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Analytical methodology

# Operationally defined species characterization and bioaccessibility evaluation of cobalt, copper and selenium in Cape gooseberry (*Physalis Peruviana* L.) by SEC-ICP MS

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ABSTRACT

*Physalis peruviana* could attract great interest because of its nutritional and industrial properties. It is an excellent source of vitamins, minerals, essential fatty acids and carotenoids. *Physalis Peruviana* is also known to have a positive impact on human health. Unfortunately, still little is known about trace elements present in *Physalis Peruviana* and their forms available for the human body. Thus, the aim of this study was to estimate bioaccessibility and characterization of species of cobalt, copper and selenium in *Physalis Peruviana* fruits.

Total and extractable contents of elements were determined by mass spectrometer with inductively coupled plasma (ICP MS). In order to separate the different types of metal complexes *Physalis peruviana* fruits were treated with the following solvents: Tris–HCl (pH 7.4), sodium dodecyl sulfate (SDS) (pH 7.4) and ammonium acetate (pH 5.5). The best efficiency of extraction of: cobalt was obtained for ammonium acetate (56%) and Tris–HCl (60%); for copper was obtained for SDS (66%), for selenium the best extraction efficiency was obtained after extraction with SDS (48%).

To obtain information about bioaccessibility of investigated elements, enzymatic extraction based on *in vitro* simulation of gastric (pepsin) and intestinal (pancreatin) digestion was performed. For copper and selenium the simulation of gastric digestion leads to the extraction yield above 90%, while both steps of digestion method were necessary to obtain satisfactory extraction yield in the case of cobalt.

Size exclusion chromatography (SEC) coupled to on-line ICP MS detection was used to investigate collected metal species. The main fraction of metal compounds was found in the 17 kDa region. Cobalt and copper create complexes mostly with compounds extracted by means of ammonium acetate and SDS, respectively. Cobalt, copper and selenium were found to be highly bioaccessible from *Physalis Peruviana*. Investigation of available standards of cobalt and selenium allows confirming the presence of vitamin $B_{12}$ and probably selenomethionine in the fraction bioaccessible by human body (obtained during enzymatic extraction). It should be noted that the presence of small seleno-compounds in Cape gooseberry was performed for the first time.

The results show that the combination of SEC and ICP MS could provide a simple method for separating of soluble element species.



## 1. Introduction

In recent years berries are becoming more popular due to their nutritional value and health benefits [1,2]. Cape gooseberry (*Physalis peruviana* L.), also known as goldenberry is a promising exotic fruit that could be a subject of many novel foods. It belongs to the *Solanaceae* family and originated in the South America. Nowadays Colombia is the largest Cape gooseberry exporter in the world [3–5]. *Physalis Peruviana* forms a dome-shaped shrub that can grow to 1 m and the fruits with an approximate weight of 4–5 g are covered by a shiny yellow peel. The flowers, produced in winter, are yellow with purple blotches [5,6]. The pulp is nutritious, containing particularly high levels of carotenoids, minerals and vitamin C [7]. The golden berry is also an excellent source of the vitamin B-complex and essential fatty acids. The protein content is exceptionally high for a fruit [8,9]. *Physalis Peruviana* is becoming more and more popular, it has increased interest worldwide

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because of its nutritional composition and the presence of biologically active compounds that provide health benefits and reduce the risk of various diseases such as cancer, malaria, asthma, hepatitis, dermatitis and rheumatism [10–13]. Although a lot of available publications focus on C. gooseberry and its properties there is a lack of information about speciation analysis of metals present and their bioavailability by human organism. Carrying out further studies, *Physalis Peruviana* could become a fruit of particular interest to the world's up-scale food industry.

The aim of the study was to separate complexes of selected elements with different bioligands and characterization of species of chosen metal. Determination of total concentration of metals in food does not provide information about their bioavailability in the human organism. Knowledge about content of element in the bioaccessible fraction is necessary to evaluate bioavailability. The information on the bioaccessibility of important nutrients in food and food supplements seems to be essential. For that reason the second objective of the study was to estimate elements bioaccessibility in C. gooseberry by *in vitro* simulation of gastrointestinal digestion using two-step model with pepsin as a gastric juice and pancreatin as an intestinal juice. To the best of our knowledge this is the first attempt to carry out characterization of species of chosen metal and bioaccessibility evaluation of metals in C. gooseberry by SEC-ICP MS.

## 2. Experimental

### 2.1. Reagents and chemicals

Sodium chloride, ammonium acetate, sodium dodecyl sulphate, Tris(hydroxymethyl) aminomethane and hydrochloric acid were purchased from Sigma–Aldrich and were of analytical reagent grade. Selenomethionine and vitamin $B_{12}$ standards (assay ≥ 98%) were also purchased from Sigma–Aldrich. Pepsin from porcine gastric mucosa and pancreatin were of biological grade (Sigma-Aldrich, Buchs, Switzerland). 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).

