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Talanta 69 (2006) 1227–1233

Talanta

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Fractionation of calcium and magnesium in honeys, juices and tea infusions by ion exchange and flame atomic absorption spectrometry

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Received 14 September 2005; received in revised form 16 December 2005; accepted 22 December 2005

Abstract

An analytical procedure was proposed to study the operational fractionation of Ca and Mg in bee honeys, fruit juices and tea infusions. The protocol devised was based on the solid phase extraction of distinct metal fractions on different sorbents, namely strong acidic cation exchanger Dowex 50Wx4, weak acidic cation exchanger Diaion WT01S and strong basic anion exchange resin Dowex 1x4. For the evaluation of the amounts of the metal fractions distinguished, a flame atomic absorption spectrometry was used off-line prior to the determination of Ca and Mg concentrations in the effluents obtained. It was established that Ca and Mg are mostly present in the analysed samples in the form of cationic species (96–100%). The accuracy of the entire fractionation scheme and sample preparation procedures involved was verified by the performance of the recovery tests.

Keywords: Fractionation; Calcium; Magnesium; Honeys; Fruit juices; Tea infusions; Flame atomic absorption spectrometry

1. Introduction

The investigation of the composition of dietary products is of special importance and significances for the assessment of food quality, in terms of its wholesomeness and acceptability [1]. That type of analysis is relevant in the identification of the botanical or geographical origins of foodstuff and beverages, in addition to the verification of their authenticity. But first and foremost, it enables the estimation of the value of the dietary products as the source of nutritionally important elements, including such physiologically essential constituents as calcium and magnesium, which play a crucial role in different vital processes occurring in the human body.

Both mentioned metals are present in almost all food in relatively high amounts. In particular, it concerns such foodstuff and beverages of natural origin as bee honeys [2–5], fruit juices [6–8] and, to a less degree, herbal teas [9–11], which all are commonly consumed and contribute to the daily dietary requirements for Ca and Mg.

But as a rule, in the overwhelming majority of the contributions devoted to the analysis of food and beverages, comprising especially honeys, juices and teas, the total concentrations of Ca and Mg are determined. Typically, it is performed by complete destruction of the organic matrices of the samples using the wet digestion in concentrated acids [3,6,8,10], the ashing in a furnace followed by the dissolution of the resulting ash in moderately concentrated solutions of acids [2,4,5], or much rarely by direct measurements, e.g. of tea infusions [9] or solutions of honeys [12]. Although, the information retrieved by that kind of assay is valuable for the reasons stated above, but it does not provide any apprehensions to the function of the existing metal species or their impact on the organism. Hence, the evaluation of the nutritional value of dietary products made upon such total concentration determinations is rather incomplete with reference to the bioavailability and the essentiality of different metal species.

Despite great progress made in the analytical instrumentation, the speciation analysis of Ca and Mg in the food is largely limited to the investigation of the associations of these metals with peptides in samples of milk and infant formulas by size exclusion and anion exchange high performance chromatography coupled to different spectrometric detectors [13,14]. Such a state possibly exists due to the diversity of calcium and magnesium species that might be present in the natural dietary products. Consequently, the determination of detailed speciation of Ca and Mg seems to be very difficult and even more impossible to carry out in some cases. For that reason, a better approach is to iden-

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doi:10.1016/j.talanta.2005.12.053

tify the fractions of these metals existing in separate groupings and differing from each other by physical and/or chemical properties, and next to determine the sum of their concentrations in each class [15]. This approach appears to be much useful and practical in view of the evaluation of the bioavailability and the accessibility of metals, as well as the assessment of the risk of a poisoning due to the exposure to metals' excess or a disease caused by their deficiency in the organism.

Previously, the operational classification of the existing analyte species according to their size, solubility, affinity or charge was performed for Al and Mn in tea infusions [16–18]; Cu, Zn and Fe in wines [19]; Cd, Co, Cu, Fe, Mn, Ni and Zn in beers [20,21]; and finally Cu and Mn in milk [22] using different preparation techniques, counting filtration, solid phase extraction on ion exchangers and adsorbents, extraction with water and organic solvents, precipitation or liquid–liquid extraction.

In the case of metals being the interest of the present study, the described approach was used so far only for distinguishing the cationic fraction of Mg in water olive extracts [23] as well as Ca and Mg in tea infusion [24]. As a result, in this contribution, the solid phase extraction using ion exchange resins, that is strong cationic Dowex 50Wx4, weak cationic Diaion WT01S and strong anionic Dowex 1x4, was used for the fractionation of Ca and Mg in samples of honeys, juices and tea infusions in order to retrieve the information convenient for better evaluation of the quality of these food products. Initially, the sorption properties of the selected cation exchangers, i.e. Dowex 50Wx4, Diaion WT01S, Amberlite IRC-718, Duolite GT-73, toward Ca and Mg were thoroughly investigated and compared in consideration of their relevance in the fractionation scheme.

