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Determination of Major-to-Trace Minerals and Polyphenols in Different Apple Cultivars

Doina TODEA[1], Oana CADAR[2*], Dorina SIMEDRU[2], Cecilia ROMAN[2], Claudiu TANASELIA[2], Iuliu SUATEAN[3], Anca NAGHIU[2]

[1] *"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Medicine, Pneumology Department, Cluj-Napoca, Romania; dtodea@umfcluj.ro*
[2] *INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath, 400293 Cluj-Napoca, Romania; oana.cadar@icia.ro (*corresponding author)*
[3] *University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur, 400372 Cluj-Napoca, Romania; isuateani@yahoo.com*

Abstract

The aim of this study was to investigate the concentration level of some major-to-trace elements and toxic minerals and polyphenols (catechin, epicatechin and quercetin) in fruits belonging to different apple cultivars ('Auriu de Cluj', 'Florina', 'Generos', 'Golden Delicious', 'Prima', 'Productiv de Cluj' and 'Starkrimson'), under specific climate conditions to northwestern Romania. The apple minerals were determined by the inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES) after a preliminary, microwave-assisted, acid digestion. The polyphenols were determined by high performance liquid chromatography (HPLC) with MS/MS detection. According to the obtained results, the edible part of the apple contains many minerals with high relative nutritional value (RNV). The major minerals (Na, K, Ca, Mg and P), minor elements (Cu, Fe, Mn, Sr and Zn) and toxic trace elements (Cd, Cr and Pb) were comparatively identified and quantified. It was found that K (31976 mg/kg) was predominant among the major elements, while Fe (3.68 mg/kg) and Cu (3.57 mg/kg) were comparable. The toxic trace metals (Cd, Cr and Pb) were below the limits of detection (0.01, 0.10 and 0.01 mg/kg, respectively) and did not determine any health risk to consumers. The main polyphenols composition (catechin, epicathechin, quercetin) differs slightly among different cultivars. In 'Idared' and 'Jonathan' cultivars, the investigated polyphenols are in higher quantities comparing to the others. In all investigated apple cultivars, quercetin was proved to be the major polyphenol, followed by epicathechin and catechin. These results suggest that, regarding only the investigated polyphenols, 'Idared' and 'Jonathan' cultivars have the highest antioxidant character in all investigated genotypes.

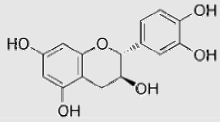
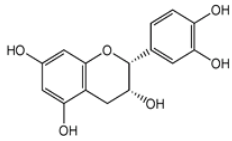
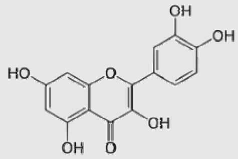
Keywords: apple fruits, multi-elements, polyphenols

Introduction

Apples are the second most produced tree fruits (apple crop is estimated at 69 million metric tonnes/year) in the world, after bananas (FAO, 2011). Apples represent an important part of the human diet because they are a good source of fiber, including the soluble fiber pectin, Vitamin A, Vitamin C, minerals and polyphenols (Wu *et al.*, 2007). Besides 75-95% water, the edible portion of apple contains an important quantity of phenolic compounds, which contribute to the flavor and can protect the human body from free radicals and reactive oxygen species (Cindrić *et al.*, 2012; Hyson, 2011; Pandey *et al.*, 2009).

Numerous studies on apples have been carried due to their nutritional importance, mainly regarding their acid, amino acid, mineral, vitamin and polyphenol contents (Auclair *et al.*, 2008; Vieira *et al.*, 2009; Ko *et al.*, 2005). Approximately 30 elements are considered essential to life. Essential nutrients which are needed in trace and ultratrace quantities (Cu, Fe, Ni, Zn, Mn, Co, Cr, and Se), are as important for life as macronutrients (Ca, K, Mg and Na) (Cindric *et al.*, 2011). However, some essential elements may become toxic when their concentration increases. The polyphenol family (phenolic acids, stilbenes, chalcones, coumarins, cromones, lignans, flavonoids, isoflavonoids, neoflavonoids and tannins) has been shown to possess significant antioxidant capacities, while they are maintaining low toxicities (Escudero *et al.*, 2008). Apple polyphenols have been registered among Japanese Standard Food Additives as antioxidants due to their strong antioxidant activity (Enomoto *et al.*, 2006). Between the apple polyphenols, flavonoids such as epicatechin, catechin and quercetin (Tab. 1) are the most important being widely distributed in natural products and medicinal herbs (Chang and Wu, 2011). Catechin and epicatechin have antioxidant,

