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Speciation analysis and bioaccessibility evaluation of trace elements in goji berries (*Lycium Barbarum*, L.)☆

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ABSTRACT

Goji berries (*Lycium Barbarum*, L.) are known for their nutritional potential as a great source of trace metals (e.g., copper, zinc and manganese) which are present in the form of highly bioaccessible compounds. In order to assess the bioaccessibility of trace elements and to identify compounds responsible for better bioaccessibility of copper and zinc, an *in vitro* simulation of gastrointestinal digestion was used in this study.

The total content of trace metals was evaluated using sample digestion followed by inductively coupled plasma mass spectrometry. Bioaccessibility of trace elements was estimated by size exclusion chromatography coupled to inductively coupled plasma mass spectrometry. These analytical methods were used to analyse samples of goji berries to determine the highest amount of elements. For total trace metal content in goji berries, Zn had the highest level of the three studied (10.6 $\mu g\, g^{-1}$), while the total content of manganese and copper was 9.9 $\mu g\, g^{-1}$ and 6.1 $\mu g\, g^{-1}$, respectively.

Additionally, the analysed metals were found to be highly bioaccessible to the human body (about 56% for Mn, 72% for Cu and 64% for Zn in the gastric extract and approximately 35% for Mn, 23% for Cu and 31% for Zn in the case of gastrointestinal extract). To obtain information about metal complexes present in goji berries, extraction treatment using different solutions (ionic liquid, HEPES, SDS, Tris-HCl, ammonium acetate, water) was performed. Enzymatic treatment using pectinase and hemicellulase was also checked. Extracts of berries were analysed by SEC-ICP-MS and μHPLC–ESI–MS/MS techniques. The ionic liquid and pectinase extraction helped efficiently extract copper (seven compounds) and zinc (four compounds) complexes. Compounds identified in goji berries are most likely to be responsible for better bioaccessibility of those elements to the human organism.

1. Introduction

Lycium barbarum L. is one of the most important plants used in traditional Chinese medicine. It has been cultivated in North West China and the Mediterranean region [1]. *Lycium barbarum* L. has been used as a functional food for centuries because of its possible beneficial effect in the prevention of chronic diseases such as age-related macular degeneration, which is supported by the presence of lutein and zeaxanthin [2]. The presence of various functional components like polysaccharides, flavonoids and carotenoids in *L. barbarum* fruits is believed to be responsible for antitumor activities, neuroprotective effect and enhancement of immunity [3–5]. The composition of polysaccharides and flavonoids has been studied extensively however quantities and varieties of some biologically active compounds still remain undetermined.

The scientific literature is particularly focusing on food with positive health effects (for example açaí, goji berries or chia seeds) presenting mostly the determination of biologically active organic compounds (phenols and flavonoids) [6,7]. Only few publications have described the content of metals in some of these fruits [8–10]. Unfortunately, at this time little is known about metal complexes with bioligands present in plants and fruits rich in biologically active compounds. Thorough research in this area is crucial because depending on chemical form of metals present in food, they may have beneficial or harmful impact on human organisms.

Numbers of studies have reported extraction of different bioligands from plant material, but literature data focusing on optimization of extraction of metal complexes with bioligands from functional foods is very limited. One of the best method to extract metal complexes with bioligands is ionic liquid extraction [11]. The presented results show that ionic liquid has ability for

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extraction of different groups of compounds and the efficiency of this techniques is much higher in comparison than with other extractants. Enzymatic treatment with pectinase and hemicellulase is another extraction method described in the literature. It was already reported that the enzymatic treatment significantly increases the efficiency of copper extraction and improves extraction of copper complexes with polyphenols from açaí and bilberry berries [12].

Studies of metals binding by pectin's were widely performed and managed to prove the binding capacity by citrus pectin. Electrostatic binding within the molecular ions Ca^{2+}, Sr^{2+}, Zn^{2+} and Cd^{2+} complexes was examined, Cu^{2+} and Pb^{2+} ions were analysed in the presence of potassium nitrate as the assistant salt [13]. A different mechanism of binding copper (very good binding capacity) was observed in comparison with lead, cadmium, nickel and calcium ions [14–16]. The aim of this study was speciation analysis of trace metals in goji berries after the extraction of metal complexes with bioligands. Copper, manganese and zinc have been chosen for this study because they are essential micronutrients for most living organisms. Determination of the total amount of metals was carried out by standalone ICP-MS. In order to confirm the presence of different metal complexes, fractions extracted from goji berries were also analysed by SEC-ICP-MS technique. Due to the importance of metal chelation for the characterization of their activities on human organism, to study the chemical nature of metal complexes with bioligands in berries by μRPLC-ESI–MS/MS method was used.

Knowledge about concentration of element in the bioaccessible fraction is necessary in order to estimate it bioavailability. Information on the bioaccessibility of nutrients in food and it supplements seems to be important. In present work, attempts have been made to estimate the bioaccessibility of metals in the goji berries by *in vitro* simulation of gastrointestinal digestion using pepsin (gastric digestion) and pancreatin (intestinal digestion). To the best of our knowledge, there is no study in the literature about speciation analysis of metal in goji berries after enzymatic treatment. The main novelty of the study was the application of μRPLC-ESI–MS/MS to identify the copper and zinc complexes in goji berries.