2. Experimental

2.1. Apparatus and materials

A single-beam, air–acetylene flame atomic absorption spectrometer Perkin Elmer 1100B (Germany) equipped with a deuterium background corrector was used throughout this work. Calcium and magnesium concentrations in the effluents, eluates and sample solutions were determined using linear calibration curve method after suitable dilutions. The upper limits of the linearity ranges for Ca and Mg were 5 and 0.5 μg ml^{-1}, respectively. The measurements were carried out at working conditions recommended by the manufacturer, i.e. acetylene flow rate—3.4 and 2.5 l min^{-1}, respectively, for Ca and Mg, air flow rate—8.0 l min^{-1}, sample aspiration rate—2.0 ml min^{-1}, spectral bandwidths—0.70 nm and analytical wavelengths for Ca and Mg—422.7 and 285.2 nm, correspondingly. The calcium and magnesium hollow cathode lamps were operated at 10 and 8 mA, respectively. The readout was performed in a time-average integration mode with the integration time of 1 s (10 replicates per sample).

Ion chromatographic measurements were performed using a Hamilton (USA) cation exchange column PRP–X200 (4.1 mm × 150 mm) with PRP–X200 guard column (2.3 mm × 25 mm). A HPIC system (Knauer, Germany) consisted of a solvent pump WellChrom Mini-Star K-500, a Knauer column thermostat Jetstream 2, a Knauer 6-port valve A0258 and a conductivity detector model 550 Alltech (USA). All measurements were carried out in isocratic mode with a conductivity range set to 10.0 μS and a time constant of 0.5 s. The flow rate of the mobile phase (1.5 mmol l^{-1} ethylenediamine water solution, pH 6.0) was 2.0 ml min^{-1}. The temperature of the column, the thermostat and the conductivity cell was set to 35 °C.

A digital pH-meter PM1 (TMS Electronics, Poland) with a combined electrode was used for measuring the pH in the sample aliquots.

Glassware employed was made from Pyrex. Before use, it was soaked in a 28% (m/v) solution of HNO_3 for 24 h and rinsed after that period for several times with deionised water.

The following sorbents were used for the solid phase extraction (in brackets, the particle size and the functionality of the resins are given): strong acidic gel-type cation exchanger Dowex 50Wx4 (200–400 mesh, sulfonic acid), weak acidic highly porous cation exchanger Diaion WT01S (100–200 mesh, carboxylic acid), chelating macroreticular resin Amberlite IRC-718 (16–50 mesh, iminodiacetic acid), chelating macroporous resin Duolite GT-73 (16–50 mesh, thiol), and strong basic gel-type anion exchanger Dowex 1x4 (100–200 mesh, trimethylbenzyl ammonium). All the resins were purchased from Supelco (USA).

2.2. Reagents and solutions

Standard solutions of Ca(II) and Mg(II) (1000 μg ml^{-1}) were obtained from Merck (Germany). Ethylenediamine (99% pure) was purchased from Aldrich (USA). Other analytical grade chemicals applied, i.e. concentrated nitric acid (HNO_3), concentrated hydrochloric acid (HCl), 30% (m/v) solution of hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), potassium dihydrogen phosphate (KH_2PO_4) and potassium hydrogen phthalate ($C_8H_5KO_4$) were supplied by POCh (Poland).

Deionised water obtained from a Bernstead (USA) D7033 EASYpure^{TM} apparatus was used for the preparation of all the solutions.

Two-element working solutions of Ca and Mg cations were prepared daily by the dilution of bulk solutions of these metals with water. For buffering, 10 ml of two-component solutions, i.e. 0.05 mol l^{-1} solutions of $C_8H_5KO_4$ containing NaOH at the concentration of 1.3, 8.7, 23 and 37 mmol l^{-1}, respectively, for adjusting to a pH of 4.0, 4.5, 5.0 and 5.5; and 0.05 mol l^{-1} solutions of KH_2PO_4 comprising NaOH at the concentration of 5.6 and 14 mmol l^{-1} for a pH of 6.0 and 6.5, respectively, were added to the solutions of Ca(II) and Mg(II), and after mixing, they were completed with water to the mark.

2.3. Samples

Six liquid honeys, two fruit juices and two herbal teas available on the market in Poland were analysed. Honeys, i.e. monofloral acacia (MF1, MF2), floral (F1, F2) and honeydew (H1, H2), apple juice (J1) and black currant nectar (J2) were entirely produced in Poland. The teas were green China leaf tea Loyd (T1) and black India granulated tea (T2). Until analysis, all the products were kept at room temperature in the original packings.

