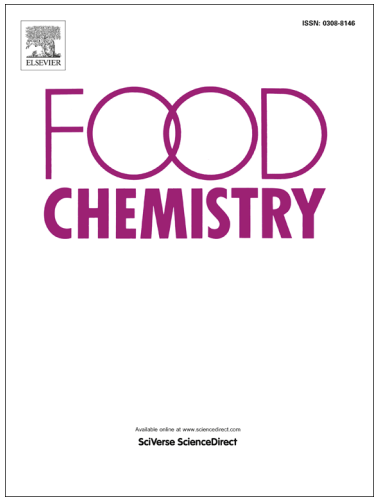

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Original Research Paper

Potential health benefits and quality of dried fruits: goji fruits, cranberries and raisins

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Abstract

Dried fruits are important snacks and additives to other foods due to their taste and nutritional advantages. Therefore there is an important goal to control the quality of the food on the market for consumer's safety. Antioxidant activity of goji fruits (*Lycium barbarum*), cranberries (*Vaccinium macrocarpon* and *oxycoccus*) and raisins (*Vitis vinifera*) were studied using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin-Ciocalteu assays. Cu, Mn and Ge influencing antioxidant activity were determined together with selected toxic metals (Cd, Ni and Pb). Contamination with fungi was studied by quantification of their marker - ergosterol and important mycotoxins (aflatoxins B1, B2, G1 and G2, and ochratoxin A) were also determined. Antioxidant activity of all tested dried fruits was confirmed with goji fruits being the most profitable for consumers. Contamination of the tested fruits with toxic metals and mycotoxins was low.

keywords: antioxidant activity; dried fruits; elements; ergosterol; LC-MS/MS; mycotoxins

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1. Introduction

Nowadays dried fruits become more and more popular in diet due to their pro-healthy compounds. Dried goji fruits (*Lycium spp.*), dried cranberries (*Vaccinium spp.*) and raisins (*Vitis spp.*) are added to cereals, snacks and other healthy foodstuffs. These fruits contain carbohydrates such as fiber and monocarbohydrates and antioxidants as flavonoids, phenolic acids, carotenoids and vitamins in concentrated form compared to fresh fruits (Bennett et al., 2011; Donno, Beccaro, Mellano, Cerutti, & Bounous, 2015). Regular consumption of appropriate quantity of dried fruits may reduce glycaemia and cardiovascular risk factors. These fruits showed antioxidant activity in a number of *in vitro* and *in vivo* studies (Anderson et al., 2011; Anderson & Waters 2013; Ishiwata, Yamaguchi, Takamura, & Matoba, 2004; Joung, Jung, Kim, & Ho-Kyung, 2008; Karadeniz, Durst, & Wrolstad, 2000; Williamson & Carughi, 2010).

Goji (goji berry or wolfberry) is the fruit of *Lycium barbarum* L. that is used in Asian countries as a traditional medicine food (Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013; Nogueira Nascimento, Menezes Silvestre, de Oliveira Leme, Seimi Nomura, & Naozuka, 2015). They possess a range of biological activates in *in vivo* studies such as exhibiting antiaging effects, neuroprotection, promotion of endurance, increasing metabolism, improved control of glucose and other diabetic symptoms, antiglaucoma effects, immunomodulation, antitumor activity, and cytoprotection (Amagase, Sun, & Borek, 2009; Zhong, Shahidi, & Naczk, 2013). Goji fruits are the source of polysaccharides, vitamin C, B complex and E as well as amino acids including the eight essential exogenous amino acids and polyphenols (catechins or hyperoside) and organic acids such as ferulic or chlorogenic acid and its derivatives (Donno et al., 2016a; Yang, Zhao, Chen, Chan, & Wu, 2015). They

also contain trace elements including zinc, iron, copper, calcium, selenium, and germanium (Nogueira Nascimento, Menezes Silvestre, de Oliveira Leme, Seimi Nomura, & Naozuka, 2015). The fruits are usually sold in open boxes and small packages in sun-dried form, although juices, concentrated extracts and infusions as well as capsules are also available (Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013).

Cranberries (*Vaccinium* genus) according to some reports have positive effects on the human health and additionally are very popular due to their specific taste (Neto, 2007; Vollmannova et al., 2014). They are sold mainly as dried and sweetened fruits and used to make juices, sauces and jellies. Cranberries are a rich source of polyphenols and due to their strong antioxidant and antimicrobial properties, cranberries can inhibit the growth of some pathogenic bacteria and other pathogens (Vollmannova et al., 2014). They are also a rich source of phenolic acids and flavonoids, which inhibit oxidative processes (Neto, 2007; Vinson, Zubik, Bose, Samman, & Proch, 2005).

Raisins are processed grapes dried with the use of heat of the sun, natural air drying or a mechanical process of oven drying (Fang et al., 2010). Some studies inform that the fruits may be a source of some vitamins such as vitamin B, minerals for example potassium, iron, calcium (Fang et al., 2010) as well as fiber and antioxidants (Karadeniz, Durst, & Wrolstad, 2000; Yeung, Glahn, Wu, Liu, & Miller, 2003; Zhao, & Hall, 2008). Additionally they are fat and cholesterol free, containing high amount of pure fructose that is easily digestible (Fang et al., 2010). Some Authors reported detectable levels of pesticide residues and mycotoxins (Aung, & Jenner, 2004; Juan, Zinedine, Moltó, Idrissi, & Mañes, 2008) as well as chemical pollutants such as heavy metals, which may contaminate raisins during production processes (Fang et al., 2010).

Therefore, the quality of the dried fruits available on the market can be different. The food which has been sold on the market should be safe for the consumers and possess high quality. The quality of fruits is measured by determination of the antioxidants by HPLC analyses (Donno et al., 2013, 2016b; Peev, Vlase, Antal, Dehelean, & Szabadai, 2007). Antioxidant activity of food products rich in antioxidants such as

phenolics or vitamins measured by DPPH assay could be a quick, cheap and easy method to check the quality of the product. It is also very important to determine such components of food as micro- and macro-elements. During the study on metals intakes it is necessary to distinguish between metals which positively affect human health such as Cu and Mn and those which are toxic and undesirable such as Pb or Cd.

Next compounds with high toxicity for human are mycotoxins. Microscopic fungi can infect agricultural crops at each stage of plant growth, storage and processing. Ergosterol - constituent of fungal cell wall, not occurring in higher plants - could be a good indicator of fungal presence (Horbik, Łowińska-Kluge, Górski, Stanisz, & Zgoła-Grześkowiak, 2013; Saxena, Munimbazi, & Bullerman, 2001; Stanisz, Zgoła-Grześkowiak, Waśkiewicz, Stępień, & Beszterda, 2015). Mycotoxins - secondary metabolites of microscopic fungi can occur in food products and influence on their quality (Wild, & Gong, 2010). Their toxicological effects on humans and animals can be either acute or chronic. Mycotoxins most often found in fruits and their processed products are aflatoxins and ochratoxin A. Among aflatoxins, aflatoxin B1 is the most toxic causing malignant tumors, mainly liver. The International Agency for Research on Cancer (IARC) classifies this toxin as a group I carcinogen for human (IARC, 2002). In turn, ochratoxin A as a possible human carcinogen (group 2B) exhibits hepatotoxic, genotoxic, teratogenic and mutagenic effects (IARC, 1993).

The first aim of the study was to analyze the antioxidant activity of methanol-water extracts prepared from dried goji fruits, cranberries and raisins. The next aim was to determinate the important elements including Cu, Mn, Ge, Se as well as toxic metals such as Cd, Ni and Pb. Ergosterol as microscopic fungal marker and mycotoxins as a secondary metabolite of fungi were also determined. As a result, both positive and negative aspects of the dried fruits consumption as a functional food in healthy diets were also shown.

