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Fractionation analysis of potentially toxic elements in apples for evaluation of their availability to humans

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Abstract

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The main objective of this work was to get information on the Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn content in different apple fractions and their digestion in the human gastrointestinal tract. A sequential analytical approach was applied to two apples varieties from the commercial network of Bulgaria, focusing on their total element concentrations, extractability in water, ethanol and acetone. The oral bioaccessibility of the elements was evaluated using the PBET and extraction with pepsin+HCl and n-octanol. The element concentrations were measured using ICP-MS. The obtained results showed the highest percentage of extraction for all analyzed elements in the water fraction and similar low extractable concentrations in the ethanol and acetone fractions for both varieties. The data from the in vitro extraction procedures showed high bioaccessibility in human gastrointestinal tract of Cu, Mn, Zn and Cd, while for Ni and Pb only 20÷30% of the total concentration were bioavailable.

Keywords: fractionation analysis; toxic elements; ICP-MS; oral bioaccessibility; Physiologically-Based Extraction Test (PBET)

Introduction

Apples make a significant part of the diet of humans and represent a good source of dietary fiber, pectins, potassium and vitamin C (Wu et al., 2007). Apples contain different classes of phenolic compounds, which can protect the human body against oxidative stress (Auclair et al., 2008; Ko et al., 2005; Kundari Vieira et al., 2009). These compounds possess ability to reduce oxidative stress by several mechanisms, i.e. direct scavenging of reactive oxygen species (Panzella et al., 2013), complexation of metal ions (Flora, 2009; Michalak, 2006) and modulation of cellular response (Birt et al., 2001).

As a non-digestible component of plants, lignin is the main constituent of the dietary fibre, with beneficial nutritive and human protective effects (Ayala-Zavala et al., 2011). Lignin from different sources has been widely studied because of its antioxidant, anti-inflammatory, antibacterial or anti-carcinogenic propertiesand prebiotic behavior (Amendola et al., 2012; Garcia et al., 2010; Hollman, 2001; Ugartongo et al., 2009). Thus lignin is being widely investigated for new and value-added applications related to its bio-activity, such anti-inflammatory, antibacterial or anti-carcinogenic action (Hollman et al., 2001). Studies are focused mainly on the polyphenolic and lignin composition and their antioxidant activity and less on the potentially toxic elements that may bound with them thanks to their chelating properties (Michalak, 2006) in spite of the limited information that some metals (e.g. Cu and Fe) have affinity for bonding with polyphenols thus increasing their antioxidant activity (Hadi et al., 2007; Huyut et al., 2017; Perron & Brumaghim, 2009). The trace metals Zn and Cd can interact with polyphenols and thus affect metallothionein level in human intestinal cells (Kuo et al., 1998).

Considering dissimilar biological activity and functionality of different physicochemical forms of metals, their total concentrations are not sufficient to provide reliable information to predict their accessibility, availability and toxicity (Jedryszka et al., 2017). In order to assess the potential intoxication risk of metals included in food and/or their nutritional value, it is essential to identify and determine the fraction of metals that is unbound and mobile and therefore bioavailable. Speciation analysis of metals in food is nowadays a fundamentally important issue which will certainly be of increasing significance in future in relation to safety and nutritional value of daily diet products (Ibanez et al., 2008).

Applying different extraction techniques it is possible to isolate a particular group of compounds or individual chemicals and then test their bioavailability (Kalinowska et al., 2014). Different schemes for extraction of polyphenols from plants have been reported (Ge & Jin, 1994; Hu et al., 1997; Spingo & Faveri, 2009). Pan et al. (2003) used ethanol extraction of polyphenols from green tea leaves. Their research, however, is directed to the identification of polyphenol compounds but not to potentially toxic elements related to them (Pan et al., 2003). It has been found that the concentration of polyphenols increases as a protective mechanism against increasing concentrations of Cd and Pb in the plant (Pandey &Tripathi, 2011). The extraction of lignin in *Prunus amygdalus* with acetone-water mixture at specific conditions (extraction time and temperature) has been investigated (Quesada-Medina et al., 2010), but no data on chelating properties are available. Some authors have used two steps extraction with HCl+pepsin and n-octanol to extract metals, which can be adsorbed in the gastrointestinal tract (Gregorova et al., 2010; Mueller-Harvey et al., 2007).