Tab. 1. Basic information of selected polyphenols

Chemical name	Chemical class	Structural formula	Molecular weight (g/mol)
Catechin	Flavan-3-ol		290.27
Epicatechin	Flavan-3-ol		290.27
Quercetin	Flavonol		302.23

anticancer, antiangiogenic, antimutagenic, hypocholesterolemic, anti-ageing, anti-diabetic, antibacterial, anti-HIV and anti-inflammatory effects (Chang and Wu, 2011). Quercetin exhibits a variety of biological activities, including cardiovascular protection and anticancer, anti-inflammatory and antioxidant activities (Wang *et al.*, 2005).

In order to obtain good quality data, several aspects such as: sample preparation, techniques used to identify the targeted compounds and the variables which influence the quality of the apple, must be considered.

Sample preparation is an important step in elemental analysis. Microwave-assisted digestion is characterized by short digestion time, small volume of reagent consumption, lower losses of volatile metals, good recoveries, accuracy, reproductibility and enhanced operator safety (Krushevska *et al.*, 1993; Lamble *et al.*, 1998; Bocca *et al.*, 2007). ICP-AES is a rapid and accurate technique for the simultaneous determination of the minor and major element contents in apples and apple juices as well. ICP-MS technique has become to be considered as the most powerful analytical tool for simultaneous trace elemental analysis of food samples due to its extreme sensitivity, selectivity and last but not the least due to its multielemental and isotopic capability (Barnes, 1999; Davidowski *et al.*, 2009; Froes *et al.*, 2009; Zeiner *et al.*, 2010; Cindrić *et al.*, 2012).

Liquid chromatography tandem mass spectrometry (LC/MS/MS) in the multiple reaction monitoring (MRM) mode has been recognized as a powerful analytical tool due to its high sensitivity and short run time (Hossain *et al.*, 2010). Through this technique, less toxic organic solvents are used as mobile phase and is the most free of interferences compared to other chromatographic techniques (Hossain *et al.*, 2010; Weingerl *et al.*, 2009).

The chemical composition of the apples is a good indicator of their quality, consumer acceptability and the health status of consumers. There are many factors that influence the chemical composition and the nutritional value of apples, such as: apple variety, soil quality, production area, farming practices, the quality of irrigation water, local climate conditions, storage and commercialization conditions. In order to avoid the influence of these factors, the samples were collected from only one farm, during one harvest/year and not representative for the market. In Central Transylvania, Romania, the most famous and the most consumed apple cultivars were: 'Golden Delicious', 'Gustav', 'Jonathan', 'Kaltherer', 'Starkrimsom'. These old apple cultivars have become well adapted to the soil and climate conditions producing good yields and high-quality fruits. The renewal process of apple variety seems to be slow and difficult both due to high costs of a new apple orchard and the attitude of farmers/consumers. The most well-known and spreaded cultivars of apples created at Fruit Research Station Cluj-Napoca were 'Aromat de vara', 'Ardelean', 'Ancuta', 'Feleac", 'Rosu de Cluj', 'Auriu de Cluj', 'Estival' and 'Productiv de Cluj' (Sestras *et al.*, 2006; Militaru *et al.*, 2013).

The aim of this study was to determine the content of macro- (Na, K, Ca, Mg and P), micro- (Cu, Fe, Mn, Sr and Zn) and toxic trace elements (Cd, Cr and Pb) and polyphenols (catechin, epicatechin and quercetin) of ten cultivars of apple fruits, under the climate conditions of Cluj-Napoca area, Romania.