2. Materials and methods

2.1. Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). Ergosterol, aflatoxins (B1, B2, G1 and G2) and ochratoxin A standards were purchased with a standard grade certificate from Sigma-Aldrich (Steinheim, Germany). MS-grade acetonitrile, methanol and pentane were supplied by POCH (Gliwice, Poland) and MS-grade formic acid was obtained from Sigma-Aldrich.

Working standard solutions of the elements were obtained by appropriate dilution of the stock standard solutions (1000 mg/L solution of Cd, Cu, Ge, Mn, Ni, Pb and Se in 0.5 mol/L nitric acid, Merck, Darmstadt, Germany). All working standard solutions were prepared daily; the appropriate stock solution was diluted with high-purity water. Chemical modifier solutions: palladium 10.0±0.2 g/L $Pd(NO_3)_2$, magnesium 10.0±0.2 g/L $Mg(NO_3)_2$ and phosphate 100.0±2 g/L $NH_4H_2PO_4$ were purchased from Merck. 65% (v/v) HNO_3 and 30% (v/v) H_2O_2 of the highest quality (Suprapur, Merck) were used for digestion of the samples.

High purity water: deionized and doubly distilled water (quartz apparatus, Bi18, Heraeus, Hanau, Germany) was also used throughout the experiments. The resistivity of the water was 18 MΩ cm.

2.2. Material

Sixteen dried fruit samples from different regions of the world were purchased in local shops. The geographical origin of the samples and their types were confirmed according to the labels of products. All goji fruits (*Lycium barbarum*) (samples G1-G5) were from China, additionally G5 came from organic (bio) farming. Cranberries came from Poland (*Vaccinium oxycoccus*) (samples C1, C2, C5), from the USA (*Vaccinium macrocarpon*) (samples C3, C4) and from Canada (*Vaccinium macrocarpon*) (sample C6 - from organic farming). Raisins (*Vitis vinifera*) were from Chile (sample R1), from Iran (samples R2, R3) and from Turkey (samples R4, R5). Raisins R5 came from organic farming. All samples were stored in a dark and dry place until analyses.

2.3. Determination of antioxidant activity

2.3.1. Extraction process for antioxidant activity assays

1 g of fruits was extracted by 10 mL of solvent mixture (methanol with double distilled water in proportion 1:1; v/v) for 30 minutes in ultrasound bath (Sono Swiss AG, Ramsen, Switzerland). After that the solution was decanted, and filtered through 0.45 µm polytetrafluoroethylene syringe filter from Agilent Technologies (Santa Clara, CA, USA) and finally diluted to proper volume (according to the antioxidant assay). The extracts of dried fruits were prepared directly before analysis.

2.3.2. DPPH assay

The ability of extracts from dried fruits to scavenge stable DPPH radicals was measured according to the method of Jeszka-Skowron, & Zgoła-Grześkowiak (2014). Briefly, the extract was diluted in methanol (1:10; v:v), and 1.0 mL of 0.5 mmol/L methanol solution of DPPH was mixed with 3 mL of the diluted extract. Then the mixture was incubated for 30 minutes at room temperature in the dark. The absorbance of samples against a reagent blank was measured at 516 nm. Antioxidant activity was expressed as percentage DPPH scavenging relative to control solution (1 mL of DPPH with 3 ml of methanol) using the following equation:

DPPH scavenging activity (%) =

((Absorbance of control - Absorbance of sample) / (Absorbance of control)) x 100 %

Methanol with water (1:1; v:v) as a blank sample was used. Trolox (20; 30; 40; 50; 60; 70; 80; 100; 120; 130 µg/ml) was used as a standard for the calibration curve (the linearity R^2 = 0.999; y = 0.6758x – 1.5205). The precision (RSD) was estimated as the ratio of the standard to the mean, RSD=2.94%. The results were expressed as mg of Trolox equivalent per g of dried fruits. The limits of detection and quantification were LOD=0.016 µg/ml and LOQ=0.050 µg/ml.

2.3.3. Folin-Ciocalteu assay

Reducing compounds/phenolic content of dried fruits extracts were determined using Folin-Ciocalteu reagent and slightly modified previously (Jeszka-Skowron, Sentkowska, Pyrzyńska, & Paz De Peña, 2016). Gallic acid (7; 14; 28; 35; 70; 105; 140 µg/ml) as an external standard was used (the linearity R^2 = 0.999; y = 0.0086x – 0.0069). Precision (RSD) estimated for this method amounted 4.03%.

The absorbance of samples against a reagent blank was measured at 754 nm after 1 hour of incubation in the dark. The results were expressed as gallic acid equivalent (GAE) in mg/g of dried fruits. The limits of detection and quantification were: LOD=3.65 µg/ml and LOQ=10.96 µg/ml.

All spectrophotometric determinations were performed with the use of Beckman UV-Vis Spectrophotometer 7500DU (Brea, CA, USA) with glass cuvettes of 1 cm length. Spectra were recorded in the range from 380 to 800 nm with 0.2 nm resolution. All determinations were carried out in triplicate.

2.4. The determination of micro- and macroelements

2.4.1. Instrumentation

The determination of selected elements (Cd, Cu, Mn, Ni, Pb and Se) was performed with the AAS 5EA spectrometer (ET AAS) (Analytik Jena GmbH, Jena, Germany) equipped with deuterium source background correction, a transversely heated graphite atomizer and the MPE5 autosampler. Pyrolytically coated graphite tubes were employed exclusively. Appropriate hollow cathode lamps (Photron, Australia) were used as the radiation sources.

The determination of Ge was conducted using the ContrAA 700 high-resolution atomic absorption spectrometer (HR-CS ET AAS) (Analytik Jena, Germany). The spectrometer was equipped with a transversely heated graphite atomizer, 300 W xenon short-arc lamp as a continuum radiation source, a compact high-resolution double echelle monochromator and a charge-coupled device (CCD) array detector.

Compressed argon of UHP 5.5 purity obtained from Air Products (Warsaw, Poland) was employed as a protective and purge gas. For wet digestion of certified reference materials as well as goji fruits,

cranberries and raisins samples a UniClever focused microwave sample preparation system (Plazmatronika, Wrocław, Poland) operating at 2450 MHz and 300 W maximum output was used. The computer-controlled system with continuous temperature, pressure and microwave power monitoring was equipped with high-pressure modified-PTFE vessel and water cooling system. The vessel capacity was 110 mL and the maximum pressure and maximum temperature were 100 atm and 300 °C, respectively. Fruits samples were ground in a laboratory mill IKA (Staufen, Germany) A11 basic designed with a grinding chamber made of Tefcel (PTFE, glass fiber-reinforced) with a stainless steel inlet.

2.4.2. Certified reference materials

The accuracy of the elements determination procedure was studied using several certified reference materials (CRMs) with the certified reference values of analytes (Cd, Cu, Mn, Ni and Pb) (Table 1). Due to the fact that Se has not been determined in the fruits samples (values below the LOD) Table 1 does not present the results of the determination of this element in the CRMs.

The following materials were chosen: SRM 1549 (Non-fat Milk Powder) from the National Institute of Standards and Technology (Gaithersburg, USA) and INCT-MPH-2 (Mixed Polish Herbs), INCT-PVTL-6 (Polish Virginia Tobacco Leaves), INCT-SBF-4 (Soya Bean Flour), INCT-TL-1 (Tea Leaves) from Institute of Nuclear Chemistry and Technology (Warsaw, Poland). All solid reference materials were used as bottled, without further grinding and sieving.

The dried fruit samples were dried in a laboratory (85°C, 24 hours), ground in a laboratory mill and digested without sieving (according to the procedure described in the section 2.4.3.)