The World Health Organization (WHO) announced agreement on recommendations regarding safe intake levels for a variety of different chemicals occurring in food, especially for potentially toxic elements (WHO 1993; 1996). Because of possible health risk it is necessary to measure not only the total concentration of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in fruits, but it is important to investigate the distribution of those elements in different plant compounds as well.

Knowledge of the bioavailability of toxic elements is useful for estimating potential human health risk. In vivo studies are both expensive and laborious, and the possibility of measuring certain parameters during the experiments is often limited (Cabanero et al., 2004), the data are normally determined in an in vitro environment and represent the amount of contaminants absorbed in the gastrointestinal tract.

A simulated gastric juice extraction procedure was used by Hocquellet & L'Hotellier (1997). Several studies on the bioavailability and bioaccessibility of essential and toxic metals in foodstuff have been performed (Intawongse & Dean, 2008; Moreda-Piñeiro et al., 2011). A few investigations were focused on nuts (Arpadjan et al., 2013; Ovca et al., 2011), but most of these studies were conducted mainly to determine bioavailability of essential, not potentially toxic elements.

The aim of this study is to propose generalized extraction procedures for fractionation analysis of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn with organic solvents in edible fruits using ICP-MS in order to understand their deposition in different plant compounds and their bioavailability. The physiologically-based extraction test is used to assess the oral bioaccessibility of metals in apple samples.

Material and Methods

Apparatus and materials

Microwave Reaction System (Anton Paar, Perkin Elmer, Multiwave 3000) was used for the microwave digestion of the samples. A controlled temperature shaking water bath SBS40 by Stuart was employed for the PBET experiment.

All ICP-MS measurements were carried out with an inductively coupled plasma mass spectrometer Perkin Elmer SCIEX DRC-e. The spectrometer was optimized (RF, gas flow, lens voltage) to provide minimal values of the ratios CeO^+/Ce^+ and Ba^{2+}/Ba^+ and maximum intensity of the analytes. External calibration by a multi-element standard solution was performed. The calibration coefficients for all calibration curves were at least 0.99. The measurement conditions for ICP-MS are presented in Table 1.

Table 1. Measurement conditions for ICP-MS (Perkin Elmer SCIEX DRC-e)

Instrument	Operating conditions
Argon plasma gas flow	15 L min^{-1}
Auxiliary gas flow	1.20 L min^{-1}
Nebulizer gas flow	0.90 L min^{-1}
Lens voltage	6.00 V
ICP RF power	1100 W
Pulse stage voltage	950 V
Dwell time	50 ms
Acquisition mode	Peak hop
Peak pattern	One point per mass at maximum peak
Sweeps/reading	8
Reading/replicates	1
Sample uptake rate	2 mL min^{-1}
Number of runs	6
Rinse time	180 s
Rinse solution	3% HNO_3 (v/v)
Isotope monitored	^{112}Cd, ^{59}Co, ^{52}Cr, ^{63}Cu, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{66}Zn

Reagents and solutions

Single element standards of Cd, Co, Cr, Cu, Mn, Ni, Pb, Zn (Fluka) with initial concentration 10 µg mL^{-1} were mixed and used for calibration after appropriate dilution to obtain the following concentrations: 0.5, 1.0, 5.0, 10.0, 25.0 and 50.0 ng mL^{-1}. HNO_3 (67-69%, Fisher Chemicals, TraceMetal Grade) and H_2O_2 (30% Fisher Chemicals, Trace Analysis Grade) were used for the digestion of the samples. Standard Reference Material NIST 1547 (Peach leaves, National Institute of Standards and Technology, USA) was used throughout the investigation to assess the accuracy of the analytical method. The applied extraction solvents were ethanol (Sigma-Aldrich, extra pure), acetone (Merck, extra pure), HCl (35-37%, Fisher Chemicals, TraceMetal Grade), pepsin (Sigma-Aldrich) and n-octanol (Merck, extra pure). All chemicals used for PBET experiment were of analytical grade. Acetic acid and sodium bicarbonate were from Merck (extra pure), pepsin and pancreatin powder were from Sigma-Aldrich, while bile salts, sodium malate, sodium citrate and lactic acid were provided by Fluka.

All solutions were prepared with double deionized water.

Two apples varieties from the commercial network of Bulgaria (Golden Delicious and Breaburn) were analyzed.