Materials and methods

Sampling

The fruit samples, 10 cultivars of apple ('Auriu de Cluj', 'Florina', 'Generos', 'Golden Delicious', 'Idared', 'Jonagold', 'Jonathan', 'Prima', 'Productiv de Cluj' and 'Starkrimson'), were collected from a private orchard, located near Cluj-Napoca, during September 2012 and 2013. Cluj-Napoca, being located in the northwestern part of Romania, has a continental climate, characterized by warm, dry summers and cold winters.

Reagents and CRMs

All reagents used for this research, were of p.a. grade and purchase from Merck, Darmstadt (H_2O_2 30%, HNO_3 65%, ICP Multielement standard IV). The certified reference material (NIST-SRM 1515 apple leaves) was obtained from

LGC Standards GmbH, Wessel, Germany. Catechin, epicatechin and quercetin were purchased from AppliChem (USA). Methanol LC-MS Optigrade (≥99.8%), Ethanol LC-MS Optigrade (≥99.8%) and Ortophosphoric acid ULC-MS Optigrade (99-100%) were acquired from LGC Standards. All glassware was cleaned with nitric acid prior to use. For all dilutions ultrapure water (18.2 MΩ/cm) obtained from a Millipore Direct-Q3 UV system (Millipore, Molsheim, France) was used.

Sample preparation

Fruits were picked at harvest maturity as it is recommended for fruits that are marked out for storage. Fruit samples consisted of five individual apple fruits picked from the same tree of the investigated orchard. The fruit samples were washed first, several times with deionized water to remove the dust particles and then dried at 105 °C for 24 h and homogenized. The dried samples were grounded, homogenized using a metal-free mortar and stored in polyethylene bags until acid digestion.

Per sample, 0.5 g was weighted accurately in a dry, clean PTFE vessel; 6 mL of HNO_3 65% and 3 mL of H_2O_2 30% were added to each sample and the vessel content was kept 4 h at room temperature before digestion. After this, the samples were digested using a four-step digestion program (time [min] / power [W] / T [°C]: 10/700/170; 15/1000/200; 10/1000/160; 10/1000/100; 10 min ventilation). After mineralization, clear solutions were cooled at room temperature, and then quantitatively transferred to 25 mL volumetric flasks and diluted to the mark with deionized water. Certified reference material NIST-SRM 1515 apple leaves and blank, consisting of deionized water and reagents, were prepared in the same way as the samples. Three replicate measurements were made for each sample.

In order to determine the polyphenol content, the edible portion of the fruit was sliced into small pieces with a ceramic knife and lyophilized to avoid the degradation of the samples. 100 g of each dried vegetable product were extracted for 5 hours with $EtOH:H_2O$ (90/10, v/v) using a Soxhlet. The extract was dried under constant flow of compressed nitrogen gas, and reconstituted in mobile phase before injection. The recovery degree for all three polyphenols was situated in 77-94% range and was measured using internal standard addition.

Instrumentation

For the microwave digestion of samples, a closed vessel microwave system Berghof MWS-3+ (Eningen, Germany) was used. The contents of Ca, Mg, Na, K, Fe and P were determined by ICP-OES (OPTIMA 5300 DV, Perkin Elmer, Norwalk, USA), while those of Cd, Cu, Mn, Pb, Sr and Zn by ICP-MS (ELAN DRC II, SCIEX, Perkin Elmer, Toronto, Canada). The calibration standards were prepared by diluting the stock multi-elemental standard solution (1000 mg/L) in 2% HNO_3 and the calibration ranges were modified according to the expected concentration values of investigated elements.

The LC/MS/MS analysis were carried out by a HPLC Agilent 1200 Series coupled with an Applied Biosystems API 3200 QTRAP mass spectrometer. The LC (liquid cromatograph) parameters are: Injection volume: 50 µl; Flow rate: 500 µl/min; Chromatographic column: Synergi Hydro RP 150 ×4.6 mm, 4 µm, Phenomenex, Torrance, California, USA; Column temperature: 33 °C; Mobile phase: A: H_2O+0.1% ortophosphoric acid; B:MeOH; Gradient program (Tab. 2). The MS/MS parameters are presented in Tab. 3.