2.4.3. Microwave-assisted digestion of CRMs and dried fruit samples

A microwave-assisted digestion procedure was carried out, in order to achieve a short digestion time and high efficiency of procedure (Altundag, & Tuzen, 2011; Jeszka-Skowron, Stanisz, & De Peña, 2016). Approximately 500 mg of powdered certified reference material or ground dried fruits sample was placed

in the vessel of the microwave digestion system and 1 mL of 30% H_2O_2 and 5 mL of 65% HNO_3 were added. For determination of Ge (absent in the CRMs) and Ni (absent in the SRM 1549) the appropriate amounts of analytes (in the form of standard solutions) were added to the materials in the vessel, before digestion. The heating time was applied as 20 minutes at 300 W. After digestion, the clear digested solution was transferred into 10 mL calibrated flask and diluted to volume with high-purity water. Before further analysis this solution was appropriately diluted depending on the concentration levels of the elements. A corresponding blank was also prepared according to the above microwave-assisted digestion procedure. It was performed to correct possible contamination from the reagents used for the sample preparation.

2.4.4. Atomic absorption spectrometry determination procedure

In the course of the study a graphite furnace was used for atomization of the analytes. In order to determine the elements in the fruit samples after digestion, 20 μL (or 30 μL for Se and Ge determination) of the solution was injected into the graphite tube for ET AAS determination under the optimized conditions (Table 2). In order to improve the removal of matrix without losing the analyte in the pyrolysis stage the selected chemical modifiers were used. Calibration was performed by the method of standard additions. The mean blank value, if necessary, was subtracted from the sample value after all calculations. Operating conditions of the ET AA spectrometer used for determination of elements in the samples after microwave-assisted digestion are shown in Table 2. The concentration detection limits were defined as 3 times the standard deviation of the blank signal (n = 10) divided by the slope of each calibration graph. LODs as well as the precision, expressed as relative standard deviation (%), are shown in Table 2.

2.5. The determination of ergosterol

The determination of ergosterol in dried fruits was done as described previously (Horbik, Łowińska-Kluge, Górski, Stanisz, & Zgoła-Grześkowiak, 2013; Stanisz, Zgoła-Grześkowiak, Waśkiewicz, Stępień, & Beszterda, 2015). Briefly, 200 mg of goji fruits, cranberries or raisins were weighed into 12 mL glass

culture tube. Then, 2 mL of methanol and 0.5 mL of 2 mol/L NaOH were added. The tube was closed with a rubber septa screw cap and inserted into a 200 mL screw-capped bottle made of high-density polyethylene (Bel-Art Products, Wayne, USA). The samples were irradiated in a Microchef 460 microwave oven (Moulinex, Caen, France) operating at 2450 MHz and 300 W for 20 s. After cooling down, the sample was extracted four times with 1 mL of pentane. Each time pentane was separated by one minute centrifugation at 4000 rpm and the combined extracts were evaporated with a gentle stream of nitrogen. After reconstitution in 0. 5 mL of methanol the samples were filtered using the 0.2 µm PTFE syringe filter.

Analysis of the samples was performed with the use of the UltiMate 3000 RSLC chromatographic system supplied by Dionex (Sunnyvale, CA, USA) connected to the API 4000 QTRAP triple quadrupole mass spectrometer from AB Sciex (Foster City, CA, USA). Exactly 10 µL of the samples were injected onto a Gemini-NX C18 column (10 cm × 2.0 mm I.D.; 3 µm) from Phenomenex (Torrance, CA, USA) thermostated at 35°C. The mobile phase consisted of a mixture of methanol:water (95:5, v/v) flowing isocratically at a flow rate of 0.4 mL/min. The chromatographic system and mass spectrometer were interfaced using the atmospheric pressure chemical ionization (APCI) source operated in a positive ion mode under the following conditions: curtain gas – nitrogen at 10 psi, nebulizer gas – nitrogen at 20 psi, temperature 400°C, nebulizing current 3 µA and declustering potential 65 V. Nitrogen at 10 psi was used as the collision gas in the second quadrupole working as the collision cell. The determination of ergosterol was conducted using the quantitative transition from 379.3 to 69.1 m/z at collision energy set to 45 V. The confirmatory transition was from 379.3 to 145.1 m/z at collision energy set to 22 V. The dwell time for data collection was set to 200 ms.

The method was revalidated. Linearity was tested in a range from 5 to 5000 ng/g and correlation coefficient R^2 was found 0.9981. Limits of detection (LOD) and quantification (LOQ) were assessed on the basis of signal to noise (S/N) ratio with S/N=3 for LOD and S/N=10 for LOQ. LOD was found at concentration 2 ng/g and LOQ at 5 ng/g. Recovery was tested by spiking 0.2 g berries with 0.5 mL standard solution at concentration 0.5 µg/mL. The result from 5 spiked samples was 95.0% with precision

expressed as relative standard deviation equal to 3.5%. The matrix effect was evaluated on the basis of quotient of two calibration curves slopes (Ferrer, Lozano, Aguera, Giron, & Fernandez-Alba, 2011; Zgoła-Grześkowiak, Jeszka-Skowron, Czarczyńska-Goślińska, & Grześkowiak, 2016). The calibration curve slope for the curve constructed from a sample fortified at various concentrations was divided by the slope obtained for the calibration curve obtained for a set of standards. The quotient equal to 0.90 was obtained which indicates slight signal suppression.

2.6. The determination of mycotoxins

2.6.1. Extraction of mycotoxins

From ground fruit samples (2 g) mycotoxins (aflatoxins and ochratoxin A) were extracted with 10 mL of adequate solvent mixtures (methanol:water - 80:20, v/v for aflatoxins and acetonitrile:water - 60:40, v/v for ochratoxin A). After filtration (Whatman No. 5 paper) extracts were collected and applied on the top of an immuneaffinity Afla Test™ or Ochra Test™ column according to manufacturer instructions (IAC, Vicam, USA). Mycotoxins were eluted from columns with methanol.

2.6.2. Determination of mycotoxins

The solutions were evaporated and the residues were reconstituted with 1 mL of methanol for chromatographic determination of mycotoxins. The occurrence of aflatoxins and ochratoxin A was analyzed using the Waters Aquity ultra-performance liquid chromatography (UPLC) system coupled to a tandem quadrupole (TQ) mass detector (Waters, Milford, MA, USA), equipped with the Empower™ 2 software for data processing. The mass spectrometer was operated in the positive electrospray ionization (ESI^+) mode using multiple reaction monitoring (MRM). High purity nitrogen was used as an ESI nebulizing and desolvation gas and argon was used as the collision gas for collision induced dissociation. The mycotoxins were separated on the Aquity BEH C18 column (2.1 mm x 100 mm, 1.7 μm particle size) using the mobile phase consisting of aqueous 0.1% formic acid in deionized water (line A) and 0.1%

formic acid in acetonitrile (line B), at a flow rate of 0.3 mL/min. The volume of each injected sample was 3 µL. The ESI+ source had the following parameters: capillary voltage 3.5 kV, cone voltage 35-50 V, source temperature 150°C, desolvation temperature 350°C, cone gas flow 50 L/h , desolvation gas flow rate of 800 L/h. The parameters on the m/z and collision energy of precursor ions, and m/z of product ions selected for the determination of mycotoxins are shown in Table 3.