Sample preparation for total element determination

About 10 kg of two apple varieties (Golden Delicious and Breaburn) each were purchased from the commercial network of Bulgaria. Whole apples were cut with a plastic knife. The edible fruit parts (flesh and peel) were obtained from six randomly selected apples in each trial and variety to minimise variation. All samples were dried at 105°C. 0.5 g of the CRM 1547 sample and 0.3 g of dried apple fruits were mixed with 10 mL HNO_3 and 4 mL H_2O_2 in a PTFE vessel and MW digestion was directly applied. The program for microwave digestion of the samples is presented in Table 2. Once the samples were completely digested and transparent they were evaporated to about 1 mL. The digested solutions were then diluted to 50 mL with double deionized water. The concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in the solutions were determined by ICP-MS.

Table 2. Program for microwave digestion of the apple samples (Tmax = 180°C)

Steps	Time, min	Power, W	P, bar
1	10	400	60
2	15	900	110
3	15	300	40
4	30	vent	10

Fractionation studies using solvent extractions

To determine the water-soluble fraction of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in apple samples 0.5 g of apple fruits were added to 10.00 mL double deionized water. The mixture was shaken for 16h at room temperature. After centrifugation and filtration the samples were analyzed by ICP-MS.

Ethanol extraction of apple samples was used to determine polyphenol-bound elements. A definite amount (0.5 g) of apple fruits was added to 10.0 mL ethanol (75% V/V) and the mixture was shaken for 16 h at room temperature. After centrifugation the supernatant was decanted, followed by filtration and solvent evaporation to 1 mL. The residue was dissolved in double deionized water and analyzed by ICP-MS.

Extraction with acetone was used to determine the elements included in lignin. 0.5 g of the apple samples were mixed with 10 mL acetone (75% V/V) and then the mixture was shaken for 40 min at 210°C (Quesada-Medina et al., 2010). After centrifugation the supernatant was decanted, followed by filtration and solvent evaporation to 1 mL. The residue was dissolved in double deionized water and analyzed by ICP-MS.

Extraction with 0.1M HCl+pepsin solution and n-octanol solvent were used to investigate the amount of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn absorbed in the stomach and intestines respectively. The pepsin-containing HCl extract of apples was obtained by adding 0.32 g pepsin in 25 ml 0.1M HCl to 0.5 g of apple samples and shaking for 4 h at 37°C. After filtration the elements of interest were determined by ICP-MS.

Extraction with n-octanol solvent was carried out adding 10.0 mL of solvent to 0.5 g sample. The mixture was shaken for 4 h at 37°C. After centrifugation the supernatant was decanted, followed by filtration and solvent removing by distillation. The residue was dissolved in double deionized water and analyzed by ICP-MS. The operating conditions for the fractionation studies are presented in Table 3.

Physiologically-Based Extraction Test (PBET)

The PBET experiment was carried out using the procedure described in Intavongse & Dean (2008) with some modifications. The procedure includes two sequential processes, a gastric and an intestinal digestion, each one carried out employing simulated human conditions. In the first stage, 0.5 g (accurately weighed) of dried apple material was ground and placed into a 50 mL polyethylene tube and treated with 40 mL of gastric solution (1.25 g pepsin, 0.50 g sodium malate, 0.50 g sodium citrate, 420 mL lactic acid and 500 mL acetic acid were diluted to 1 L with deionised water, adjusted to pH 2.5 with conc. HCl). The mixture was then shaken at 100 rpm in a thermostatic bath maintaining 37°C. After 1 h, the solution was centrifuged at 3000 rpm for 10 min and a 5 mL aliquot was removed and filtered through 0.45 µm cellulose

Table 3. Operating conditions for fractionation studies

Experiment	Fraction	Reagent/material	Experimental conditions
Digestion	Total element concentrations	Concentrated HNO_3+H_2O_2	4 h at 200°C
Extraction with water	Water-soluble elements	Deionized water	16 h at 25°C
Extraction with ethanol	Polyphenol-bound	C_2H_5OH	16 h at 25°C
Extraction with acetone	Lignin-bound	CH_3COCH_3	40 min at 210°C
Extraction with HCl+pepsin	Absorbable in stomach	Pepsin in 0.1M HCl	4 h at 37°C
Extraction with n-octanol	Absorbable in intestine	n-Octanol	4 h at 37°C

filter. 5.0 mL of the original gastric solution was then back flushed through the filter into the sample tube to retain the original solid: solution ratio, i.e. 0.5:40 g mL^{-1}.