2.4. Column operation

The resins as received (approximately 1.2 g) were slurried into the glass columns (internal diameter of 1.0 cm) equipped with frits and stopcocks (Supelco, USA) using deionised water. It resulted in bed heights between 2.5 and 5.0 cm, depending on the kind of the resin used. After packing the columns, a pre-conditioning treatment was proceeded to remove any metal impurities originated from the manufacturing processes. The resin beds were flushed at first with 25 ml of deionised water and next with 10 ml of a 2.0 mol l^{-1} solution of HCl. After that, the columns were rinsed with 25 ml portions of deionised water followed by 10 ml of a 1.0 mol l^{-1} solution of NaOH. Finally, the columns were washed successively with deionised water using from 25 to 75 ml, dependently on the type of the resin studied. In the case of anion exchange resin Dowex 1x4, the conditioning treatment applied included flushing with deionised water (25 ml), rinsing with the acid solution (10 ml of 2.0 mol l^{-1} HCl) and then washing with another portion of deionised water (25 ml).

For the investigation of the retention behaviour of Ca and Mg cations on the cation exchange resins, two-element solutions of Ca(II) and Mg(II) of volume of 100 ml, containing the analytes at the concentration of 10 mg ml^{-1} and buffered to the respective pH ranging between 4.0 and 6.5, were driven through the columns at a given flow rate with the aid of a two-channel peristaltic pump (LabCraft, France). After passing of a 4/5 of the volume of the test solutions, about 20 ml portions of the effluents from the columns were collected and then analysed on the content of the metals of interest by means of the F-AAS method. Under each experimental conditions, the procedure was completed three times, the procedural blank was run in addition and taken into account in the calculations. The retention efficiencies of the analytes were assessed by the comparison of the Ca and Mg concentrations determined in the effluents (mean values) with those in the loaded solutions.

2.5. Sample preparation and analysis

For all the preparation procedures performed, blank solutions were simultaneously run to assess the degree of the contamination at any step of the sample treatment and analysis. For each type of samples analysed, three replicated samples were prepared and measured. The final results were given as the average values with the repeatability expressed as 1 S.D.

2.5.1. Honeys

Solutions of honeys were prepared by dissolving 2.5 g portions of honey in about 20 ml of deionised water. The resulting liquids were next quantitatively transferred to the volumetric flasks and filled with water to the volume of 100 ml. The solutions obtained in such a way were filtered through the 0.45 μm pore size Nylon 66 filters (Supelco, USA). The filtrates were saved and passed through the chosen chromatographic columns at the flow rate of 2 ml min^{-1}. The effluents from the columns (approximately 20 ml) were collected and subjected to the analysis on the content of Ca and Mg by means of the F-AAS method.

The filters were digested in the mixture of concentrated HNO_3 and 30% (m/v) H_2O_2 using glass beakers covered with watch glasses and heating on a hot plate. For that aim, 10 ml of HNO_3 were used until the digest solutions were clear and light coloured. After cooling, 10 ml of H_2O_2 were added and the heating was gently continued. After evaporation to near dryness, the samples were taken up with deionised water. The resulting liquids were quantitatively transferred to the volumetric flasks (25 ml) and completed with water to the mark prior to the analysis using the F-AAS method.

The total concentrations of Ca and Mg in honeys were determined directly by the FAAS method adopting, with a little modification, the procedure proposed by Lopez-Garcia et al. [12]. For that purpose, the solutions of honeys were prepared as described above. Before topping up with water, they were acidified with concentrated HNO_3 to the final concentration of 5% (m/v) and measured.

2.5.2. Juices

After opening, the juices were passed through the 0.45 μm Nylon 66 filters. Fifty millilitre portions of the filtrates were passed through the chromatographic columns at the flow rate of 2 ml min^{-1} for solid phase extraction. The effluents collected (about 10 ml) were analysed using F-AAS to determine the amounts of Ca and Mg.

The filters were digested (see the description in the section above) and the resulting sample solutions were subjected to the analysis.

The total contents of Ca and Mg in juice filtrates were measured directly by the F-AAS method, after their preliminary acidification with HNO_3 to the concentration of 5% (m/v).

2.5.3. Teas

The tea infusions were prepared in 1 l beakers by pouring 5.0 g of tea leaves or granulates with 400 ml portions of boiling deionised water. After stirring, the teas were left to brew for 15 min. After that time, the infusions were filtered through the 3W paper filters (Filtrak, Germany). The grounds remained on the filters were rinsed several times with small portions of deionised water. Finally, the filtrates and washings were merged and diluted with water to the volume of 500 ml in the volumetric flasks. Fifty millilitre portions of such tea infusions were driven through the chromatographic columns with the flow rate of 2 ml min^{-1}. The F-AAS method was applied for the determination of the amounts of Ca and Mg in the collected effluents (20 ml) as well as in the infusions after their acidification with HNO_3 to the final concentration of 5% (m/v).