For linearity, six-point (0.1, 0.2, 0.5, 1.0, 2.0, 4.0 ng/g) calibration curves were separately prepared for each mycotoxin (AFB1, AFB2, AFG1, AFG2 and OA) and they were obtained using the linear least squares regression procedure of peak area versus concentration. The linearity of the standard curves was reliable between 0.9891 (for AFB2) and 0.9993 (for OA). LOD was 0.3 ng/g for all tested aflatoxins and 0.1 ng/g for OA. LOQ was calculated as three-fold LOD. The recovery experiment was performed on mycotoxin-free cranberries samples, spiked with three different levels of each mycotoxin separately at a concentration of 0.1, 0.5 and 2.0 ng/g. On the basis of these experiments recovery rates were 96.7-97.5%, 86.2-89.7%, 85.2-88.9%, 91.3-94.5% and 97.9-101.6% for AFB1, AFB2, AFG1, AFG2 and OA, respectively.

2.7. Statistical analysis

The results were expressed as mean ± standard deviation (at least three replicates). The analysis of variance and significant differences among the mean values were performed with one-way ANOVA (Tuckey's test). The significance level was based on a confidence level of 95.0%. The Pearson's correlation between the results was also determined. The experimental data was analyzed by using Statistica 12.5 program (StatSoft Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Antioxidant activity of dried fruits

Antioxidant activity of dried fruits was measured by DPPH radical scavenging activity and with the use of the Folin-Ciocalteu reagent. These simple and useful methods are widely used to determine *in vitro* antioxidant activity/capacity of food products (Bennet et al., 2011; Jeszka-Skowron, & Zgoła-Grześkowiak 2014; Jeszka-Skowron, Sentkowska, Pyrzyńska, & Paz De Peña, 2016; Stelmach, Pohl, & Szymczycha-Madeja, 2015).

The antioxidant activity of all extracts prepared from dried goji fruits was at least 4-fold higher than the extracts obtained from dried cranberries or raisins ($P \leq 0.05$) (Figure 1). The averages of DPPH and Folin-Ciocalteu assays (± SD) for goji berries extracts were: 40.6 ± 3.7 mg Trolox/g dried fruits and 11.5 ± 1.3 mg GAE/g dried fruits; for cranberries 9.9 ± 1.9 mg Trolox/g dried fruits and 2.5 ± 0.6 mg GAE/g dried fruits and for raisins: 9.4 ± 0.8 mg Trolox/g dried fruits and 1.9 ± 0.3 mg GAE/g dried fruits.

The extracts of dried goji No 4 and 5 (*bio* product – from an organic farm) showed the highest antioxidant activity in DPPH assay (43.6 ± 0.5 mg Trolox/g dried fruits and 45.0 ± 1.0 mg Trolox/g dried fruits) as well as in Folin-Ciocalteu assay (13.4 ± 0.5 mg GAE/g dried fruits and 12.0 ± 0.2 mg GAE/g dried fruits) (Figure 1). These results were 10-fold higher than the phenolic content in methanol extracts obtained for goji fruits (of fresh matter) (Zhao, & Hall, 2008). Endes, Uslu, Özcan, & Er (2015) showed that the dried goji fruit water extract contained 3.44 ± 0.37 mg GAE/100 mL of total phenol content and its antioxidant activity in DPPH assay was equal to: 20.78 % ± 1.29. These results are difficult to compare with the results from this study because of different extraction solvents (water vs. methanol-water) used, slightly different preparation of assays and not the same calculations of assays.

The antioxidant activity of dried cranberries extracts and raisins extracts were not significantly different ($P > 0.05$) (Figure 1). Ishiwata, Yamaguchi, Takamura, & Matoba (2004) measured reducing compounds of dried fruits with Folin-Ciocalteu reagent and the highest level of total phenolic content was determined for raisins. Vinson, Zubik, Bose, Samman, & Proch (2005) showed that the extracts of cranberries possessed higher level of phenolics (in the range of 593-1147 mg catechin equivalents/100 g fresh weight) than the extracts from raisins (505-597 mg catechin equivalents/100 g fresh weight). Vollmannova et al.

(2014) found that cranberries possessed higher level of total phenolic (in the range 1488-3049 mg GAE/kg) than blueberries.

No correlation was found between higher antioxidant activity of fruits and their origin from organic farms (Figure 1).

The Pearson's correlation between DPPH and Folin-Ciocalteu assays of dried fruits was very high (R^2 = 0.975). Similar results were gathered for extracts obtained from tea leaves and coffee beans (Jeszka-Skowron, & Zgoła-Grześkowiak, 2014; Jeszka-Skowron, Sentkowska, Pyrzyńska, & Paz De Peña, 2016). Bennett et al. (2011) found linear regression between ferric reducing/antioxidant power assay (FRAP) and total phenolic content in dried fruits, included raisins (R^2 = 0.81). Pearson's correlations between total phenolic content vs. DPPH (R^2 = 0.943), total phenolic content vs. FRAP (R^2 = 0.756) and DPPH vs. FRAP (R^2 = 0.668) in dried fruits from India were determined by Reddy, Sreeramulu, & Raghunath (2010).

3.2. Concentration of selected elements in the CRMs and dried fruits

All element concentrations in the CRMs and real dried fruit samples were expressed on a dry weight basis. Due to the fact that in the laboratory there were no available CRMs compatible with the samples matrix (Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013), to study the reliability of the results, CRMs of other plant or food samples have been used. This kind of materials is supposed to represent the fruits matrices the best (Altundag, & Tuzen, 2011; Fang et al., 2010). The precision and accuracy of the ET AAS method was studied by analyzing five certified reference materials with different matrices: non-fat milk powder, mixed herbs, tobacco leaves, soya bean flour and tea leaves. The results obtained for digested CRMs were in a good agreement with certified values according to the *t*-test at a 95% confidence level (Table 1). Precision expressed as relative standard deviation for 5 replicated measurements of CRMs solutions varied from 2% to 18%. Recoveries achieved

were satisfactory and equal to: 95-105% for Pb, 96-110% for Cd, 96-104% for Ni, 90-105% for Ge, 95-104% for Mn and 97-104% for Cu.

The concentrations of selected elements in the dried fruit samples were determined and the analytical results, as averages of five determinations with standard deviations around mean values, are given in Table 4. As can be seen some similarities between concentrations of particular elements within samples of different origin and brands can be observed. In general, the elemental concentrations in all samples decreased in the following order: Mn > Cu > Ni > Pb > Cd > Ge for goji fruits; Mn > Cu > Ni > Cd > Ge > Pb for cranberries and Cu > Mn > Ni> Pb > Cd > Ge for raisins. Se was not found in the tested samples.

The lowest average contents were achieved for Ge: 0.015 mg/kg (<0.0010-0.013) in goji fruits; 0.010 mg/kg (<0.0010-0.012) in cranberries and 0.011 mg/kg (<0.0010-0.015) in raisins. The average values for Ge show that concentrations in the analyzed fruits did not present a major difference between the species. Therefore, it is difficult to specify which of the tested fruits can be the best source of the element. Ge is less determined in fruit samples because of its relatively low concentrations, and thus the requirement for low limits of detection. Thus it is difficult to compare the results with literature.