The second stage, 70–73 mg bile salts and 20–25 mg pancreatin were added to the sample tube and the mixture was adjusted to pH 7.0 with $NaHCO_3$ solution (10 g/100 mL). The sample was then shaken at 100 rpm in a thermostatic bath maintaining 37 °C for further 4 h when a second 5.0 mL aliquot was removed and filtered. To prevent unwanted sorption of analyzed metals to container walls all PBET extracts were acidified with 1% V/V HNO_3. This made additional centrifugation and filtration of the small intestinal phase extracts necessary as bile salts precipitate at pH = 2. The sample residue was further digested by nitric acid and hydrogen peroxide (residual fraction). Measurements of different PBET fraction (gastric, intestinal and residual) were performed according the operating conditions given in Table 1.

Calculation of bioaccessibility

The bioaccessibility measurements are reported as relative bioaccessibility expressed as a percentage and calculated per digestion according to the following equation (Oomen et al., 2002):

$$\text{Bioaccessibility (\%)} = \frac{\text{metal mobilized from plant sample during digestion}}{\text{metal present in plant sample before digestion}} \times 100$$

Results and Discussion

Total element concentration

The accuracy of the ICP-MS measurement data was evaluated by analyzing the standard reference material – NIST 1547. The concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn found in apple samples are given in Table 4. All results from the analysis were average of three parallel determinations characterized with the respective standard deviation. In all cases the precision is better than 10%.

The evaluation of the results in Table 4 indicates a very good agreement between our data and certified/information/ values for the measured elements in SRM NIST-1547.

The results in Table 4 show that the concentrations of Cd, Cr, Mn, Ni and Zn in Golden delicious were higher than in Breaburn, while Pb and Co was higher in Breaburn. Cu had similar concentrations in both apple varieties. The concentrations of all determined elements in the investigated apple varieties were significantly lower than those that can cause risk to human health (WHO 1993; 1996).

Fractionation analysis

To define better the content of potentially toxic elements in different apple compounds like polyphenols and lignin analytical fractionation analysis was performed based on water, ethanol and acetone extractions.

The evaluation of the possible losses during the sample preparation steps and the eventual presence of matrix

Table 4. Concentration (μg g^{-1}) of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in the apple samples and CRM-1547

Element	Fruit-Golden Delicious	Fruit –Breaburn	CRM 1547	
			Experimental value	Certified value
Cd	0.031±0.002	0.014±0.002	0.027±0.002	0.026±0.003
Co	0.014±0.003	0.020±0.04	0.065±0.003	0.07[a]
Cr	0.61±0.04	0.48±0.03	1.02±0.03	1[a]
Cu	2.84±0.02	2.65±0.03	3.5±0.2	3.7±0.4
Mn	3.99±0.03	1.35±0.04	99±4	98±3
Ni	0.22±0.02	0.11±0.02	0.62±0.05	0.69±0.09
Pb	0.20±0.02	0.55±0.03	0.88±0.02	0.87±0.03
Zn	10.1±0.2	3.48±0.04	17.5±0.3	17.9±0.4

[a] – Information value, determined but not certified; Mean± S.D. (n = 3)

Table 5. Elements fractionation results for Golden Delicious apple samples

Elem.	Water-soluble		Polyphenol fraction		Lignin fraction		Total extracted percentage, %
	Concentration, μg g^{-1}	Extracted, %^{a}	Concentration, μg g^{-1}	Extracted, %^{a}	Concentration, μg g^{-1}	Extracted, %^{a}	
Cd	12.7±0.5^{b}	41	1.1±0.4^{b}	3.2	1.0±0.2^{b}	2.4	46.6
Co	2.49±0.03^{b}	17.8	0.5±0.2^{b}	3.6	0.22±0.04^{b}	1.6	23
Cr	0.40±0.02	65	0.090±0.003	14	0.071±0.003	11.7	90.7
Cu	1.09±0.04	38	0.49±0.02	17.2	0.34±0.02	12	67.2
Mn	1.08±0.05	27	0.25±0.03	6.2	0.504±0.003	5.0	38.7
Ni	0.050±0.004	23	0.012±0.004	5.5	0.016±0.005	7.3	35.8
Pb	0.046±0.002	23	0.007±0.001	3.5	0.42±0.04*	0.24	26.7
Zn	5.43±0.03	54	0.25±0.02	2.5	0.504±0.006	5.1	61.6