The total concentrations of Ca and Mg in the studied teas were determined after their complete decomposition by the wet digestion method in the open vessel system. At first, tea leaves or granulates were ground in an agate mortal. Next, 0.5 g portions of the resulting sample powder were placed in glass beakers, followed by the addition of 5 ml of concentrated HNO_3, and left for overnight. Subsequently, another 5 ml portions of concentrated

HNO_3 were added. Then, the beakers were covered with watch glasses and heated on a hot plate until there were no fumes of nitrogen dioxide (approximately 45 min). After cooling, 10 ml of 30% (m/v) H_2O_2 solution were added and the heating was carried on to obtain colourless solutions. At last, the solutions were evaporated to few millilitres and filtered through the quantitative 3H paper filters (Filtrak, Germany). The filtrates and washings were transferred to the volumetric flasks and diluted with water to the volume of 50 ml. The resulted sample solutions were analysed by the F-AAS method.

2.6. *Fractionation scheme*

For the operational fractionation of Ca and Mg in the samples of food and beverages, the filtrates of solutions of honeys, juices and tea infusions were passed with the flow rate of 2 ml min^{-1} through the following sorbents: strong cation exchanger Dowex 50Wx4, weak cation exchanger Diaion WT01S and strong anion exchanger Dowex 1x4. Each time, the respective column effluents were collected and subjected to the analysis by the F-AAS method to determine the amounts of Ca and Mg species not retained by the particular resin in use. The concentrations of the fractions distinguished were calculated from the difference between the total contents of Ca and Mg in the samples and their quantities found in the respective effluents.

The metal groupings classified through that protocol were two cationic fractions (Dowex 50Wx4, Diaion WT01S) comprising free cations of Ca and Mg, stable cationic complexes of both metals with inorganic and organic ligands, and possibly labile (cationic, anionic and/or neutral) complexes of Ca(II) and Mg(II), which dissociate on the resins during the passage of the samples through the columns, and anionic fraction (Dowex 1x4) containing stable anionic complexes of Ca(II) and Mg(II).

3. Results and discussion

Preliminarily, four different ion exchange resins, that is Dowex 50Wx4, Diaion WT01S, Amberlite IRC-718 and Duolite GT-73, were examined in order to compare their abilities for the retention of Ca(II) and Mg(II) cations under various experimental conditions. Accordingly, the effect of the flow rate and the pH of the solutions loaded onto the columns was investigated on the retention efficiency of Ca and Mg using the artificial solutions comprising these metals at concentration of 10 $\mu g\ ml^{-1}$. Such relatively high concentration of the analytes was applied for two main reasons: to increase the accuracy of measurements of metal contents in the effluents coming from the columns, as well as to match the concentrations of Ca and Mg in the studied samples of honeys, juices, tea infusions. In addition, the influence of the concentration of Ca and Mg, in the range from 1 to 200 $\mu g\ ml^{-1}$, was examined on retention of the metals in question by the resins.

3.1. *Flow rate of solution passage*

The influence of the flow rate of the solutions on the retention efficiency of Ca and Mg was investigated using 100 ml solutions of the metals (10 $\mu g\ ml^{-1}$) buffered to pH of 5.0. The solutions were passed through the columns filled with the resins with the flow rates corresponding to 1, 2 and 4 ml min^{-1}.

It was found that the sorption of Ca(II) and Mg(II) cations by the resins considered were predominantly attributed to the size of their particles, which is recognised to affect the attainment of the equilibrium of the solid phase with the surrounding solutions [25]. Hence, for finer particle size resins (Dowex 50Wx4 and Diaion WT01s), the sorption of metals took place very fast even at relatively higher flow rates of the solutions through the resin beds. For strong cation exchanger Dowex 50Wx4, the average metal retention was equal to 100% with uppermost R.S.D. of 1% for both metals and for all flow rate settings. Similar behaviour was observed for weak cation exchanger Diaion WT01S with exception of the retention efficiency achieved for Ca at the flow rate of 4 ml min^{-1} and equalled to 98 ± 2%.

In the case of lower mesh number resins, i.e. chelating Amberlite IRC-718 and Duolite GT-73 exchangers, Ca(II) and Mg(II) cations were retained quantitative only for the flow rate of 1 ml min^{-1}. Under these conditions the following retention efficiencies were attained 98 ± 2% for Ca and 99 ± 2% for Mg, and 98 ± 2% for Ca and 98 ± 2% for Mg, respectively, for Amberlite IRC-718 and Duolite GT-73. For higher flow rates, a decrease in the retention efficiencies was observed for both metals. And thus, for Amberlite IRC-718, the retention efficiencies established for Ca were equal to 95 ± 1 and 92 ± 3%, while for Mg they reached the values of 94 ± 1 and 91 ± 2%, correspondingly for the flow rate of 2 and 4 ml min^{-1}. For Duolite GT-73, the effect of the flow rate with which the solutions were passed through the columns was the most significant as its increase to 4 ml min^{-1} led to deterioration of the retention efficiency up to 82 ± 5% for Ca and 79 ± 4% for Mg.