There is no information about EU legislation settings for the maximum concentrations of selected elements in the dried exotic fruits. The exception is the concentration of Cd and Pb in so-called “fruits” and ”small fruits”, respectively (Regulation of European Commission, 2008). The values defined for fruits by the law are 0.05 and 0.20 mg/kg for Cd and Pb, respectively. The results show that the levels of Cd and Pb were relatively low and were below the maximum allowable limits specified by the European Food Legislation (Regulation of European Commission, 2008). In the presented study average contents of the elements were quantified as: 0.046 mg/kg (0.026-0.057) and 0.109 mg/kg (<0.0022-0.154) in goji fruits; 0.019 mg/kg (0.015-0.027) and 0.007 mg/kg (<0.0022-0.007) in cranberries; 0.016 mg/kg (0.013-0.020) and 0.119 mg/kg (0.009-0.363) in raisins for Cd and Pb, respectively (Table 4). The highest average value for Cd was achieved in goji fruits (0.046 mg/kg) and for Pb in raisins (0.119 mg/kg). Additionally, the

highest amount of Pb was determined in raisin sample from organic farming R5 (0.363 mg/kg), which exceeds the 0.2 mg/kg limit. The concentrations found stayed in accordance with the values reported in the literature (Fang et al., 2010; Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013; Vollmannova et al., 2014). Other Authors established Cd and Pb concentrations in goji fruits as <0.035-0.090 mg/kg and 0.035-0.095 mg/kg (Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013), respectively; in cranberries as 0.007-0.021 mg/kg and 0.031-0.073 mg/kg (fresh matter) (Vollmannova et al., 2014), respectively and in raisins as 0.004-0.010 mg/kg and 0.007-0.014 mg/kg (Fang et al., 2010), respectively.

In the study the maximum average Ni content was found for goji fruits as 2.61 mg/kg (1.28-5.42) and the highest concentration was noted for fruits from organic farming (G5): 5.42 mg/kg. The results for Ni show that concentrations in cranberries and raisins did not present a major difference between the fruits. The average values determined were: 0.087 mg/kg (0.048-0.132) and 0.129 mg/kg (0.016-0.235) in cranberries and raisin, respectively. The concentrations found in goji fruits were higher or were comparable (for raisins) to the values reported in the literature (Fang et al., 2010; Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013). The Authors informed that Ni concentration were in the range of 0.33-0.90 mg/kg in goji (Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina, 2013) and in the range of 0.125-0.243 mg/kg in raisins (Fang et al., 2010). It is difficult to find any reports on the determination of Ni in cranberries.

The levels of the elements in goji fruits ranged between 5.21-10.5 mg/kg and 5.14-11.8 mg/kg for Cu and Mn, respectively. The mean values were quite comparable for the elements: 7.78 mg/kg for Cu and 8.55 mg/kg for Mn. Very similar average concentration of Cu was established in raisins: 7.98 mg/kg (3.98-14.20). The Mn average content in raisins was lower than in goji: 3.34 mg/kg (2.03-5.19). As in the case of Ni, also in the sample R5 (from organic farming) the highest amount of Cu was found (14.20 mg/kg). In the study the lowest average concentrations of the elements were determined in cranberries: 0.681 mg/kg (0.186-1.82) and 1.49 mg/kg (1.15-1.94) for Cu and Mn, respectively. The results obtained for goji fruits

are comparable to Llorent-Martínez, Fernández-de Córdova, Ortega-Barrales, & Ruiz-Medina (2013) studies in which the authors reported concentration of Cu in the range of 1.5-8.5 mg/kg and Mn in the range of 6-12 mg/kg as well as to Nogueira Nascimento, Menezes Silvestre, de Oliveira Leme, Seimi Nomura, & Naozuka (2015) results for Cu (5.2-6.9 mg/kg). In the case of raisins the presented Cu and Mn values are also in a good agreement with literature, for Cu: 3.70-7.37 mg/kg and for Mn: 3.99-6.32 mg/kg according to Fang et al. (2010). There are no reports in the literature on the Mn concentrations in cranberries. Only Vollmannova et al. (2014) inform about Cu concentration in the range of 0.491-0.825 mg/kg of fresh matter. These values stayed close to the presented average value (0.681 mg/kg).

For the first time, a high correlation between Mn and antioxidant activity of dried fruit samples was determined. The Pearson's correlation between Mn and DPPH assay was obtained ($R^2 = 0.762$) as well as between Mn and Folin-Ciocalteu assay ($R^2 = 0.772$). These correlations could be essential for human health - these elements are involved in antioxidative defense system of the organism. Cu and Mn are the part of endogenous enzymes and radical scavengers such as superoxide dismutase: SOD1, SOD2 and SOD3 (Neto, 2007).

3.3. Ergosterol and mycotoxins content

The quality of food depends not only on bioactive compounds but also on potential toxic substances and microorganisms. The LC-MS/MS was used to determine the mycotoxins: aflatoxin B1, B2, G1, G2 and ochratoxin A as well as ergosterol content.

Ergosterol which occurs in fungi cell walls can be used as an indicator of fungal contamination (Saxena, Munimbazi, Bullerman, 2001; Stanisz, Zgoła-Grześkowiak, Waśkiewicz, Stępień, & Beszterda, 2015). Thus, even in dead fungal biomass it can be isolated from a sample and quantified by LC-MS/MS method. In this study ergosterol content was determined in every tested goji fruits (Table 5) in the range 236.2 – 971.9 ng/g with one exception (< 2 ng/g). In three of them (one with the highest level of ergosterol) ochratoxin A (1.11-2.08 ng/g) was determined. Goji fruits did not contain any aflatoxins. The cranberry

samples contained ergosterol but at low level (maximum level 55.9 ng/g) and did not contain any mycotoxins. In the case of the raisins, two samples with the highest level of ergosterol also contained aflatoxin B1 and ochratoxin A.

In the sample of raisin (4R) aflatoxin B1 was detected at the highest level – 2.35 ± 0.52 ng/g. According to European Commission Regulation the acceptable, maximum level of sum of aflatoxins (B1, B2, G1, G2) in raisins is 4 ng/g, while the maximum ochratoxin A content for dried grape fruits (currants, raisins and sultanas) - 10 ng/g (Regulation of European Commission, 2008). In all analyzed samples mycotoxins content did not exceed the acceptable, maximum level. In available literature there is a lack of reports about occurrence of aflatoxins and ochratoxin A in cranberries and goji fruits, whereas mycotoxins content in raisins was demonstrated. Azaiez, Font, Manes, & Fernandez-Franzon, (2015) found that the mean mycotoxin concentrations for the dried grape fruits were for: aflatoxin B1 -16.5 ng/g (<0.2-17.5 ng/g); aflatoxin G1 - 2.35 ng/g (<0.5-3.1 ng/g) and ochratoxin A - 3.03 ng/g (not specified - 5.5). Thirteen out of 228 samples (dates and dried fruits) contained aflatoxins at the levels that exceed the maximum limits established in the EU legislation. In similar studies, Juan, Zinedine, Moltó, Idrissi, & Mańes (2008) showed that aflatoxin B1 content of dried raisins was on average 10.7 ± 2.3 ng/g and maximum level for these fruits was 13.9 ng/g.

The Pearson's correlation between ergosterol content and ochratoxin A (R^2 = 0.464) was found. On the other hand the correlation between ergosterol and aflatoxin B1 was not significant (R^2 = 0.029). This weak correlation could be because ergosterol was detectable before aflatoxin B1 formation at high inoculum level (Saxena, Munimbazi, & Bullerman, 2001).

4. Conclusion

Due to the fact that dried fruits are relatively cheap and easy to store they are increasingly incorporated into other foods, beverages and recipes. According to some studies they may be used as healthy alternative to sweet snacks and may be a valuable component of the diet. All analyzed dried fruits

showed antioxidant activity and contained important for human health micronutrients such as Cu and Mn as well as some amounts of Ge. Additionally the results obtained for trace toxic elements (Cd and Pb) in analyzed dried fruits were acceptable to human consumption for the most of the tested samples. The level of mycotoxins was also acceptable in the analyzed samples. However, there was no correlation between the content of aflatoxins and ergosterol and only a slight correlation between the content of ochratoxin A and ergosterol which diminishes a possibility of using ergosterol as a marker of mycotoxin contamination.

Notes

The authors declare no competing financial interest.

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References

Altundag, H., & Tuzen, M. (2011). Comparison of dry, wet and microwave digestion methods for the multi element determination in some dried fruit samples by ICP-OES. *Food and Chemical Toxicology, 49*, 2800-2807.