Mean± S.D. (n = 3)
^{a} – the percentage is calculated relative to the total concentration
^{b} —concentration in ng g^{-1}

Table 6. Elements fractionation results for Breaburn apple samples

Elem.	Water-soluble		Polyphenol fraction		Lignin fraction		Total extracted percentage, %
	Concentration, μg g^{-1}	Extracted, %^{a}	Concentration, μg g^{-1}	Extracted, %^{a}	Concentration, μg g^{-1}	Extracted, %^{a}	
Cd	6.3±0.3^{b}	45	0.6±0.2^{b}	4.3	1.2±0.6^{b}	7.1	56.4
Co	3.9±0.4^{b}	19.7	0.42±0.05^{b}	2.1	0.2±0.1^{b}	1.0	22.8
Cr	0.35±0.02	70	0.060±0.005	11.4	0.096±0.003	19	100.4
Cu	1.28±0.04	48	0.52±0.03	19.5	0.22±0.04	8.2	75.7
Mn	0.58±0.05	43	0.083±0.004	19.5	0.20±0.03	14.8	77.3
Ni	0.027±0.004	24.5	6.7±0.5*	6.1	8.4±0.5	7.6	38.2
Pb	0.148±0.004	27	0.055±0.003	9	0.11±0.01	1.8	37.8
Zn	2.21±0.06	63	0.115±0.05	2.9	0.20±0.03	5.8	71.7

Mean± S.D. (n = 3)
^{a} – the percentage is calculated relative to the total concentration
^{b} – concentration in ng g^{-1}

interferences, 10 ng ml $^{-1}$ of each of the analyzed elements were added to all extract solutions (ethanol, acetone and n-octanol). The model solutions were analyzed using the same ICP-MS method like the real samples. The results of the recovery tests did not show significant losses of the analyzed elements regardless of the complicated sample preparation and were in the range of 97-100%.

The analytical results obtained for Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn concentrations in different fractions are presented in Table 5 and Table 6.

According to the results in Tables 5 and 6 the elements are mainly included in the water soluble fraction which indicates that they are predominantly inorganically bound. Worth mentioning is that the percentage of water soluble forms in Breaburn is higher than in Golden Delicious while the total concentrations were lower or similar (Table 4). For both apples varieties the highest percentage of water soluble fraction has Cr, followed by Zn, Cd, Cu, Mn and Ni.

Breaburn apples have higher percentage of elements in the polyphenol fraction as well. For both varieties Cu and Cr are most included in the fraction followed by Mn in Breaburn. The results for Zn do not support the conclusions in Zhang & Zhao (2015) about the affinity of Zn towards polyphenols. Most probably the polyphenol composition in the apples is different from those in tea leaves.

Similar results are obtained for the lignin forms. Cr is again with highest participation followed by Cu in Golden Delicious and Mn in Breaburn.

The total percentage of elements included in the three fractions is given in the last columns of Tables 5 and 6. Naturally highest percentage of incorporation has Cr followed by Cu and Zn. The lowest percentage is established for Co and Pb which indicates that they are probably present in insoluble

Table 7. Concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in 0.1 M pepsin-containing HCl and n-octanol fraction of fruit samples

Elem.	Fruit-Golden delicious		Fruit- Breaburn	
	0.1 M pepsin+ HCl	n-octanol	0.1 M pepsin+ HCl	n-octanol
	µg g^{-1}	µg g^{-1}	µg g^{-1}	µg g^{-1}
Cd	0.0061±0.0003	0.0146±0.001	0.003±0.001	0.007±0.001
Co	0.0045±0.0002	0.0035±0.0002	0.0061±0.0003	0.0031±0.0002
Cr	0.104±0.005	0.276±0.019	0.084±0.004	0.26±0.02
Cu	1.142±0.017	0.607±0.042	0.93±0.05	0.46±0.03
Mn	1.145±0.022	1.173±0.082	0.42±0.02	0.227±0.012
Ni	0.029±0.001	0.012±0.008	0.013±0.007	0.013±0.001
Pb	0.033±0.001	0.0092±0.0006	0.146±0.007	0.045±0.003
Zn	3.60±0.05	1.333±0.093	1.83±0.017	0.432±0.002

inorganic compounds. The fact that Cr is the element with highest percentage in the investigated fractions deserves attention. The role of Cr(III) as an essential element required for carbohydrate metabolism and beneficial for cardiovascular diseases (Cefalu & Hu, 2004) is seriously argued lately (Cefalu, 2007) since it is still unknown which is the useful form in which the element enters the body and performs the beneficial functions. Therefore the result that nearly 100% of the Cr in the apples is included in these three fractions (water soluble, polyphenol and lignin) may add useful information towards the problem.