Tab. 2. Gradient flow for LC column separation of catechin, epicatechin and quercetin

Total time (min)	Flow rate (µl/min)	A (%)	B (%)
0.00	500	90.0	10.0
25.00	500	10.0	90.0

Tab. 3. Detection parameters (compound and source dependent) for catechin, epicatechin and quercetin

Compound	Q1 Mass (u)	Q3 Mass (u)	DP (V)	EP (V)	CE (V)	CXP (V)
Catechin	288,8	190,9	-45	-5	-10	-4
Epicatechin	288,9	244,9	-40	-7.5	-22	0
Quercetin	300,9	150,8	-45	-5.5	-30	-2

FIA parameters
CUR:10.00 psi; CAD:Medium; IS:-4500.00V; TEM:600 °C; GS1:35 psi; GS2:20 psi.

Tab. 4. Analysis of certified reference material (NIST-SRM 1515-Apple Leaves)

Element	Certified value[a] (µg/g)	Obtained value (µg/g)	Recovery (%)
Cd	0.013 ± 0.002	0.012 ± 0.002	92.3
Cu	5.64 ± 0.24	5.41 ± 0.07	95.9
Fe	83.0 ± 5.0	81.5 ± 1.1	98.2
Mn	54.0 ± 3.0	49.2 ± 1.0	91.0
Pb	0.470 ± 0.024	0.442 ± 0.024	94.0
Zn	12.5 ± 0.3	10.9 ± 1.0	87.2
Ca (%)	1.526 ± 0.015	1.432 ± 0.102	93.8
K (%)	1.61 ± 0.02	1.42 ± 0.07	88.2
Mg (%)	0.271 ± 0.008	0.272 ± 0.081	101

[a]Information values in brackets

Results and discussions

Major, minor and toxic trace elements

The quality of the analytical procedure was assured using a Standard reference material (NIST-SRM 1515-Apple Leaves). The obtained values (Cd, Cr, Cu, Fe, Pb, Zn, Ca, K, Mg and Na) of CRM draw against certified values indicating that the measured values correlate well with certified values. Limits of detection (LOD) of every studied element were experimentally calculated as three times the standard deviation of ten measurements of independent reagent blank solutions. The obtained values (Cd, Cu, Fe, Mn, Pb, Zn, Ca, K and Mg) of CRM draw against certified values indicate that the measured values correlate well with certified values (Tab. 4).

Apples are considered a good source of dietary minerals. The range of concentration (mean ± SD, mg/kg d.w.) of major and minor elements in 10 apple cultivars is given in Tabs. 5 and 6. According to the obtained results, in the edible

Tab. 5. Mean concentration of major elements in apple samples (mg/kg d.w.)

Sample	Year	Na	K	Ca	Mg	P
'Auriu de Cluj'	2012	9.22±1.23	25660±2356	286±112	271±67	653±111
	2013	7.90±1.56	22530±3242	190±103	276±89	665±102
'Florina'	2012	9.30±2.12	29731±3258	347±98	295±78	861±211
	2013	8.18±1.78	32442±2345	423±87	312±65	745±278
'Generos'	2012	10.9±2.12	30123±3215	321±110	286±110	521±113
	2013	8.58±1.78	28855±3012	413±102	428±142	730±234
'Golden Delicios'	2012	14.9±2.12	41340±4012	274±113	284±113	877±235
	2013	11.6±1.12	40620±3121	294±110	345±123	759±243
'Idared'	2012	10.2±2.31	38630±1211	356±102	340±78	661±213
	2013	8.61±1.11	32030±1989	330±87	276±56	673±214
'Jonagold'	2012	10.8±2.11	32441±2312	206±110	266±145	582±225
	2013	8.00±1.87	31130±3211	604±236	467±213	593±235
'Jonathan'	2012	10.3±1.45	33450±1987	267±87	285±110	666±236
	2013	8.63±2.01	35300±2113	256±98	433±134	678±235
'Prima'	2012	8.64±1.89	21122±2345	246±134	285±108	475±114
	2013	8.46±2.11	28671±2345	145±98	401±132	483±235
'Productiv de Cluj'	2012	10.3±1.11	32035±2112	246±45	306±213	533±245
	2013	9.93±1.23	38834±3121	414±87	435±211	543±213
'Starkrimson'	2012	9.38±1.23	31126±2345	267±110	271±108	711±325
	2013	8.60±2.01	33440±2356	359±104	365±98	724±112
Mean values		9.62	31976	312	331	657
Standard dev.		1.63	5366	102	66.4	113

Tab. 6. Mean concentration of minor elements in apple samples (mg/kg d.w.)