Anderson, J. A., Huth H. A., Larson, M. M., Colby, A. J., Krieg, E. J., Golbach, L. P., Simon, K. A., Wasmundt, S. L., Malone, C. J., & Wilson T. (2011). Glycaemic and insulin response to raisins, grapes and bananas in college aged students. *FASEB Journal, 25*, 587.4.

Anderson, J. W., & Waters, A. R. (2013). Raisin consumption by humans: Effects on glycemia and insulinemia and cardiovascular risk factors. *Journal of Food Science, 78,* A11–A17.

Aung, L. H., & Jenner, J. F. (2004). Detection of 2,4,6-trichloroanisole in microorganism-free irradiated raisins by solid-phase microextraction and GC-MS. *Journal of Stored Products Research, 40*, 451-459.

Azaiez, I., Font, G., Manes, G. J., & Fernandez-Franzon, M. (2015). Survey of mycotoxins in dates and dried fruits from Tunisian and Spanish markets. *Food Control, 51*, 346-343.

Donno, D., Beccaro, G.L., Mellano, M.G., Cerutti, A.K., & Bounous G. (2015). Goji berry fruit (Lycium spp.): antioxidant compound fingerprint and bioactivity evaluation. *Journal of Functional Foods, 18B*, 1070-1085.

Donno, D., Mellano, M.G., Raimondo, E., Cerutti, A.K., Prgomet, Z., & Beccaro, G.L. (2016a). Influence of applied drying methods on phytochemical composition in fresh and dried goji fruits by hplc fingerprint. *European Food Research and Technology,* In press: first online, 1-14.

Donno, D., Boggia, R., Zunin, P., Cerutti, A.K., Guido, M., Mellano, M.G., Prgomet, Z., Beccaro, G.L. (2016b). Phytochemical fingerprint and chemometrics for natural food preparation pattern recognition: An innovative technique in food supplement quality control. *Journal of Food Science and Technology*, *53,* 1-13.

Donno, D., Cavanna, M., Beccaro, G.L., Mellano, M.G., Torello-Marinoni, D., Cerutti, A.K., Bounous, G. (2013). Currants and strawberries as bioactive compound sources: Determination of antioxidant profiles with HPLC-DAD/MS. *Journal Of Applied Botany And Food Quality. 86*, 1-10.

Endes, Z., Uslu, N., Özcan, M. M., & Er, F. (2015). Physico-chemical properties, fatty acid composition and mineral contents of goji berry (*Lycium barbarum* L.) fruit. *Journal of Agroalimentary Processes and Technologies, 21*(1), 36-40.

Fang, Y. L., Zhang, A., Wang, H., Li, H., Zhang, Z. W., Chen, S. X. & Luan, L. Y. (2010). Health risk assessment of trace elements in Chinese raisins produced in Xinjiang province. *Food Control, 21*, 732-739.

Ferrer, C., Lozano A., Aguera A., Giron J., & Fernandez-Alba A.R. (2011). Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. *Journal of Chromatography A, 1218,* 7634–7639.

Horbik, D., Łowińska-Kluge, A., Górski, Z., Stanisz, E., & Zgoła-Grześkowiak, A. (2013). Microwave-assisted extraction combined with HPLC-MS/MS for diagnosis of fungal contamination in building materials. *Journal of the Brazilian Chemical Society*, *24*, 1478-1486.

International Agency for Research on Cancer (IARC). (1993). Ochratoxin A. IARC Monographs on the evaluation of carcinogenic risks to humans: Some naturally occurring substances, food items and constituents, heterocyclic aromatic amines and mycotoxins. Vol. 56, pp. 26–32.

International Agency for Research on Cancer (IARC). (2002). IARC monographs on the evaluation of carcinogenic risks to humans. Vol. 82. IARC, Lyon, France.

Ishiwata, K., Yamaguchi, T., Takamura, H., & Matoba, T. (2004). DPPH radical-scavenging activity and polyphenol content in dried fruits. *Food Science and Technology Research, 10*, 152–156.

Jeszka-Skowron, M., & Zgoła-Grześkowiak, A. (2014). Analysis of antioxidant activity, chlorogenic acid, and rutin content of *Camellia sinensis* infusions using response surface methodology optimization. *Food Analytical Methods, 7,* 2033-2041.

Jeszka-Skowron, M., Sentkowska, A., Pyrzyńska, K., & De Peña, M. P. (2016). Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: influence of green coffee bean preparation. *European Food Research and Technology*, *242*, 1403–1409.

Jeszka-Skowron, M., Stanisz, E., & De Peña, M. P. (2016). Relationship between antioxidant activity, chlorogenic acids and elemental composition of green coffee. *LWT - Food Science and Technology, 73,* 243–250.

Joung, K. M., Jung, H. J., Kim, K. N., & Ho-Kyung, K. (2008). Effects of cranberry powder on serum lipid profiles and biomarkers of oxidative stress in rats fed an atherogenic diet. *Nutrition Research and Practice, 2(3),* 158–164.

Juan, C., Zinedine, A., Moltó, J. C., Idrissi, L., & Mañes, J. (2008). Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. *Food Control*, *19*, 849-853.

Karadeniz, F., Durst, R.W., & Wrolstad, R.E. (2000). Polyphenolic composition of raisins. *Journal of Agricultural and Food Chemistry, 48,* 5343-5350.

Llorent-Martínez, E. J., Fernández-de Córdova, M. L., Ortega-Barrales, P., & Ruiz-Medina, A. (2013). Characterization and comparison of the chemical composition of exotic superfoods, *Microchemical Journal, 110*, 444-451.

Neto, C. C. (2007). Cranberry and blueberry: Evidence for protective effects against cancer and vascular diseases. Review. *Molecular Nutrition & Food Research, 51*, 652-664.

Nogueira Nascimento, A., Menezes Silvestre, D., de Oliveira Leme, F., Seimi Nomura, C. & Naozuka, J. (2015). Elemental analysis of goji berries using axially and radially viewed inductively coupled plasma-optical emission spectrometry. *Spectroscopy, 30,* 36-41.

Peev, C.I., Vlase, L., Antal, D.S., Dehelean, C.A., & Szabadai, Z. (2007). Determination of some polyphenolic compounds in buds of Alnus and Corylus species by HPLC. *Chemistry of Natural Compounds*. 43(3):259-262.

Reddy, C. V. K., Sreeramulu, D., & Raghunath, M. (2010). Antioxidant activity of fresh and dry fruits commonly consumed in India. *Food Research International, 43*, 285–288.

Regulation (EC) No 629/2008: Commission Regulation (EC) No 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, L 173/6. Official Journal of the European Union, Brussels 3.7.2008.

Saxena, J., Munimbazi, C., & Bullerman, L.B. (2001). Relationship of mould count, ergosterol and ochratoxin A production. *International Journal of Food Microbiology*, *71*, 29-34.

Stanisz, E., Zgoła-Grześkowiak, A., Waśkiewicz, A., Stępień, Ł., & Beszterda, M. (2015). Can ergosterol be an indicator of fusarium fungi and mycotoxins in cereal products? *Journal of the Brazilian Chemical Society*, *26,* 1-8.

Stelmach, E., Pohl, P., & Szymczycha-Madeja A. (2015). The content of Ca, Cu, Fe, Mg and Mn and antioxidant activity of green coffee brews. *Food Chemistry*, *182*, 302-308.

Vinson, J.A., Zubik, L., Bose, P., Samman, N., & Proch, J. (2005). Dried fruits: excellent in vitro and in vivo antioxidants. *Journal of the American College of Nutrition, 24,* 44–50.