Although all values were higher for Breaburn the most significant differences between the apple varieties were established for Mn (38.2 against 77.3%), Pb and Zn. This means that although similar, at least some of the elements in the apple varieties have different behavior.

Bioavailability analysis

Measurements of total elements concentration are not enough to predict bioavailability. The bioavailability of metal-containing species in food has been correlated with the extractability obtained by enzymolys is with gastric juice and intestinal juice (Özdemir & Güçer, 1998). Bioavailability depends largely on the ability of the ultimate physicochemical forms in which elements reach the absorption site to cross the intestinal barrier (Hocquellet & L'Hotellier, 1997).

To investigate the bioavailability of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn extractions with 0.1 M pepsin-containing HCl and n-octanol solvent were performed to establish the absorbed fraction of these elements in the stomach and intestine, respectively. The results are presented in Table 7. The calculations of the percentage absorbed Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in the stomach and intestine from Golden delicious and Breaburn apple samples are shown on Figure 1 and Figure 2.

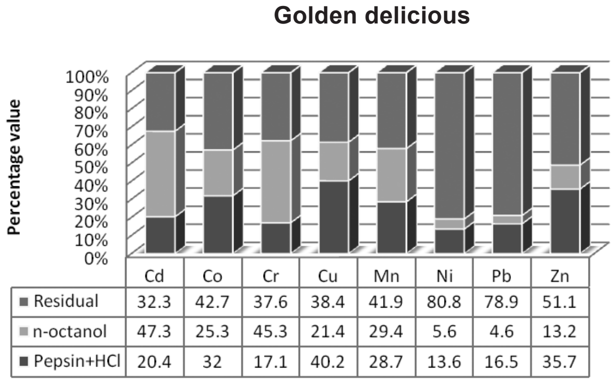

Fig. 1. Percentage absorbed Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in the gastrointestinal tract from Golden delicious apple sample using pepsin+ HCl and n-octanol
*Residual is calculated relative to the total concentration

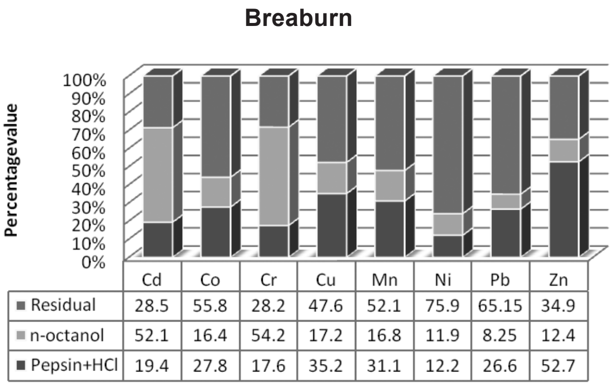

Fig. 2. Percentage absorbed Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in the gastrointestinal tract from Breaburn apple sample using pepsin+ HCl and n-octanol
*Residual is calculated relative to the total concentration

Table 8. Concentration of the analyzed elements in gastric and intestinal phases obtained by PBET experiment

Elem.	Friut- Golden delicious			Fruit- Breaburn		
	Gastric phase	Intestinal phase	Residual phase	Gastric phase	Intestinal phase	Residual phase
	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$	$\mu g\ g^{-1}$
Cd	0.008±0.001	0.014±0.006	0.009±0.001	0.003±0.001	0.007±0.001	0.0046±0.0002
Co	0.004±0.001	0.003±0.001	0.006±0.002	0.005±0.001	0.003±0.001	0.010±0.001
Cr	0.10±0.02	0.26±0.03	0.24±0.06	0.086±0.004	0.20±0.02	0.187±0.009
Cu	1.21±0.06	0.97±0.04	0.64±0.02	1.197±0.059	0.85±0.04	0.59±0.03
Mn	1.26±0.06	1.57±0.07	1.16±0.04	0.37±0.02	0.34±0.02	0.62±0.03
Ni	0.032±0.001	0.021±0.009	0.166±0.007	0.014±0.001	0.0094±0.0002	0.08±0.01
Pb	0.036±0.008	0.006±0.001	0.157±0.003	0.146±0.007	0.033±0.001	0.37±0.02
Zn	3.66±0.18	2.66±0.09	3.77±0.05	1.62±0.08	0.77±0.04	1.08±0.05

The results indicate that over 65 % of Pb and 75% of Ni are not absorbed in the gastrointestinal tract. The absorption in Golden Delicious is even less than in Breaburn. Over 50% of Co and Mn in Breaburn and around 40 % in Golden Delicious are also not absorbed.

