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Determination of the Total Content of Calcium and Magnesium and Their Bioavailability in Ripened Bee Honeys

Iwona Sergiel[†] and Pawel Pohl*[,‡]

[†]Department of Biotechnology, Faculty of Biological Sciences, University of Zielona Gora, Prof. Z. Szafrana 1, 65-516 Zielona Gora, Poland, and [‡]Department of Analytical Chemistry, Faculty of Chemistry, Wroclaw University of Technology, Smoluchowskiego 23, 50-372 Wroclaw, Poland

An analytical scheme for the fractionation and the determination of Ca and Mg in different bee honeys is presented. Using tandem column solid phase extraction based on a nonionic macroreticular adsorbing resin Amberlite XAD-16 and a gel-type strong cation exchange resin Dowex 50W×8-200, three different groups of the Ca and Mg species were separated, including the hydrophobic, the cationic, and the residual species fractions. The concentrations of Ca and Mg were measured by means of flame atomic absorption spectrometry without any special chemical preparation of the analyzed samples. When regarding the total concentrations of Ca and Mg in the analyzed ripened floral and honeydew honeys, it was assessed that the most abundant group of the metal species was the cationic fraction, contributing for some honeys up to 96.3 and 97.0% of Ca and Mg, respectively. A relatively significant contribution of the hydrophobic fraction was also found, i.e., up to 20.1% for Ca and 23.0% for Mg.

KEYWORDS: Calcium; magnesium; fractionation analysis; flame atomic absorption spectrometry; food analysis

INTRODUCTION

The speciation of metals in food products is a subject of increasing scientific interest since the metals complexed by different classes of bioligands may differentially reduce their bioavailability and change the toxicity (*1*). In fact, that type of analysis enables a much better estimation of the nutritional value of the food products as compared to the total analysis (*2*). It is especially important in the case of Ca and Mg that are the gold standards when discussing supplements, mineral ratios, paired cell receptors, or many other nutrition-related health issues and are present in relatively high amounts in almost all food products (*3*, *4*).

Unfortunately, in the overwhelming majority of the contributions devoted to the analysis of food products, including honey, usually, the total concentrations of Ca and Mg are determined (*5*−*7*), which may not directly correspond to the availability of these metals (*8*). However, a detailed speciation analysis of Ca or Mg, aiming at the exact definition of their molecular forms and the structure of bioligands complexing these metal ions, seems to be rather impractical and even impossible (*9*). A better approach would be to identify the fractions of the Ca and Mg species existing in the separate groupings and differing from each other by the physical and/or chemical properties, and next to determine the sum of their concentrations in the respective operationally distinguished and defined classes of the chemical forms.

Unfortunately, there are a limited number of studies concerning the speciation analysis of metals in honey. The fractionation analysis of Ca and Mg has been reported earlier (*10*), but it was established that these metals are almost completely present in the form of the cationic species, likely to be the simple ions of Ca and Mg and/or their stable complexes with the low molecular mass organic species. However, such an operational fractionation pattern could be a consequence of the sorbents used, that is, the ion exchangers only. Nevertheless, more and more, there is experimental evidence that Ca and Mg can be complexed by the phenolic and polyphenolic compounds (*11*, *12*). Hence, it would be interesting to verify whether Ca and Mg are bound by this class of bioligands in such natural food products as honey, containing relatively high amounts of the phenolics as well as Ca and Mg.

The objective of the present work was to investigate the possibility of flame atomic absorption spectrometry (FAAS) for the rapid analysis of honeys on the content of Ca and Mg by direct measurements of the solutions prepared by dissolving the samples of honey only in water. In addition, the suitability of a tandem column solid phase extraction (SPE) procedure, developed in our laboratory for the fractionation of Cu, Fe, and Mn in beer (*13*), to provide information on the extent of organic complexation of Ca and Mg and their bioavailability in honey was examined. In the adapted approach to the chemical fractionation, a nonionic macroreticular adsorbing resin Amberlite XAD-16 coupled to a gel strong cation exchange resin Dowex 50W×8-200 was used to discriminate and determine the Ca and Mg species among three operationally defined groupings differing in hydrophobicity and charge.