Vollmannova, A., Musilova, J., Toth, T., Arvay, J., Bystricka, J., Medvecky, M. & Daniel, J. (2014). Phenolic compounds, antioxidant activity and Cu, Zn, Cd and Pb content in wild and cultivated cranberries and blueberries. *International Journal of Environmental Analytical Chemistry, 94*, 1445-1451.

Wild, C. P, & Gong, Y. Y. (2010). Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis, 31,* 71-82.

Williamson, G., & Carughi, A. (2010). Polyphenol content and health benefits of raisins. *Nutrition Research, 30,* 511–519.

Yang, R., Zhao, C., Chen, X., Chan, S., & Wu, J. (2015). Chemical properties and bioactivities of Goji (*Lycium barbarum*) polysaccharides extracted by different methods. *Journal of Functional Foods, 17*, 903–909.

Yeung, C. K., Glahn, R., Wu, X., Liu, R. H., & Miller, D. (2003). In vitro iron bioavailability and antioxidant activity of raisins. *Journal of Food Science, 68*, 701-705.

Zgoła-Grześkowiak, A., Jeszka-Skowron, M., Czarczyńska-Goślińska, B., & Grześkowiak, T. (2016) Determination of parabens in Polish river and lake water as a function of season. *Analytical Letters, 49,* 1734-1747.

Zhao, B., & Hall, C. A. III. (2008). Composition and antioxidant activity of raisin extracts obtained from various solvents. *Food Chemistry, 108*, 511-518.

Zhong, Y., Shahidi, F., Naczk, M. (2013). Phytochemicals and health benefits of goji berries. In: Dried fruits: phytochemicals and health effects. Blackwell Publishing Ltd., pp 133–14

Figure Capture

Figure 1. Antioxidant activity of dried fruit extracts measured by DPPH and Folin-Ciocalteu (F-C) assays. Chinese goji fruits (G1-G5; G5 organic farming); Polish cranberries (C1, C2, C5), USA (C3, C4) and

Canadian cranberries (C6 – the organic farming); raisins from Chile (R1), from Iran (R2, R3) and from Turkey (R4; R5 - came from the organic farming).

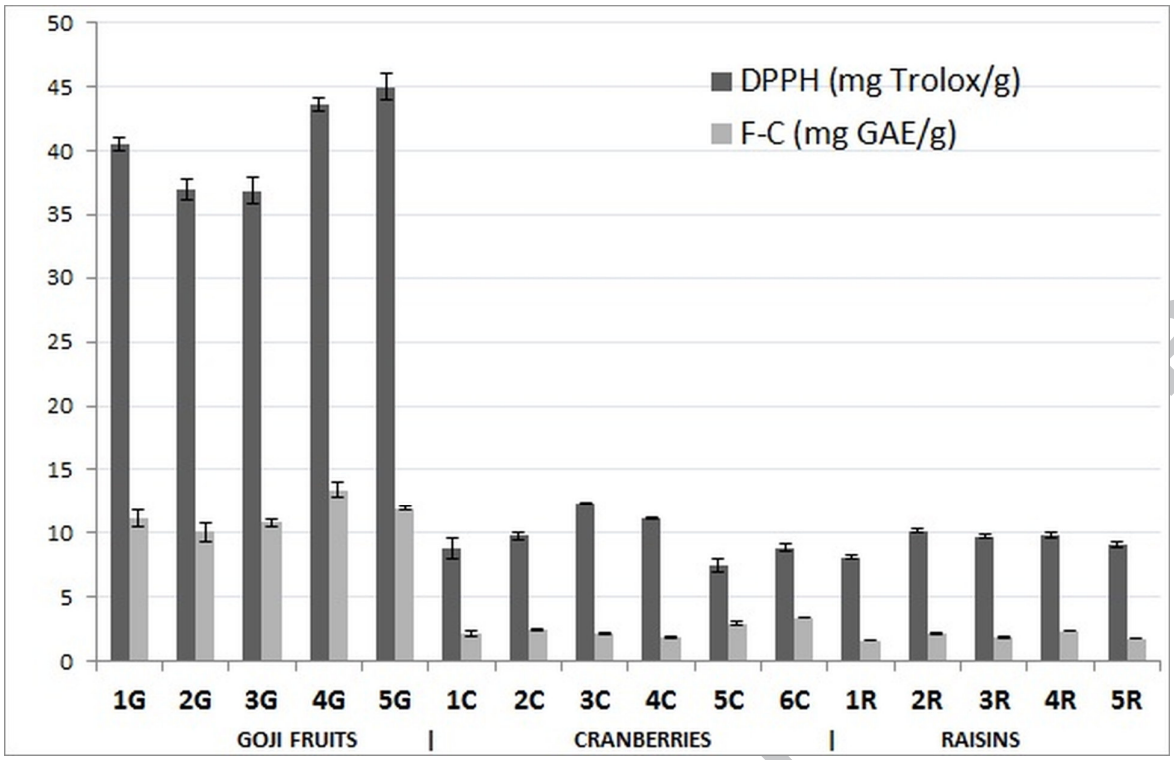

Fig. 1

Table 1. The determination of selected elements in CRMs with the use of ET AAS technique.

Certified reference material		Element (mg/kg)					
		Pb	Cd	Ni	Ge[4]	Mn	Cu
SRM 1549 Non-fat Milk Powder	Found	0.018±0.002	<0.0014[2]	0.52±0.05	0.045±0.005	0.27±0.04	0.73±0.09
	Certified	0.019±0.003	0.0005 ±0.0002	0.50[4]	0.050	0.26±0.06	0.70±0.10
INCT-MPH-2 Mixed Polish Herbs	Found	2.18±0.21	0.192±0.012	1.51±0.11	0.023±0.023	189±10	7.71±0.55
	Certified	2.16±0.23	0.199±0.015	1.57±0.16	0.025	191±12	7.77±0.53
INCT-PVTL-6 Tobacco Leaves	Found	998±95[1]	2.29±0.15	1.42±0.07	0.106±0.005	138±3	4.98±0.20
	Certified	972±147[1]	2.23±0.12	1.49±0.14	0.100	136±5	5.12±0.23
INCT-SBF-4	Found	0.076±0.014	0.032±0.005	3.15±0.21	0.047±0.005	30.8±1.6	14.80±0.60

Soya Bean Flour	Certified	0.083[3]	0.029[3]	3.12±0.18	0.050	32.3±1.1	14.30±0.46
INCT-TL-1	Found	1.87±0.15	0.029±0.002	5.98±0.23	0.098±0.006	0.159±0.009[5]	20.2±0.9
Tea Leaves	Certified	1.78±0.24	0.030±0.004	6.12±0.52	0.100	0.157±0.011[5]	20.4±1.5

Obtained data are presented as mean ± SD (n=5)
[1] µg/kg,
[2] below limit of detection (see Table 2),
[3] information value,
[4] CRMs were spiked with the element,
[5] %.

Table 2. Operating conditions of the ET AA spectrometer used for determination of elements in CRMs and goji berries, cranberries and raisins after microwave-assisted digestion.