Highest absorption in the stomach is established for Cu and Zn while Cd and Cr are predominantly absorbed in the intestines. Therefore it might be presumed that Cu and Zn are present mainly in polar and ionic groups while Cd and Cr are mainly as molecular and complex structures.

Bioaccessibility of metals from apple fruits using PBET test

The bioaccessibility of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in the human body was evaluated using PBET experiment. The apple fruits were treated with gastric and intestinal juices and then the gastric and intestinal fractions were collected and analyzed by ICP-MS. The bioaccessible concentrations together with the metal concentrations in the residual phase are reported in Table 8. The data are also presented as the percentage bioaccessibility of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in Figure 3 and Figure 4 for Golden Delicious and Breaburn, respectively.

The PBET experiment established that Ni, Pb and Co are least absorbed in the gastrointestinal tract. The highest percentage for gastric absorption have Cu and Zn while highest intestinal absorption was found for Cd and Cr. Zn has almost equal distribution. According the total percentage of absorption in the gastrointestinal tract the elements may be arranged in the following order: Cu > Zn ≥ Cd >Mn ≥ Cr > Co > Ni ≥ Pb. Similar results were published in Intawongse & Dean (2008) who analyzed bioaccessibility of Cr, Cd, Cu, Fe, Mn, Mo, Ni, Pb and Zn from vegetables in the gastrointestinal tract.

Figure 5 presented the calculated total percentage bioavailability of analyzed elements in the human body using PBET. It shows that very high bioaccessibility in human gastrointestinal tract was observed for Cu, Mn, Zn and Cd, while for Ni and Pb only 20÷30% of the total concentrations were bioavailable.

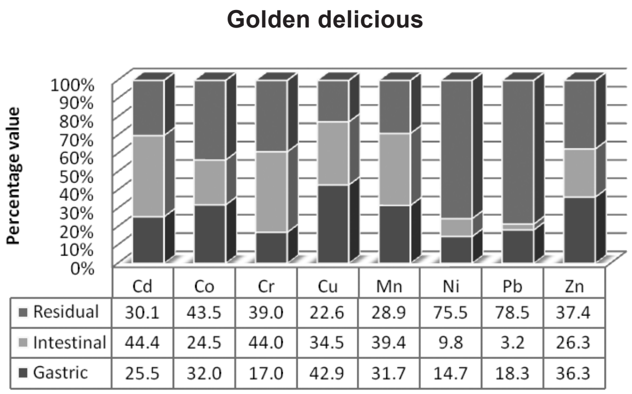

Fig. 3. Bioaccessibility of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in Golden delicious apple sample

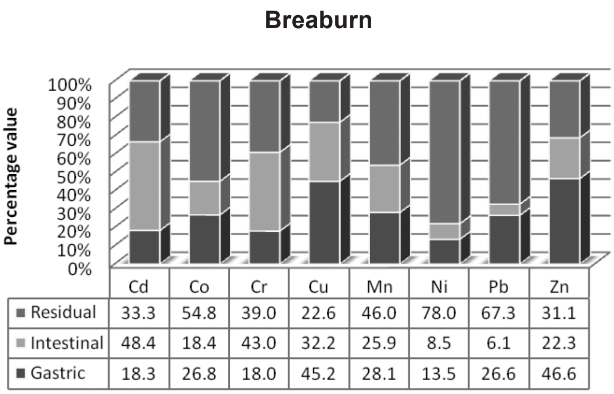

Fig. 4. Bioaccessibility of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in Breaburn apple sample