MATERIALS AND METHODS

Chemicals. All reagents used in the experiments were of analytical grade. Thirty-seven percent (m/v) HCl and 65% (m/v) HNO_3 were

*Corresponding author. Tel: +48-71-320-3445. Fax: +48-71-320-2494. E-mail: pawel.pohl@pwr.wroc.pl.

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Table 1. Operating Conditions Used for the Determination of Ca and Mg in Honeys by Means of FAAS

	Ca	Mg
fuel flow rate (L min^{-1})	1.5	1.5
oxidant flow rate (L min^{-1})	8.0	8.0
wavelength (nm)	422.7	285.2
slit width (nm)	0.7	0.7
lamp current (mA)	10	6
linearity range (mg L^{-1})	0.1−5.0	0.02−1.0
detection limit (mg L^{-1})	0.002	0.0005
precision[a](% (n = 3))	0.6−5.3	0.4−6.4

[a] As RSD.

purchased from J. T. Baker (USA). Other chemicals were supplied by POCH (Poland). Ultrapure water obtained with a PRO-11G (WIGO, Poland) reverse osmosis water purification system was used throughout. Single-element stock standard solutions of 1000 mg L^{-1} of Ca and Mg (Merck, Germany), and glucose (POCH, Poland) were used to prepare the working standard solutions (50 mL) containing 2.00 mg L^{-1} of Ca, 0.50 mg L^{-1} of Mg, and 40.0 g L^{-1} of glucose that corresponds to an average content of these constituents in a 5.0% (m/v) solution of honey. In a similar way, the working standard solutions with tartaric acid (500 mg L^{-1}) added to complex Ca and Mg were also prepared. The resulting working standard solutions were adjusted to pH 3.5, 4.0, 4.5, and 5.0 using 2.0 mL of the suitable 0.10 mol L^{-1} acetic acid−sodium acetate buffering solutions. These solutions were applied to characterizing the sorption properties of the polymeric sorbents used in the tandem column SPE fractionation assembly.

Apparatus. The concentrations of Ca and Mg in all solutions were determined using a Perkin-Elmer (Germany) atomic absorption spectrometer, model 1100B. It was equipped with a 10 cm single slot burner head for the C_2H_2-air combustion flame. A sampling unit integrated with the burner comprised a standard mixing chamber, a flow spoiler, and an end-cap with a drain assembly. The solutions were continuously aspirated through a stainless steel nebulizer. The operating conditions used for the measurements are given in **Table 1**. All sample solutions were analyzed against the external standard solutions of Ca and Mg.

The Supelco (USA) glass columns (10 mm ID) with coarse frits and Teflon stopcocks were applied to solid phase extraction (SPE). A Cole-Parmer (USA) 4-channel MasterFlex L/S peristaltic pump was employed to control the flow rates of the solutions passed through the columns.

Preparation of Resins. The Amberlite XAD-16 resin (Sigma-Aldrich, Germany) was dried in an oven at 110 °C for 2 h prior to use. Its 1.2 g portions were wetted with methanol and poured into the columns. The resin beds formed were washed with 20 mL of water, followed by 10 mL of a 1.0 mol L^{-1} HCl solution, and again with 20 mL of water. The 1.2 g portions of the Dowex 50W×8-200 resin (Sigma-Aldrich, Germany) were wetted with water and poured into the columns. The resin beds formed were washed with 10 mL of a 1.0 mol L^{-1} HCl solution, followed by 20 mL of water. Next, 10 mL of a 1.0 mol L^{-1} NaOH solution was passed through the columns. To remove the excess NaOH, the resin beds were rinsed with 20 mL of water. Water and the conditioning solutions were passed through the columns at a flow rate of 1.0 mL min^{-1}.

Total Analysis. Thirteen ripened honeys, including acacia (A), buckwheat (B), multiflower (F1, F2), goldenrod (G1, G2), goldenrod-buckwheat (GB), heather (H1, H2), honeydew (HD), linden (L), and rape (R1, R2), were obatined for the analysis. The samples of honeys were supplied by two beekeepers, whose apiaries are located in the suburbs of Wroclaw, the Lower Silesian region, Poland. The samples were stored in precleaned plastic containers in a dark place. Before sampling, they were mixed using glass stirring rods.