3.2. *pH of solution*

Due to moderate or low acidity reported in the literature for honeys, some fruit juices and tea infusions [1,2,4,16,17], the effect of the pH of the solutions on the retention of Ca and Mg by the resins investigated in this study was examined in the range from 4.0 to 6.5. For that reason, 100-ml solutions of Ca(II) and Mg(II) cations (10 $\mu g\ ml^{-1}$), buffered to the respective pH values, were passed through the resin beds with the flow rate of 2 ml min^{-1}. For Duolite GT-73 resin, the flow rate of 1 ml min^{-1} was used.

It was established that cations of Ca and Mg were completely retained from the solutions in the whole pH range investigated and by all the exchangers used. In the case of Dowex 50Wx4, Diaion WT01S and Duolite GT-73 resins, the average retention efficiencies for Ca as well as for Mg, ascertained for each of these resins as the mean values of the retention percentages found for separate pH adjustments, were equal to 100%. The uncertainty of single results, expressed as relative standard deviation, varied from 0.5 to maximally 3%. For Amberlite IRC-718 resin, the average retention efficiency found for Ca was equal to 98%, while for Mg it was 99%. The relative standard deviations of the separate results were within 1–3%.

Elucidating the possible mechanisms of sorption of Ca and Mg cations, the classification of metals due to the type of their possible binding to the active sites of different resins was considered [26]. In view of that categorisation, Ca^{2+} and Mg^{2+} belong to the group of "hard" cations which retention on the resins takes place via the electrostatic interactions with the functional groups. Particularly, strong cation exchanger Dowex 50Wx4 with sulphonic acid functional groups, being dissociated in a wide pH range, retained quantitatively the cations of Ca and Mg (generally M^{2+}) within the investigated pH range presumably owing to the reversible ion exchange reaction $2R{-}SO_3{}^- + M^{2+} = (R{-}SO_3)_2M$ [27,28].

For weak cation exchanger Diaion WT01S containing carboxylic acid functional groups of pK_a somewhat close to 4.5, it was expected that the retention of Ca and Mg cations would rather occur above the pH of 6, which is under the conditions of the complete dissociation of −COOH groups. However, both metal cations were completely sorbed by the resin at lower pH, i.e. between 4.0 and 6.5, and such a behaviour could be likely associated to the ion exchange reaction $2R{-}COOH + M^{2+} = (R{-}COO)_2M + 2H^+$, which was presumed by Pesavento et al. on the basis of the study of sorption properties of weak cation exchange carboxylic resin Amberlite CG-50 using Gibbs-Donnan model [29].

Sorption of bivalent cations of Ca and Mg by iminodiacetic acid group containing resin Amberlite IRC-718, observed at the pH values lower than those predicted from the complexation properties of iminodiacetate ligand (−LH(H)), could be related to binding these metal cations by two carboxylic groups in line with the reaction $2R{-}LH(H) + M^{2+} = (R{-}LH)_2M + 2H^+$, which was also assumed on the basis of Gibbs-Donnan model in the description of sorption properties of Chelex-100 resin [30].

Unfortunately, for the resin Duolite GT-73, containing −SH groups, there was no adequate literature, which could clarify the sorption mechanism of Ca(II) and Mg(II) cations in the studied pH range. Possibly, due to the pK_a of thiol group, which is around 7, both metals could be retained through the interactions similar to those established for the carboxylic groups.

3.3. Concentration of Ca and Mg

As the contents of Ca and Mg in the analysed bromatological samples are relatively high [1], the effect of the concentration of these metals on their retention by the resins applied was investigated as well. Accordingly, two-component 100-ml solutions containing metal cations at concentrations corresponding to 1, 10, 20, 50, 100 and 200 $\mu g\,ml^{-1}$, and adjusted to pH 5.0, were passed through the columns filled with the cation exchangers at the flow rate of 2 or, in the case of Duolite GT-73, 1 $ml\,min^{-1}$.

The retention efficiencies found for Ca and Mg on the basis of the analysis of column effluents were close to 100% in the whole studied analyte concentration range. It indicated that all the resins have reasonably high binding capacity toward the metals under consideration and practical usefulness in the operational fractionation analysis.