2. Experimental

2.1. Chemicals and materials

Pepsin from porcine gastric mucosa and pancreatin were of biological grade (Sigma-Aldrich, Buchs, Switzerland). Ammonium acetate, formic acid, sodium dodecyl sulphate, tris(hydroxymethyl)aminomethane, HEPES for molecular biology, pectinase from *Aspergillus Niger*, hemicellulase from *Aspergillus Niger,* hydrochloric acid, sodium phosphate monobasic, 1-bromobutane and 1-methylimidazole were purchased from Sigma Aldrich and were of analytical reagent grade. Methanol and hydrogen peroxide were purchased from POCh (Gliwice, Poland). Nitric acid was the product of Fluka (Switzerland) of purity for trace metals analysis. Deionized water (18 MΩ cm) prepared with a Milli-Q system (Millipore Elix 3, Millipore, Saint- Quentin, France) was used throughout. The SEC column was calibrated using size exclusion standard (BIO-RAD, Warsaw, Poland). The calibration curves were prepared using solution of Environmental Spike Mix (1000 mg L^{-1} of Fe, K, Ca, Na, Mg and 100 mg L^{-1} of Ag, Al, As, Ba, Be, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Tl, V, Zn, U; matrix 5% HNO_3) purchased from Agilent Technologies.

The dried goji berries were obtained from *Sante* (Poland).

The ionic liquid used in this study was 1-*n*-buthyl-3-methylimidazolium bromide ([C_4mim]Br), synthesized as described on references [11]. The solution of ionic liquid used for extraction was prepared by dilution in MQ water.

2.2. Instrumentation

Chromatographic separations were performed using Agilent 1100 gradient HPLC pump (Agilent Technologies, Waldbronn, Germany) as the sample delivery system. All connections were made of PEEK tubing (0.17 mm i.d.). Agilent 7500a ICP Mass Spectrometer (Agilent Technologies, Tokyo, Japan) was used as an element-specific detector for quantification of metal content in goji berries and as on-line HPLC detector. Ni-skimmer was installed in the interface, the position of torch and nebulizer gas flow was adjusted daily with special emphasis to decrease the level of CeO/Ce below 0.2% with the aim to minimize the risk of polyatomic interferences caused by oxides. The working conditions were optimized daily using a 10 μg L^{-1} solution of $^{7}Li^{+}$, $^{89}Y^{+}$ and $^{209}Bi^{+}$ in 2% (v/v) HNO_3.

The screening for the metal complexes was performed by means of size exclusion chromatography coupled to ICP-MS. Metal species were eluted from SEC Superdex200 10/300GL (GE Healthcare Life Sciences, Freiburg, Germany) column with 10 mM ammonium acetate buffer (pH 7.4) as a mobile phase. The column was calibrated with a mixture of thyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa) and vitamin B_{12} (1.35 kDa) before the analysis.

Operational parameters are summarized in Table 1.

A Bandelin Sonorex Model 1210 ultrasonic bath (Bandelin, Berlin, Germany), MPW Model 350R centrifuge (MPW Warsaw, Poland) and water bath with thermostatically controlled temperature (Mammert, Germany) were used for extraction procedures. Microwave digestion Speedwave® four Berghof, (Berghof, Chemnitz, Germany) was used for samples' mineralization.

2.3. Sample preparation

2.3.1. Samples digestion toward total metal content determination in goji berries

The dried goji berries were grounded in liquid nitrogen using agate mortar and pestle until a homogenous powder was formed. In order to determine the total amount of elements, samples (0.2 g dry mass) were digested by microwave assisted mineralization with a mixture of 5 mL of HNO_3 and 3 mL of H_2O_2. The digests were diluted with MQ water to a final volume of 25 mL.

2.3.1.1. Inductively coupled plasma mass spectrometry. Further dilutions toward ICP-MS analysis were prepared using 2% nitric acid solution and 10 ng mL^{-1} of yttrium (^{89}Y) as an internal standard. The quantification of metal content was carried out by use of calibration curves (relevant dilutions of multi-element standard solution with internal standard addition). Curves were linear in the investigated range from 2.0 μg L^{-1} to 100.0 μg L^{-1} with r^2 above 0.999. Limits of detection (LOD) were calculated for standard deviations (SD) of 10 measurements for blank and it was found to be 0.05-0.9 μg L^{-1}.

2.3.1.2. X-Ray fluorescence analysis (XRF). The dried goji berries were analysed by X-ray fluorescence spectrometer Spectro Midex (Spectro, Ametek, Kleve, Germany). The energy-dispersive XRF equipped with a Midex/VITUS drift (SDD) high-resolution detector was applied. As an X-ray source, a 30 W Be-tube (maximum 45 kV and 0.3 mA) was used. A set of collimators with quadratic cross-sections of 2.4 mm (with and without Zr-filter) results in spot sizes on the sample of 2 mm. The time of acquisition was 180 s. All measurements were carried out in air.

2.3.2. Extraction procedure

An initial step was carried out to determine the best solvent for extraction of metal complexes. To find the most suitable sol-