To directly analyze the honeys, their samples were dissolved in water to obtain 5.0% (m/v) sample solutions. Accordingly, 2.5 g portions of honeys, weighed into 100 mL beakers, were dissolved first in about 10 mL of water. Next, the fluids achieved were transferred to 50 mL volumetric flasks and made to the volume with water. The samples of honeys were digested as well in the open vessel system using 65% (m/v) HNO_3 and 30% (m/v) H_2O_2 solutions as the oxidizing reagents. For that purpose, 2.5 g portions of honeys, weighed into 250 mL beakers, were dissolved in 10 mL of a diluted (1 + 1) HNO_3 solution. Next, the beakers with the sample fluids achieved were covered with cover glasses and placed on a hot plate. After 3 h of refluxing at 90−110 °C, the cover glasses were removed, and the liquids were evaporated almost to dryness. After cooling, 10 mL of a 30% (m/v) H_2O_2 solution was added to the residues, and the resulting liquids were again evaporated to near dryness. Finally, the remains were redissolved with water, transferred to 50 mL volumetric flasks, and diluted with water to the desired volume.

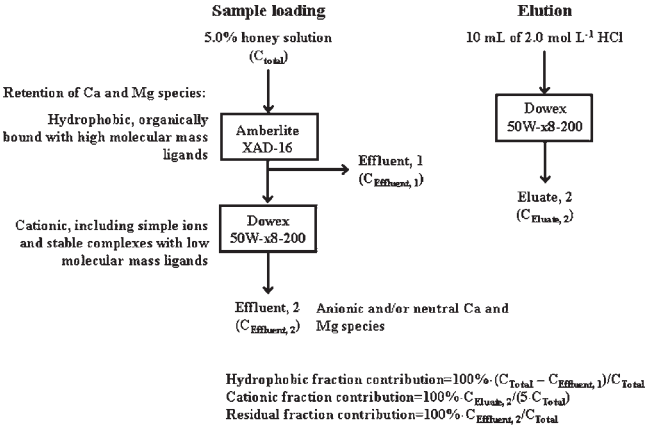

Figure 1. Scheme of the fractionation procedure based on solid phase extraction with Amberlite XAD-16 and Dowex 50W×8-200 used for partitioning Ca and Mg in honeys.

The total concentrations of Ca and Mg in all resulting sample solutions were determined using FAAS against the external standard solutions and suitable reagent blanks.

Fractionation Analysis. To fractionate Ca and Mg in honeys, 50 mL of their 5.0% (m/v) solutions was passed at a flow rate of 1.0 mL min^{-1} through the first column packed with the Amberlite XAD-16 resin. The effluents of this column were immediately passed through the second column with the Dowex 50W×8-200 resin at a flow rate of 1.0 mL min^{-1} as well. When 40 mL of the sample solutions was driven through the linked columns, 5.0 mL portions of their effluents were collected to assess the concentrations of Ca and Mg not retained by the adsorbent (first column, $C_{Effluent, 1}$) and determine the content of Ca and Mg not retained by both the columns used (second column, $C_{Effluent, 2}$). Finally, the columns were disconnected, and the metal species retained by the second column were recovered using 10 mL of a 2.0 mol L^{-1} HCl solution at a flow rate of 1.0 mL min^{-1}. The respective 10-mL eluates were collected and analyzed for the content of Ca and Mg sorbed by the Dowex 50W×8-200 resin ($C_{Eluate, 2}$). The concentrations of Ca and Mg in all effluents and eluates were measured against the suitable external standard solutions and the procedural blanks. With this procedure (a scheme of the manifold is shown in **Figure 1**) three operationally defined fractions of Ca and Mg species were separated.

RESULTS AND DISCUSSION

Determination of Ca and Mg Content. A possibility of the direct determination of Ca and Mg in honeys by analyzing the sample solutions resulting from the simple dissolution of certain portions of honey in water against the calibration with the external standard solutions was investigated to find a rapid method of analysis, an alternative to those based on dry or wet oxidative decompositions.