Taking into account the sorption properties of the resins established under the conditions investigated in the present study along with their capacities given by the suppliers, for further researches Dowex 50Wx4 (4.8 meq/g) and DiaionWTO1S (9.0 meq/g) were selected and applied in the fractionation experiments.

3.4. Fractionation analysis

There are only a few papers published on the fractionation of Ca and Mg in foodstuff and beverages. Usually, the information on the total contents of these metals is available. So far, the operationally defined fractionation of Mg in olives was proposed by Yasar and Gucer [23]. In that fractionation protocol, Dowex 50Wx8 cation exchange resin was used for the retention of magnesium cations and other cationic magnesium species from water extracts of olives. Cation exchange separation based on solid phase extraction cartridges was also applied for study of the charge of dissolved species of Ca and Mg in a tea infusion [24].

Here, two cation exchangers, i.e. strong acidic Dowex 50Wx4 and weak acidic Diaion WT01S, binding differently the cations of metals of interest, were used for distinguishing the cationic fractions of Ca and Mg in honey solutions and fruit juices (dissolved fraction obtained after filtration of the samples through 0.45 μm pore size filters) and tea infusions. The fractions separated were operationally defined, and therefore the results obtained were strictly related to the separation properties of the resins used [31]. In the light of the available knowledge [1] on the composition of analysed samples, and possible inorganic and organic molecules able to complex calcium and magnesium cations, it was presumed that the cationic fractions should most likely contain free Ca and Mg cations and stable cationic complexes of the metals in question with low molecular mass ligands, mainly organic acids such as citric (H_3Cit), malic (H_2Mal), oxalic (H_2Oxa) and tartaric (H_2Tar), for which, at pH 4–5 typical for studied samples, the following forms are predominant, i.e. H_2Cit^-, $HMal^-$, $HOxa^-$ and $HTar^-$. These fractions could also contain any cationic, anionic and/or neutral unstable complexes of Ca and Mg with organic ligands of different lability. The significance of the evaluation of the amounts of the fractions of cationic species of Ca and Mg, regarded as highly assimilable by the organisms [23], was related to a more comprehensive assessment of quality and dietary value of the studied samples of food and beverages.

Strong basic anion exchange resin Dowex 1x4 was employed in this fractionation protocol for the estimation of stable anionic complexes of Ca and Mg (anionic fraction) as well as for the examination of the reliability of the results obtained using the cationic exchangers.

The results of the fractionation analysis of Ca and Mg in the studied samples are given in Tables 1 and 2, respectively. In the case of samples of honeys, no particulate fraction of Ca and Mg (defined as >0.45 μm) was found in the honey solutions; the contents of the metals in the digests of the filters were below the level of the detection limits that is 0.01 and 0.001 $\mu g\,ml^{-1}$, respectively, for Ca and Mg. In the dissolved fraction, both metals were predominantly present in the form of the cationic species; the contribution of the cationic fraction for Ca was from 96 to

Table 1
Total contents of Ca and concentrations of metal fractions distinguished in honeys (in $\mu g\,g^{-1}$), fruit juices (in $\mu g\,ml^{-1}$) and tea infusions (in $\mu g\,ml^{-1}$)

Sample	Sample pH	Total content	Cationic fraction[a]	Cationic fraction[b]	Anionic fraction[c]
MF1	4.8	29.55 ± 0.55 (100 ± 2)	28.67 ± 0.71 (97.0 ± 2.4)	29.33 ± 0.36 (99.2 ± 1.4)	1.71 ± 0.45 (5.8 ± 1.5)
MF2	4.6	27.44 ± 0.41 (100 ± 1)	27.30 ± 0.43 (99.5 ± 1.6)	27.35 ± 0.43 (99.7 ± 1.6)	0.70 ± 0.46 (2.6 ± 1.7)
F1	5.0	79.12 ± 0.58 (100 ± 1)	78.74 ± 0.58 (99.5 ± 0.7)	78.63 ± 0.59 (99.4 ± 0.7)	1.20 ± 0.89 (1.5 ± 1.1)
F2	5.1	76.68 ± 1.15 (100 ± 1)	76.39 ± 1.15 (99.6 ± 1.5)	76.44 ± 1.15 (99.7 ± 1.5)	–[d]
H1	4.8	42.10 ± 0.64 (100 ± 2)	41.47 ± 0.64 (98.5 ± 1.4)	41.64 ± 0.66 (98.9 ± 1.4)	1.34 ± 1.17 (3.2 ± 0.5)
H2	4.9	29.34 ± 0.44 (100 ± 1)	29.24 ± 0.45 (99.6 ± 1.5)	29.18 ± 0.45 (99.4 ± 1.5)	–[d]
J1	3.8	102.8 ± 1.0 (100 ± 1)	102.7 ± 1.0 (99.9 ± 1.0)	95.17 ± 1.13 (92.6 ± 1.1)	1.30 ± 2.66 (1.3 ± 2.6)
J2	4.0	336.0 ± 3.4 (100 ± 1)	335.9 ± 3.4 (100 ± 1)	302.0 ± 3.8 (89.9 ± 1.1)	47.00 ± 7.84 (14.0 ± 2.3)
T1	5.2	5.35 ± 0.05 (100 ± 1)	5.31 ± 0.05 (99.2 ± 0.9)	5.32 ± 0.05 (99.4 ± 0.9)	–[d]
T2	5.2	1.39 ± 0.01 (100 ± 1)	1.33 ± 0.01 (95.7 ± 0.7)	1.34 ± 0.01 (96.4 ± 0.7)	–[a]