Sample	Year	Cu	Fe	Mn	Sr	Zn
'Auriu de Cluj'	2012	4.02±1.02	3.24±0.96	1.69±0.59	0.857±0.331	1.15±0.63
	2013	2.34±1.12	2.84±0.89	1.05±0.75	0.451±0.292	2.12±0.56
'Florina'	2012	3.49±1.13	2.55±0.45	1.98±0.65	0.640±0.451	1.85±0.78
	2013	2.99±1.23	3.30±0.89	1.45±0.45	0.384±0.112	2.75±0.98
'Generos'	2012	3.54±0.98	5.00±1.11	1.80±0.78	0.944±0.653	2.20±1.12
	2013	2.94±0.99	2.84±1.23	0.70±0.72	0.406±0.122	1.98±0.89
'Golden Delicios'	2012	6.14±1.24	5.44±0.89	1.63±0.65	0.856±0.234	3.05±1.25
	2013	4.84±1.35	3.05±0.67	1.10±0.48	0.454±0.112	2.55±1.23
'Idared'	2012	2.09±0.78	2.70±0.56	2.89±0.96	1.16±0.34	1.00±0.56
	2013	3.94±1.12	3.26±0.78	1.15±0.45	0.505±0.235	1.88±1.01
'Jonagold'	2012	3.11±1.56	3.24±1.12	1.95±0.65	0.665±0.112	0.88±0.23
	2013	3.24±0.75	3.81±1.10	2.10±0.43	0.353±0.098	1.98±1.12
'Jonathan'	2012	3.24±0.68	3.11±0.89	1.78±0.65	0.577±0.101	1.05±0.89
	2013	4.54±1.13	5.41±1.13	2.25±0.78	0.534±0.089	2.47±1.23
'Prima'	2012	2.32±0.78	4.98±0.75	2.00±0.99	0.748±0.231	1.48±0.75
	2013	4.23±1.89	4.92±1.14	2.01±0.78	0.751±0.320	1.49±0.97
'Productiv de Cluj'	2012	3.12±1.12	2.57±1.03	1.68±0.45	0.486±0.114	1.47±0.56
	2013	5.04±1.13	3.69±0.89	3.20±0.59	0.490±0.105	2.33±1.12
'Starkrimson'	2012	2.14±0.87	3.81±0.92	1.94±1.00	0.872±0.212	2.45±0.78
	2013	4.12±1.14	3.95±1.12	1.95±0.89	0.873±0.321	2.42±1.25
Mean values		3.57	3.68	1.81	0.65	1.93
Standard dev.		1.05	0.96	0.58	0.22	0.62

part of the apple, the concentration for the major-elements varies in the following way: K > Ca > P > Mg > Na and Fe > Cu > Zn > Mn > Sr for the micro-elements, respectively.

Major elements

Among the major elements examined in apple fruits, K was the most abundant with an overall mean concentration of 31976 ± 5366 mg/kg. This result was comparable with the data reported for apple fruits by Cindric *et al.* (2012) (38600 mg/kg) and higher than those determined by Hamurcu *et al.* (2010) - 863 mg/kg, Horsley *et al.* (2014) - 10226 mg/kg, and Ozcan *et al.* (2012) - 7000 mg/kg. The obtained values for Na (9.62 ± 1.63 mg/kg) were similar to those reported by Cindric *et al.* (2012) - 9.92 mg/kg, and lower than those determined by Humurcu (37.0 mg/kg) and Ozcan (600 mg/kg). Adults should consume 4700 mg K/day and 1500 mg Na/day (deMan, 1999). Apple fruits may be a good source of potassium, which plays an important role in the human body, especially in the transmission process of nerve signals, but also in fluid balance and proper function of heart, muscles, kidney and hormones. Sodium plays a key role in muscle and nerve function and work with potassium to coregulate ATP and fluids.