Effect of Honey Matrix. For that reason, at the outset of this work, the effect of the presence of the honey matrix in solutions on the analytical performance of FAAS was examined. Accordingly, the 1.0, 2.5, 5.0, and 10.0% (m/v) solutions of multiflower honey (F1) were prepared and directly analyzed by FAAS for the concentrations of Ca and Mg using the external standard solutions. It was found that the difference between the results obtained for both metals were statistically insignificant (see **Table 2**). Using the t-test under the assumption of the 95% confidence

level, the concentrations of Ca and Mg achieved measuring the 5.0% (m/v) honey solutions appeared to match those obtained when the 1.0, 2.5, and 10.0% (m/v) honey solutions were analyzed. This implies that the effect of the honey matrix in the studied range is not profound. It was also assessed that the acidification of the sample solutions, as previously reported in the case of ICP-OES measurements (*14*), was not necessary and could be avoided. Finally, it was established using the *t*-test ($p = 0.05$, $k = 4$) that the results obtained with the proposed method (the sample dissolution in water only) were similar to those obtained after the initial digestion of the honey samples and the subsequent measurements of the resulting digests. Considering, however, that the analysis of the sample solutions containing honey dissolved at levels higher than 5.0% (m/v) was suggested to be avoided in the case of FAAS (*5*) due to a possible clogging of the nebulizer and an appearance of carbonaceous residues in the burner, it was decided here that the 5.0% (m/v) solutions of the studied honeys would be prepared for their direct analysis by FAAS for the content of Ca and Mg.

Total Concentrations of Ca and Mg in Honeys. The results of the determination of the Ca and Mg concentrations obtained with this rapid method of analysis are given in **Table 3**. They indicate that the content of Ca (24.89−115.6 μg g^{-1}) and Mg (10.34−190.0 μg g^{-1}) in the analyzed honeys is relatively high but locates within the range of the concentrations previously found in other floral and honeydew honeys collected in Poland (*15*) or other European countries (*16*−*20*).

The precision of the proposed method, expressed as the RSD from 3 independent determinations, was found to change from 0.3% (goldenrod honey, G1) to 4.1% (heather honey, H2) in the case of Ca and from 0.4% (honeydew, HD) to 6.4% (goldenrod honey, G2) in the case of Mg. Because of the lack of a relevant honey-based standard reference material, the recovery test was performed to verify the accuracy of the method. The 5.0% (m/v) solutions of previously selected honey (multiflower, F1) were spiked with 100 mg L^{-1} standard solutions of Ca and Mg at such amounts that the their concentrations doubled the original concentrations of Ca and Mg found in the 5.0% (m/v) solutions of multiflower honey, F1. The recoveries of the added Ca and Mg determined afterward ($n = 3$) were 101 ± 3% and 99.0 ± 1.0%, respectively. In the same way, the recovery test was carried out for the less concentrated goldenrod honey, G2. Accordingly, the recoveries obtained for Ca and Mg were 98.6 ± 2.2% and 100 ± 2%, respectively. Both the adequate precision and the accuracy evaluated by the recovery test proved the dependability of the described method of the direct analysis of honey. As compared to the methods in which honey is decomposed, this method has the advantage of being very simple and rapid; it can be successfully used for routine determinations.

Determination of Fractionation Forms of Ca and Mg. So far, only single-column SPE with the strongly acidic cation and basic anion exchangers has been reported for the partitioning of metals in honey. It resulted in distinguishing the operationally defined fractions of the cationic and the anionic species of Ca, Fe, Mg, and Zn (*10*, *21*). To elucidate a possible association of Ca and Mg with the high molecular mass ligands naturally present in honey, the present study was attempted to operate the fractionation procedure in which the nonionic and hydrophobic adsorbing resin Amberlite XAD-16 would be applied to retain such chemical forms of Ca and Mg.

Sorption Behavior of Amberlite XAD-16 and Dowex 50W×8-200. To investigate whether the simple ions of Ca and Mg are sorbed by the adsorbing resin, the working standard solutions of the simple Ca and Mg ions were passed through the columns with the Amberlite XAD-16 resin, while the respective effluents were collected and analyzed to determine the amounts of Ca and Mg retained by the resin. As can be seen from **Table 4**, the simple Ca and Mg ions were not sorbed at pH 3.5−5.0 by Amberlite XAD-16, although this kind of adsorbing resin was previously described to retain the simple ions of other

