromatogr., A* **2004**, *1059*, 143–148.

(23) de la Fuente, E.; Sanz, M. L.; Martinez-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.

(24) Montañes, F.; Olano, A.; Ibáñez, E.; Fornari, T. Modeling solubilities of sugars in alcohols based on original experimental data. *Thermodynamics* **2007**, *53* (9), 2411–2418.

(25) Siddiqui, I. R. The sugars of honey. *Adv. Carbohydr. Chem. Biochem*. **1970**, *25*, 285–309.

(26) Sanz, M. L.; González, M.; de Lorenzo, C.; Sanz, J.; Martínez-Castro, I. A contribution to the differentiation between nectar honey and honeydew honey. *Food Chem*. **2005**, *91*, 313–317.

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