In both years, the highest values for K and Na were observed in 'Golden Delicious' (41340 mg/kg K and 14.9 mg/kg Na - 2012) and (40620 mg/kg K and 11.6 mg/kg Na - 2013), while the lowest in 'Prima' (K: 21122 mg/kg; Na: 8.64 mg/kg – 2012) and 'Auriu de Cluj' (K: 22530 mg/kg and 7.90 mg/kg Na - 2013).

Calcium and magnesium also play an essential role in muscle function, nerve transmission, bone and teeth formation and hormone secretion; furthermore, magnesium is required for processing ATP. The Recommended dietary allowance, RDA (average daily dietary intake level sufficient to meet the nutrient requirements of nearly all, 97-98% healthy individuals in a particular life-stage or gender group) for adults is 1000 mg Ca/day, and 400 mg Mg/day (deMan, 1999). It should also have to be considered the role of calcium to the maintenance of fruit ripening and optimum quality during postharvest storage. This role is enhanced directly in the prevention of specific disorders (bitter pit) and in relationships between calcium and general quality properties (flesh firmness) (Ferguson and Watkins, 1982). The content of Ca (312 ± 102 mg/kg) and Mg (331 ± 66.4 mg/kg) were ten times lower than the reported results for apples (4410 mg/kg-Ca and 3140 mg/kg) by Cindric *et al.* (2012).

Regarding to the annual variations, the concentration of Ca and Mg were highest in 'Idared' (356 mg/kg Ca and 340 mg/kg Mg - 2012) and 'Jonagold' (604 mg/kg Ca and 467 mg/kg Mg - 2013), while the lowest was in 'Jonagold' (206 mg/kg Ca and 266 mg/kg Mg - 2012), Prima (145 mg/kg Ca-2013), 'Auriu de Cluj' and 'Idared' (276 mg/kg Mg - 2013).

Phosphorous is a very important component of the bones and cells having an important role in protein production to satisfy the needs of the human body. The RDA for daily phosphorous for adults is 700 mg P/day (deMan, 1999). The obtained values for P (657 ± 113 mg/kg) were similar to those obtained by Hamurcu *et al.* (2010) - 700 mg/kg and Horsley *et al.* (2014) - 683 mg/kg. In both years, 'Golden Delicious' exhibited the highest content of P (877 mg/kg - 2012 and 759 mg/kg - 2013), while 'Prima' exhibited the lowest content (475 mg/kg – 2012 and 483 mg/kg – 2013).

Regarding the annual variation on minor and major element concentrations, the obtained mean concentration was similar to those from both years. 'Golden Delicious' apple had the highest average content of major elements.

One apple (~300 g fresh weight or 40 g dry matter) can offer up to 1279 mg K (27.2% RDA), 0.38 mg Na (0.03% of RDA), 12.5 mg Ca (1.25% of RDA), 13.3 mg Mg (3.31% of RDA) and 26.3 mg P (3.75% of RDA). As a result, major elements were found to have good nutritional contribution in accordance to RDA.

Minor elements

The minor-elements also support important functions of the human body. Iron is necessary to produce hemoglobin and myoglobin, proteins that carry oxygen in the human body. Manganese, one of the vital important elements, enhances the absorption of calcium and plays an important role in the production of bones and connective tissues. Chromium enhances the action of insulin, making it important in blood sugar regulation processes. The human body needs copper and zinc as well, to produce enzymes involved in protection against oxidative processes. Strontium can improve the cellular make-up of bones and teeth by preventing tooth decay of soft bones (WHO, 2006; Vicente *et al.*, 2014).

In both years, the concentrations of investigated minor elements in all apple samples were: Cu (3.57 mg/kg), Fe (3.68 mg/kg), Mn (1.81 mg/kg), Sr (0.65 mg/kg) and Zn (1.93 mg/kg). According to RDA of investigated minor elements (Cu – 1.2 mg/day, Fe - 8.0 mg/day, Mn – 2.3 mg/day, Sr – not stated, Zn – 11.0 mg/day; deMan, 1999), one apple can provide 0.143 mg Cu (11.9% of RDA), 0.147 mg Fe (1.84% of RDA), 0.073 mg Mn (3.16% of RDA) and 0.077 mg Zn (0.70% of RDA).