Table 2. Total Concentrations of Ca and Mg Determined in Multiflower Honey (F1) after the Analysis of the Solutions Containing Different Sample Amounts Dissolved in Water and the Values for the *t*-Test Obtained Comparing the Metal Concentrations Found for the 5.0% (m/v) Solutions to Those Determined in Other Solutions[a]

honey content in solution (%) (m/v)	Ca		Mg	
	concentration (μg g^{-1})	$t_{computed}$	concentration (μg g^{-1})	$t_{computed}$
1.0	33.5 ± 0.5	−1.35	11.7 ± 0.2	+0.77
2.5	33.1 ± 0.6	−2.16	11.9 ± 0.2	+2.32
5.0	34.0 ± 0.4		11.6 ± 0.1	
5.0[b]	33.6 ± 0.3	−1.39	11.8 ± 0.3	+1.10
5.0[c]	33.4 ± 0.7	−1.29	11.8 ± 0.5	+0.68
10.0	33.3 ± 0.3	−2.42	11.5 ± 0.3	−0.55

[a] The average values ($n = 3$) ± SD. The critical value of |*t*| for 4 degrees of freedom and at 95% level of confidence is 2.78. [b] Dissolution with water and acidification with HNO_3 to the concentration of 0.70 mol L^{-1}. [c] Wet oxidative digestion with 65% (m/v) HNO_3 and 30% (m/v) H_2O_2.

Table 3. Total Concentrations of Ca and Mg (in μg g^{-1}) in the Analyzed Honeys after Their Dissolution in Water (I) and Wet Oxidative Digestion with 65% (m/v) HNO_3 and 30% (m/v) H_2O_2 (II)[a]

honey	Ca			Mg		
	I	II	$t_{computed}$	I	II	$t_{computed}$
A	24.89 ± 0.54	25.55 ± 1.39	−0.76	15.21 ± 0.10	15.13 ± 0.72	+0.19
B	28.35 ± 0.25	29.47 ± 1.86	−1.03	11.20 ± 0.05	11.58 ± 0.23	−2.80
F1	73.16 ± 0.28	75.83 ± 1.30	+3.48	34.79 ± 0.36	34.45 ± 0.82	+0.65
F2	56.84 ± 1.72	55.56 ± 2.78	+0.67	52.86 ± 0.41	54.00 ± 1.09	−1.69
G1	29.35 ± 0.10	29.60 ± 0.15	−2.40	10.34 ± 0.11	10.65 ± 0.80	−0.66
G2	34.54 ± 0.57	34.45 ± 0.68	+0.17	10.55 ± 0.68	11.19 ± 0.15	−1.59
GB	46.34 ± 0.50	45.15 ± 1.29	+1.49	17.45 ± 0.34	17.92 ± 0.34	−1.69
H1	47.86 ± 0.98	47.59 ± 1.79	+0.22	21.98 ± 0.21	21.81 ± 0.45	+0.59
H2	115.6 ± 4.7	116.8 ± 3.8	−0.34	190.0 ± 1.3	194.2 ± 3.2	−2.11
HD	25.26 ± 0.17	25.63 ± 0.53	−1.15	31.30 ± 0.11	31.04 ± 0.58	+0.76
L	43.20 ± 0.19	43.43 ± 1.19	−0.33	11.66 ± 0.10	11.84 ± 0.23	−1.25
R1	42.60 ± 1.60	39.70 ± 2.01	−1.96	12.14 ± 0.50	12.05 ± 0.10	+0.31
R2	33.32 ± 0.22	33.49 ± 0.48	−0.56	15.83 ± 0.26	16.07 ± 0.34	−0.97

[a] The average values ($n = 3$) ± SD. The critical value of |*t*| for 4 degrees of freedom and at 95% level of confidence is 2.78.

metals (*22*−*24*). It was also established that Amberlite XAD-16 does not sorb Ca and Mg when passing the working standard solutions (pH 3.5−5.0) containing tartaric acid; the concentrations determined in the column effluents were the same as those in the loaded solutions (see **Table 4**).