[a] Obtained after passing the sample through Dowex 50Wx4.
[b] Obtained after passing the sample through Diaion WT01S.
[c] Obtained after passing the sample through Dowex 1x4.
[d] Not possible to calculate due to the concentration in the effluent equal to or higher than the total concentration in the sample.

100% of its total concentration in the sample, while for Mg it spanned the range from 97 to 100%. For both cation exchangers used, the concentrations of the fractions of Ca and Mg in each honey samples were statistically identical (on the basis of the test "*t*" for $p = 0.05$), indicating that, besides free metal cations and stable cationic complexes of Ca and Mg with low molecular organic ligands, probably very labile and moderately labile species could be present or the donation of the labile species to that fraction was very minor. It was also ascertained using the HPIC measurements that the concentrations of free metal cations in relation to the total metal concentrations determined amounted for 11.2 (for sample F2) to 17.7% (for H2) in the case of Ca and for 35.6 (for F2) to 62.1% (for MF2) for Mg. The contribution of the anionic fraction in honey samples was rather negligible and reached maximally 5.8 and 4.6% of the total concentrations of Ca and Mg, respectively. It should be noted, however, that this fraction could be overestimated in same cases possibly due to the retention of any non-anionic species of both metals [31].

The particle fraction separated from apple juice (J1) contained $0.040 \pm 0.001\ \mu g\,ml^{-1}$ of Ca and $0.030 \pm 0.001\ \mu g\,ml^{-1}$ of Mg. In the case of black currant nectar, the concentration of Ca and Mg present in that fraction accounted for 1.63 ± 0.02 and $0.010 \pm 0.001\ \mu g\,ml^{-1}$, respectively. In the case of the dissolved fraction of samples analysed, similar pattern of fractionation was observed as for honey samples; the cationic fractions of Ca and Mg contributed to 93–100% of the total soluble contents of these two metals in the samples. For the sample of black currant nectar (J2), a difference (up to 10%) between the contributions of both cationic fractions determined for Ca appeared, simultaneously with a considerable donation (about 14%) of the anionic fraction established in relation to the total amount of that metal dissolved in the sample. Such occurrence could be probably associated with chemical modification of black currant juice prior to the nectar production. At the present stage it is difficult to connect any possible chemical modification with differences in the results obtained.

Unfortunately, the results of the fractionation analysis attained here for Ca and Mg in honey and juice samples could not be compared with the literature data because of the lack of the appropriate contributions devoted to that subject.

The total contents of metals of interest in the tea samples were as follows: $2273 \pm 19\ \mu g\,g^{-1}$ of Ca and $2653 \pm 72\ \mu g\,g^{-1}$ of Mg in leaf green tea (T1), and $2373 \pm 67\ \mu g\,g^{-1}$ of Ca and $1035 \pm 36\ \mu g\,g^{-1}$ of Mg in granulated black tea (T2). For leaf tea, the extraction efficiencies, expressed as the percentages of

Table 2
Total contents of Mg and concentrations of metal fractions distinguished in honeys (in $\mu g\,g^{-1}$), fruit juices (in $\mu g\,ml^{-1}$) and tea infusions (in $\mu g\,ml^{-1}$)