Parameter	Cd	Cu	Ge[5]	Mn	Ni	Pb	Se
Wavelength (nm)	228.8	324.8	265.1178	279.5	232.0	217.0	196.0
Spectral band width (nm)	0.8	0.8	200[6]	0.2	0.2	0.5	1.2
Lamp current (mA)	4	3.0	9[7]	3.5	5	8	10
Modifier	$NH_4H_2PO_4$ + $Mg(NO_3)_2$	-	$Pd(NO_3)_2$ +$Mg(NO_3)_2$	$Pd(NO_3)_2$	$Mg(NO_3)_2$	$NH_4H_2PO_4$	$Pd(NO_3)_2$
Modifier concentration (mg/L)	1[4] + 0.6[4]	-	2[8] + 2[8]	2	120	20	2
Modifier volume (µL)	4	-	5	5	5	5	5
Sample volume (µL)	20	20	30	20	20	20	30
Pyrolysis temp. (°C)/hold (s)	850/4	700/2	1400/10	1100/2	950/2	900/2	350 and 1050[9] /20 and 10
Atomization temp. (°C)/hold (s)	1650/2	1800/4	2600/3	2200/2	2250/5	1800/4	2100/4
LOD[1] (ng/mL)	0.07	0.06	0.05	0.03	0.04	0.11	0.19
LOD[2] (mg/kg)	0.0014	0.0012	0.0010	0.0006	0.0008	0.0022	0.0038
Precision[3] (%)	6-7	4	6-13	2-15	4-7	8-11	3-10[10]

[1] limit of detection calculated according to a 3σ blank criterion (the standard deviation of the blank, n = 10) for standard solutions,
[2] limit of detection calculated according to a 3σ blank criterion (the standard deviation of the blank, n = 10) for samples,
[3] as relative standard deviation for 5 replicated measurements of CRMs solutions containing Cd, Cu, Ge, Mn, Ni and Pb,
[4] g/L,
[5] the determination was conducted using high-resolution continuum source electrothermal atomic absorption spectrometry (HR-CS ET AAS),
[6] spectral range (pixel),
[7] A,
[8] µg/L,
[9] two-step pyrolysis was used,
[10] calculated for aqueous standard solution.

Table 3. Optimized LC-MS/MS parameters for the determination of mycotoxins.

Mycotoxins	Precursor ion	Quantification ion		Confirmation ion	
		Product ion	CE[1]	Product ion	CE[1]
Ochratoxin A	404.0	358.0	45	239.0	43
Aflatoxin B1	313.1	241.1	40	285.1	35
Aflatoxin B2	315.1	287.0	36	259.0	38
Aflatoxin G1	329.0	243.0	28	311.1	30
Aflatoxin G2	331.1	313.3	30	245.2	32

[1]Collision energy (eV)

Table 4. Concentration of selected elements in goji berries, cranberries and raisins (mg/kg). Description for the samples – Figure 1

	Sample	Pb	Cd	Ni	Ge	Mn	Cu
Goji fruits	G1	0.047±0.005	0.054±0.001	1.69±0.06	0.017±0.001	9.83±0.10	10.5±0.3
	G2	<0.0022[2]	0.053±0.002	1.77±0.07	0.016±0.001	8.96±0.18	7.93±0.16
	G3	0.154±0.012	0.057±0.001	1.40±0.03	<0.0010[2]	9.15±0.14	8.90±0.09
	G4	0.153±0.002	0.048±0.002	1.28±0.05	0.013±0.001	11.8±0.5	6.22±0.12
	G5	0.101±0.002	0.026±0.001	5.42±0.27	0.013±0.001	5.14±0.03	5.21±0.05
	Min.[1]	**0.047**	**0.026**	**1.28**	**0.013**	**5.14**	**5.21**
	Max.	**0.154**	**0.057**	**5.42**	**0.017**	**11.8**	**10.50**
	Average[1]	**0.109**	**0.046**	**2.61**	**0.015**	**8.55**	**7.78**
	SD	**0.052**	**0.014**	**1.93**	**0.002**	**2.50**	**2.29**
Cranberries	C1	0.006±0.001	0.017±0.001	0.065±0.001	<0.0010[2]	1.66±0.02	0.347±0.007
	C2	0.007±0.001	0.027±0.001	0.048±0.002	<0.0010[2]	1.54±0.02	0.239±0.005
	C3	<0.0022[2]	0.017±0.001	0.052±0.001	0.012±0.001	1.39±0.06	0.186±0.015
	C4	<0.0022[2]	0.020±0.001	0.105±0.004	0.007±0.001	1.94±0.01	1.82±0.02
	C5	<0.0022[2]	0.015±0.001	0.117±0.008	<0.0010[2]	1.16±0.02	0.202±0.010
	C6	<0.0022[2]	0.018±0.001	0.132±0.001	<0.0010[2]	1.15±0.01	0.628±0.006
	Min.[1]	**0.006**	**0.015**	**0.048**	**0.007**	**1.15**	**0.186**
	Max.	**0.007**	**0.027**	**0.132**	**0.012**	**1.94**	**1.82**
	Average[1]	**0.007**	**0.019**	**0.087**	**0.010**	**1.49**	**0.681**
	SD	**0.001**	**0.005**	**0.038**	**0.003**	**0.36**	**0.719**
Raisins	R1	0.009±0.001	0.013±0.001	0.016±0.001	0.007±0.001	3.51±0.05	7.07±0.07
	R2	0.042±0.001	0.014±0.001	0.034±0.001	<0.0010[2]	2.03±0.07	3.98±0.08
	R3	0.013±0.001	0.015±0.001	0.165±0.008	0.008±0.001	5.19±0.09	5.69±0.06
	R4	0.037±0.003	0.014±0.001	0.235±0.007	0.011±0.001	3.10±0.15	6.75±0.20
	R5	0.363±0.011	0.020±0.001	0.183±0.005	0.015±0.001	2.34±0.15	14.2±0.4
	Min.[1]	**0.009**	**0.013**	**0.016**	**0.007**	**2.03**	**3.98**
	Max.	**0.363**	**0.020**	**0.235**	**0.015**	**5.19**	**14.20**

Average[1]	**0.119**	**0.016**	**0.129**	**0.011**	**3.34**	**7.98**
SD	**0.167**	**0.003**	**0.098**	**0.004**	**1.38**	**4.42**

Obtained data are presented as mean ± SD (n=5)
[1] calculated without values below limit of detection,
[2] below limit of detection (see Table 2).

Table 5. The ergosterol and mycotoxins content in dried fruits (ng/g). Description for the samples – Figure 1

	Sample	Ergosterol	Aflatoxin B1	Ochratoxin A
Goji fruits	1G	596.3 ± 10.0^{j}	<0.3	1.11± 0.09
	2G	236.2 ± 8.8^{h}	<0.3	1.83± 0.39
	3G	359.3 ± 17.3^{i}	<0.3	<0.1
	4G	$< 2^{a}$	<0.3	<0.1
	5G	971.9 ± 18.4^{l}	<0.3	2.08± 0.37
Cranberries	1C	54.2 ± 6.4^{f}	<0.3	<0.1
	2C	46.7 ± 5.8^{f}	<0.3	<0.1
	3C	27.8 ± 2.2^{e}	<0.3	<0.1
	4C	55.9 ± 2.0^{f}	<0.3	<0.1
	5C	11.6 ± 0.4^{c}	<0.3	<0.1
	6C	5.2 ± 0.6^{b}	<0.3	<0.1
Raisins	1R	660.0 ± 6.2^{k}	1.68± 0.12	0.44± 0.07
	2R	15.1 ± 1.4^{d}	<0.3	<0.1
	3R	12.2 ± 1.4^{cd}	<0.3	<0.1
	4R	92.6 ± 6.8^{g}	2.35± 0.52	1.27± 0.18
	5R	30.9 ± 3.2^{e}	<0.3	<0.1

<LOD - below limit of detection
Groups labelled with different letters (a–l) are significantly different at $P < 0.05$

Highlights

- Antioxidant activity (AA) of dried goji fruits, cranberries and raisins was showed
- ET AAS was used to determine Ge, Mn and Cu in dried fruits
- ET AAS was used to determine toxic metals Cd, Pb and Ni in dried fruits
- The positive correlations of AA and Mn as well as AA and Cu were obtained
- Aflatoxins B1/ B2/ G1/ G2, ochratoxin A and ergosterol were determined