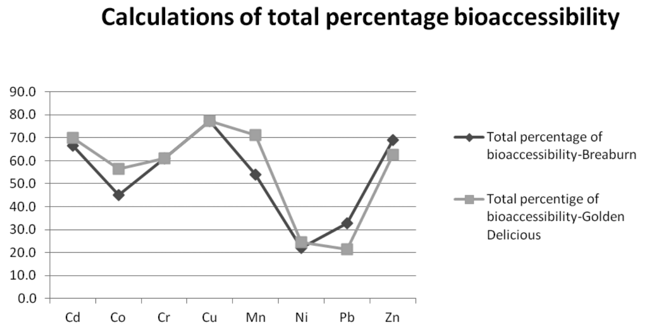

Fig. 5. Total percentage of bioaccessibility of Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn in Breaburn and Golden Delicious

Although it cannot be assumed that the elements exist in the apples only in the fractions investigated here (with the exception of Cr) a comparison between the total extraction % (Tables 5 and 6) and total absorption in the tract shows that Co and Cd have higher % of total absorption than the available quantities established during the extractions which means that they obviously exist in other than the investigated forms. Cu, Pb and Zn have similar % of availability and absorption while Cr and Ni have higher % of availability (results from extraction) than absorption.

From human health point the result that Pb in apples is mostly not absorbed in the gastrointestinal tract is important. However contrary to Pb, Cd has high bioaccessability therefore its concentration in apple fruits especially from polluted areas should be controlled.

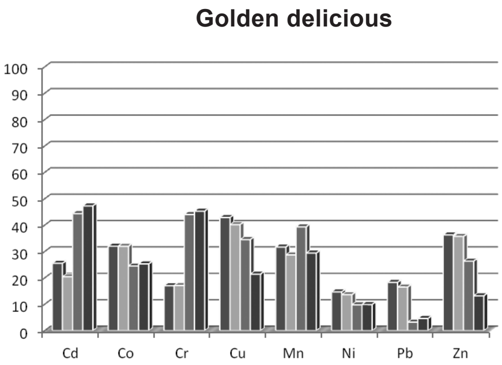

Fig. 6. Comparison of the percentage of bioavailability of the analyzed elements from Golden delicious apple fruit obtained by the extraction with pepsin-HCl, n-octanol and PBET test

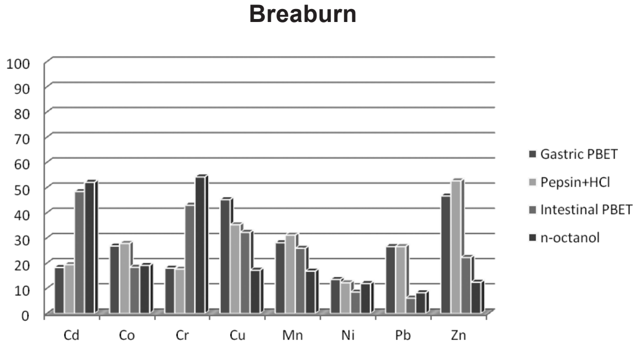

Fig. 7. Comparison of the percentage of bioavailability of the analyzed elements from Breaburn apple fruit obtained by the extraction with pepsin-HCl, n-octanol and PBET test

Comparison between PBET and extraction with 0.1 M pepsin-containing HCl and n-octanol

The comparison between the results obtained in the procedure using HCl and n-octanol and PBET test for bioaccessibility is shown in Figure 6 and Figure 7 in percentages

In both procedures there were no significant differences for the gastric absorption in the percent extraction of the analyzed elements using the gastric PBET and pepsin+ HCl. But in the second stage (intestinal absorption) when n-octanol was used results for Cu, Mn, and Zn were significantly lower than intestinal PBET. It is difficult to recommend which results is better reflecting the real situation but the existence of such difference between the tests should be kept in mind. The results for Cr for intestinal absorption only for Breaburn show that the PBET test provides 11% higher bioaccessability. Probably there is some difference in the forms of Cr in both apple varieties.

Conclusion

The results from the extraction procedures applied to two apple varieties show that the investigated elements are present mainly in the water soluble fraction. Cu and Cr are included with highest percentage in the polyphenol and lignin fractions compared to the other elements. According the results from the bioaccessability tests over 65% of Pb and Ni are not absorbed in the gastrointestinal tract. Zn and Cu are absorbed mainly in the stomach while Cr and Cd – in the intestines. Highest bioaccessability (about 70%) was established for Cu, Zn and Cd, the latter deserves special attention especially in polluted areas. The comparison between the PBET and extraction with pepsin + HCl and n-octanol indicate similar results concerning stomach absorption and higher results for intestinal absorption of Cu, Mn and Zn using PBET which should be considered in absorption evaluation studies.

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