When driving the working standard solutions of the simple Ca and Mg ions through the columns with Dowex 50W×8-200 and subsequently analyzing the collected effluents for the presence of Ca and Mg, it was assessed that these metals were completely retained by the resin from the solutions; the concentrations of Ca and Mg found in the respective effluents were below the detection limits evaluated for FAAS, i.e., 0.002 and 0.0005 mg L^{-1} in the case of Ca and Mg, respectively. When passing the working standard solutions of Ca and Mg (pH 3.5−5.0) with added tartaric acid, it was found that the retention of both metals, mostly in the form of their simple ions and the positively charged tartrate complexes, i.e., $CaHTA^+$ and $MgHTA^+$ (*25*), was also high. The respective retention efficiencies determined were in the range from 77.0% (pH 5.0) to 92.5% (pH 3.5) for Ca and from 90.0% (pH 5.0) to 100% (pH 3.5) in the case of Mg.

Finally, it was established that Ca and Mg could be quantitatively eluted from the cation exchange resin using the 1.0 and 2.0 mol L^{-1} solutions of HCl or HNO_3. The recoveries attained under these conditions were in the interval of 98.7−101% (Ca) and 99.2−100% (Mg).

Fractionation Pattern of Ca and Mg in Honeys. Considering the information about the sorption behavior of both sorbents and preventing the retention of possible Ca and Mg forms bound to the high molecular mass compounds present in honey, it was decided here that the strong cation exchanger would follow the adsorbent in the fractionation manifold applied and that 5.0% (m/v) solutions of honeys would be loaded. Since the total exchange capacity of the cation exchange resin Dowex 50W×8-200 was 1.7 meq mL^{-1}, denser solutions could also be used, i.e., 10, 15, or 20% (m/v), but they would be less comfortable when handling because of their high viscosity and stickiness. The results of this fractionation analysis with reference to the concentrations of Ca and Mg in the distinct classes of the species separated in the analyzed honeys are given in **Table 5**.

Table 4. Concentration (in mg L^{-1}) of Ca and Mg Determined in the Column Effluents Obtained after Passing the Working Standard Solutions of the Simple Ca and Mg Ions with (I) and without (II) Added Tartaric Acid through the Amberlite XAD-16 Resin[a]

	Ca		Mg	
pH	I	II	I	II
3.5	2.05 ± 0.02	1.95 ± 0.05	0.50 ± 0.01	0.53 ± 0.03
4.0	2.01 ± 0.04	1.99 ± 0.05	0.49 ± 0.02	0.50 ± 0.01
4.5	1.98 ± 0.05	2.01 ± 0.04	0.51 ± 0.02	0.50 ± 0.03
5.0	2.04 ± 0.06	1.98 ± 0.06	0.49 ± 0.01	0.51 ± 0.02

[a] The average values (n = 3) ± SD.

It was found that the cationic fraction is the predominant class of the Ca and Mg species in the studied samples. Regarding the total quantities of Ca and Mg in the analyzed honeys, it appeared that this fraction accounts for up to 96.3% (Ca) and 97.0% (Mg), which agrees well with the previously reported findings achieved with the cation exchanger Dowex 50W×4 only (*10*). Since the endogenous organic ligands present in honeys (*26*) may have certain metal binding capabilities, the cationic species fraction could be supposed to contain not only the simple ions of Ca and Mg but also the stable complexes of these metals with the low molecular mass organic compounds, mostly including organic acids and amino acids. Because of the ease of migrating through the cell membranes of these species of Ca and Mg, this fraction can be additionally regarded as the most bioavailable in honeys.

The residual species appeared to be the less abundant fraction of Ca and Mg in honey. The share of this class of the species in the total concentrations of Ca and Mg found in the analyzed honeys was in the range 0.10−6.5% in the case of Ca and 0.1−2.1% in the case of Mg. This fraction, likely to be associated with the presence of the stable anionic and/or neutral complexes of Ca and Mg with the low molecular compounds, could also be presumed as highly absorbable.

Finally, it was established that Ca and Mg are complexed by the high molecular mass compounds that expose high to moderate hydrophobicity and could be retained by the Amberlite XAD-16 resin. The hydrophobic species fraction was found to contribute 2.3 to 20.1% and 2.8 to 23.0%, respectively, to the total concentrations of Ca and Mg. Since this fraction was expected to include mostly the forms of Ca and Mg bound with phenolic acids and other phenolic compounds of honeys, it was assumed to be less available; typically, the mentioned phenolic compounds are recognized to substantially inhibit the absorbability of the metals from beverages and food products of different origin (*27*).