Sample	Sample pH	Total content	Cationic fraction[a]	Cationic fraction[b]	Anionic fraction[c]
MF1	4.8	10.98 ± 1.09 (100 ± 10)	10.71 ± 1.09 (97.5 ± 10.0)	10.76 ± 1.09 (98.0 ± 10.0)	0.51 ± 1.35 (4.6 ± 12.3)
MF2	4.6	10.73 ± 0.21 (100 ± 2)	10.71 ± 0.21 (99.8 ± 2.0)	10.65 ± 0.21 (99.2 ± 2.0)	0.17 ± 0.25 (1.6 ± 2.3)
F1	5.0	20.32 ± 0.52 (100 ± 3)	20.24 ± 0.52 (99.6 ± 2.6)	19.83 ± 0.54 (97.6 ± 2.6)	0.66 ± 0.54 (3.2 ± 2.6)
F2	5.1	37.60 ± 0.56 (100 ± 1)	37.30 ± 0.69 (99.2 ± 1.8)	36.88 ± 0.65 (98.1 ± 1.7)	0.31 ± 0.65 (0.82 ± 1.72)
H1	4.8	40.25 ± 1.98 (100 ± 5)	40.13 ± 1.98 (99.8 ± 4.9)	39.44 ± 1.98 (98.0 ± 4.9)	1.05 ± 2.11 (2.6 ± 5.2)
H2	4.9	32.52 ± 0.65 (100 ± 2)	32.42 ± 0.65 (99.6 ± 2.0)	32.08 ± 0.65 (98.6 ± 2.0)	–[d]
J1	3.8	69.40 ± 0.69 (100 ± 1)	69.40 ± 0.69 (100 ± 1)	68.36 ± 0.71 (98.5 ± 1.0)	1.15 ± 0.77 (1.6 ± 1.1)
J2	4.0	89.70 ± 0.90 (100 ± 1)	89.68 ± 0.90 (100 ± 1)	85.82 ± 0.92 (95.7 ± 1.0)	–[d]
T1	5.2	4.65 ± 0.05 (100 ± 1)	4.58 ± 0.05 (98.5 ± 1.1)	4.63 ± 0.05 (99.6 ± 1.1)	–[d]
T2	5.2	5.75 ± 0.06 (100 ± 1)	5.68 ± 0.06 (98.8 ± 1.0)	5.74 ± 0.06 (99.8 ± 1.0)	–[d]

[a] Obtained after passing the sample through Dowex 50Wx4.
[b] Obtained after passing the sample through Diaion WT01S.
[c] Obtained after passing the sample through Dowex 1x4.
[d] Not possible to calculate due to the concentration in the effluent equal to or higher than the total concentration in the sample.

the soluble metal contents relative to the respective total amounts in the sample, were equal to 23.2 ± 6.2 and 17.3 ± 5.4%, respectively, for Ca and Mg. In the case of granulated tea, the extraction efficiencies reached the values of 5.9 ± 1.6% for Ca and 54.6 ± 6.6% for Mg. These results, concerning the total contents of Ca and Mg and the extraction efficiencies in the teas, were in a reasonable agreement with those reported in Ref. [24] and the papers cited therein.

The outcomes regarding the contribution of the cationic forms of Ca and Mg to the total concentrations of these metals in the tea infusions were strictly consistent with those observed by Odegard and Lund [24] who used similar effluent approach to the evaluation of the charge of Ca and Mg species extracted from the tea leaves to the infusion during the brewing process. Correspondingly, it was found that from 96 to almost 100% of the total soluble metal concentrations of Ca and Mg were present in the form of cationic species, likely to be metal cations associated with small organic molecules (<1000 Da) or/and not associated with organic matter at all [24].

3.5. Verification of validity

The validity of the analytical procedures carried out at different steps of sample treatment and analysis, e.g. direct measurements of Ca and Mg in the honey solutions, juices and tea infusions, digestion of the tea samples prior to the determination of Ca and Mg, as well as during the fractionation protocol, e.g. retention of Ca and Mg cations on cation exchange resins, was tested by the performance of the recovery tests. For that purpose, the samples were spiked with known amounts of metals (added as Ca^{2+} and Mg^{2+}), matching the concentrations found in the analysed samples, that is from 5 to 350 $\mu g\, ml^{-1}$. Then, the respective procedures were completed and the recoveries of added quantities of Ca(II) and Mg(II) were assessed.

The recoveries found for Ca and Mg and for all procedures performed were between 96.0 and 108% (the relative standard deviations for three replicates were lower than 6%) demonstrating the appropriateness of the applied methods.

4. Conclusions

The devised fractionation protocol has allowed the classification of Ca and Mg species present in honeys, fruit juices and tea infusions according to their sorption behaviour on cation and anion exchange resins. It has been recognised that the fractionation pattern for Ca and Mg is very similar. Both metals principally or entirely exist in the analysed samples in the form of cationic species. The results achieved can provide more useful information about the quality and nutritional value of studies foodstuff and beverages than the total content analysis.

In addition, the presented scheme is inexpensive and easy in operation. The non-eluting approach used for the evaluation of the fraction concentrations has the advantage that is straightforward, has lower blank levels and involves less opportunity for the contamination associated with the solvent elution procedure.

The area of its improvement lies in application of a size exclusion resin to classify the possible metal groupings due to their molecular size as well as coupling of the sorbents used in a series of different arrangement. The effort of such works has been undertaken.

Acknowledgment

Technical assistance of Er. J. Skornowicz is gratefully acknowledged.

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