### 2.2. Instrumentation

The screening for the metal complexes was carried out by means of size exclusion chromatography coupled to ICP MS. Prepared samples were analysed on a Superdex200 10/300GL (GE Healthcare Life Sciences) exclusion column with bed volume of 24 mL. Before the analysis the column was calibrated with a mixture of thyroglobulin (670 kDa), γ-globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), vitamin $B_{12}$ (1,35 kDa). Chromatographic separations were performed using Agilent 1100 gradient HPLC pump (Agilent Technologies, Waldbronn, Germany) as the sample delivery system. Agilent 7500a ICP MS (Tokyo, Japan) was used as on-line HPLC detector. All connections were made with PEEK tubing (0.17 mm i.d.). Operational parameters are summarized in Table 1. The determination of concentration of elements in C. gooseberry samples (after mineralization, buffer and enzymatic extraction) was carried out by Agilent 7500a ICP MS as an element-specific detector. Ni/Cu-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 $CsO^+$ 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% $HNO_3$, with a dwell time of 0.1 ms for each isotope.

**Table 1**
Operational parameters for SEC and ICP MS.

| Settings | |
|---|---|
| SEC separation | |
| Pump | Agilent 1100 |
| Column | Superdex 200 (10 × 300 mm × 10 μm)-GE Healthcare Life Sciences |
| Mobile phase | 10 mM ammonium acetate buffer (pH 7.4) |
| Elution program | Isocratic |
| Flow | 0.7 mL $min^{-1}$ |
| Injection volume | 100 μL |
| Column temperature | 24 °C |
| ICP-MS | Agilent 7500a |
| RF Power | 1350 W |
| Plasma, auxiliary, nebulizer gas flow | 15.0, 1.0 and 1.05 L $min^{-1}$ |
| Cones | Sampler–Pt, Skimmer–Ni |
| Monitored isotopes | $^{55}Mn$, $^{59}Co$, $^{63}Cu$, $^{65}Cu$, $^{66}Zn$, $^{67}Zn$, $^{68}Zn$, $^{82}Se$, $^{95}Mo$ |
| Dwell time | 0.1 ms |

A Bandelin Sonorex Model 1210 ultrasonic bath (Germany) and MPW Model 350R centrifuge (MPW Warsaw, Poland) were used for extraction procedures.

### 2.3. Sample preparation

#### 2.3.1. Determination of total content of elements and buffer extraction method

The dried C. gooseberry was obtained from Kenay (Poland, imported from Peru) and stored at 4 °C until analysis.

For determination of total amount of elements in *Physalis Peruviana*, samples (0.3 g of dry mass) were digested by microwave-assisted mineralization with a mixture of 5 mL of $HNO_3$ and 3 mL of $H_2O_2$. After cooling down the digests were diluted with MQ water to the volume of 25 mL and then diluted before ICP MS analysis. Samples and standard solutions were prepared with addition of $^{89}Y$ as the internal standard. The external calibration curves were linear in the investigated range from 2 μg $L^{-1}$ to 150 μg $L^{-1}$ with $r^2$ above 0.997.

Samples of C. gooseberry (0.07 g) were extracted during 1 h in ultrasonic bath, using 1 mL of the following buffers: (1) 10 mM ammonium acetate (pH 5.5), (2) 30 mM Tris–HCl (pH 7.4), (3) 2% sodium dodecyl sulphate (SDS) in water (pH 7.4). After extraction, the obtained solutions were centrifuged for 20 min at 15 000 rpm at 15 °C. The final supernatants were filtered with 0.45 μm syringe filter (Sigma–Aldrich, Bellefonte, PA, USA), two first drops were discarded and only the remaining part of the filtrates was injected on the size exclusion column.

#### 2.3.2. In vitro *simulation of gastrointestinal digestion*

The *in vitro* digestion method was based on Luten et al. [14] modified to the studied berries. 2.5 mL of gastric juice (6% w/v pepsin in 0.15 M NaCl, acidified to pH 1.8 by means of HCl) was added to 0.07 g of C. gooseberry and then shaken and sonicated for 10 min in ultrasonic bath. In the next step the mixture was incubated in the thermostatic water bath for 3.5 h at 37 °C. After incubation the mixture was centrifuged at 4 °C for 20 min at 15 000 rpm. The supernatants were filtered through 0.45 μm syringe filters (Sigma–Aldrich, Bellefonte, PA, USA) and analyzed. For intestinal digestion, $NaHCO_3$ solution was added to the remaining part of sample to obtain the neutral pH. After that 2.5 mL of intestinal juice (1.5% w/v pancreatin in 0.15 M NaCl) was added and the mixture was incubated for 2 h at 37 °C. Following centrifugation and filtration of supernatant, the gastrointestinal extract was analyzed by SEC-ICP MS.

To collect the information about the bioaccessibility of important nutrients from *Physalis Peruviana* by human organism, the