With the proposed fractionation procedure, Ca and Mg were recovered with efficiencies of 92.5 to 103.3% (Ca) and 97.1 to 103.0% (Mg) (see the sums of the fractions related to the total concentrations in **Table 5**). This verifies the reliability of the method applied for the separation of the species fractions and the determination of the Ca and Mg concentrations. The repeatability of the results is also satisfactory; the pooled RSDs estimated

Table 5. Concentrations of Ca and Mg (in μg g^{-1}) in the Hydrophobic (I), the Cationic (II), and the Residual (III) Fractions

	Ca				Mg			
honey	I	II	III	S[a] (%)	I	II	III	S[a] (%)
A	2.69 ± 0.84	20.88 ± 0.08	0.69 ± 0.26	97.5 ± 4.1	2.81 ± 0.11	12.03 ± 0.03	<0.01	<97.6 ± 0.6
B	1.63 ± 0.30	24.79 ± 1.39	1.85 ± 0.12	99.7 ± 5.1	0.64 ± 0.15	10.37 ± 0.11	0.24 ± 0.03	97.1 ± 1.7
F1	1.67 ± 0.17	72.86 ± 1.70	1.08 ± 0.20	103.3 ± 2.3	1.07 ± 0.33	33.44 ± 0.56	0.12 ± 0.02	99.5 ± 2.1
F2	10.26 ± 0.58	44.76 ± 0.90	1.36 ± 0.23	99.2 ± 3.5	5.17 ± 0.92	46.83 ± 0.65	<0.01	<98.4 ± 2.2
G1	2.98 ± 0.42	24.78 ± 1.33	0.71 ± 0.02	97.0 ± 4.7	1.65 ± 0.38	8.57 ± 0.15	0.05 ± 0.01	99.3 ± 4.1
G2	4.28 ± 0.37	29.38 ± 0.45	0.79 ± 0.05	99.7 ± 2.3	0.58 ± 0.03	10.04 ± 0.06	0.14 ± 0.06	102.0 ± 6.6
GB	1.65 ± 0.29	40.71 ± 1.62	1.61 ± 0.36	94.9 ± 3.8	0.49 ± 0.12	16.67 ± 0.64	0.03 ± 0.01	98.5 ± 4.1
H1	1.92 ± 0.10	45.33 ± 0.49	0.06 ± 0.03	98.9 ± 2.2	3.84 ± 0.54	18.07 ± 0.06	0.03 ± 0.01	99.8 ± 2.6
H2	20.20 ± 2.70	84.40 ± 1.80	2.30 ± 0.40	92.5 ± 4.7	12.80 ± 1.20	177.9 ± 7.0	0.10 ± 0.10	100.4 ± 3.8
HD	1.91 ± 0.58	22.91 ± 0.14	0.18 ± 0.02	98.9 ± 2.4	4.65 ± 0.49	26.97 ± 0.19	0.04 ± 0.01	101.0 ± 1.7
L	5.67 ± 0.92	37.00 ± 1.09	1.57 ± 0.54	102.4 ± 3.5	0.77 ± 0.15	11.17 ± 0.28	0.08 ± 0.02	103.0 ± 2.8
R1	1.80 ± 0.30	37.90 ± 0.90	1.30 ± 0.50	96.2 ± 4.4	2.79 ± 0.47	9.33 ± 0.56	<0.01	<99.9 ± 7.2
R2	6.43 ± 0.69	24.59 ± 0.13	0.97 ± 0.26	96.0 ± 2.2	0.79 ± 0.18	15.38 ± 0.25	0.12 ± 0.03	102.9 ± 2.6

[a] The sum of the fractions (I + II + III) related to the total metal concentration with the figure behind being the pooled RSD.

for the sums of the classified groups of the species were found to change from 2.2 to 5.1% for Ca and 0.6 to 7.2% for Mg.

The investigation of FAAS in conjunction with the chemical fractionation of Ca and Mg by tandem column SPE has demonstrated the validity of such a combination in the study of ripened bee honeys. In comparison to the previously reported literature, this is the first time when the presence of the Ca and Mg species associated with the phenolic compounds in honeys has been reported. Additionally, it seems that the ripened bee honeys are a good source of Ca and Mg supplementation in a daily diet since both metals are present to a high extent in very readily bioavailable forms. Also, the present work demonstrates the very simple and rapid method of the total analysis of honey by means of FAAS.

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