Toxic trace elements

In all samples, the contents of heavy metals (Cd, Cr and Pb) were below the limit of detection (0.01, 0.10 and 0.01 mg/kg, respectively). European legislation (1881/2006/EC setting maximum levels for certain contaminants in foodstuffs) regulates the maximum admitted concentration of Pb (0.10 mg/kg wet weight) and Cd (0.05 mg/kg wet weight) in fruits. The obtained concentrations of Pb and Cd

Tab. 7. Concentrations (mg/100g d.w.) of catechin, epicatechin and quercetin, determined in different cultivars of apple fruits

Cultivar	Concentration (mg/100g d.w.)		
	Catechin	Epicatechin	Quercetin
'Auriu de Cluj'	0.55	6.02	9.53
'Florina'	0.58	6.42	12.21
'Generos'	0.68	5.93	10.44
'Golden Delicios'	0.62	6.03	10.02
'Idared'	0.83	7.83	12.69
'Jonagold'	1.04	9.87	14.33
'Jonathan'	0.71	5.88	10.69
'Prima'	0.49	5.65	9.84
'Productiv de Cluj'	0.44	6.21	8.99
'Starkrimson'	0.52	5.81	10.25

in all apple samples were lower than the values set by European legislation, indicating no harmful impact on human health by the consumption of the apples grown in this part of Romania.

Polyphenols content

Three apple polyphenols, namely catechin, epicatechin and quercetin, were determined (Tab. 7). The concentrations of polyphenols were slightly different among cultivars, with some increases for 'Idared' and 'Jonathan'. Several other studies confirmed earlier that the content of polyphenols differs by variety and is affected by different factors such as: apple variety, fruit development, conditions during long-term storage, superficial scald development in apple fruit and solar radiation (Lachman *et al.*, 2006). Taking into consideration the fact that the investigated cultivars were selected from the same orchard, were grown under similar conditions and were sampled of their positions, it can be concluded that the differences in polyphenols quantity depends only on the apple variety.

In all investigated apple cultivars, quercetin was proved to be the major polyphenol, with concentrations from 8.99 to 12.21 mg/100 g d.w. The concentrations decreased to epicatechin (from 5.88 to 9.87 mg/100 g d.w) and to catechin (from 0.49 to 1.04 mg/100 g d.w). Similar results were obtained in other studies (Manach *et al.*, 2005).

A recent study (Randall, 2012) suggest that the daily dose of 300 mg to 600 mg of apple polyphenols may be all that is needed to obtain good health benefits. The present results suggest that catechin, epicatechin and quercetin from 100 g dried apples cover only 0.666% from the necessary dose. Manach *et al.* (2005) support the idea that the most abundant polyphenols in our diet, are not necessarily leading to the highest concentration of active metabolites in target tissues, the three investigated polyphenols being of great interest for our health.

Conclusion

The results of this study provide valuable data regarding major-to-trace elements and polyphenols in different apple cultivars, under climate conditions of Cluj-Napoca area, Romania. The investigated apple samples contain significant amounts of several essential elements and polyphenolic compunds as well. Apple consumption represents a valuable and a very important source of minerals and antioxidants. Toxic element concentrations (Cd, Cr and Pb) were below the limits of detection showing no harmful effects caused by these elements through the high consumption of apples. Regarding the annual variation on minor and major element concentrations, the obtained mean concentration were similar in both years. The LC/MS/MS method was proved to be a robust and useful technique for polyphenols' determination. The results obtained are of interest especially for the medical world due to the aminoacid characteristics of the investigated polyphenols. These data should prove useful especially for the medical world when designing anti-cancer, anti-angiogenic, anti-mutagenic, hypocholesterolemic, anti-ageing, anti-diabetic, anti-bacterial, anti-HIV and anti-inflammatory treatments.

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