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# Effect of cooking on phosphorus and trace elements species in peas

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**Abstract**

Changes of solubility of phosphorus, manganese, iron, cobalt, nickel, copper, zinc and molybdenum and changes of proportions of individual element species fractions in pea seeds, occurring as a result of soaking and boiling of pea, were investigated. Speciation analyses were accomplished by size exclusion chromatography–inductively coupled plasma mass spectrometry. Total contents of elements, the respective soluble portions and portions passing SEC column, were ascertained and mass balances were done. During boiling of pea most of elements were partly dissolved. Compounds of elements remaining in the boiled pea became less soluble in most cases. Soluble high molecular weight element species, present in the original pea, were completely removed by boiling of pea. Low molecular weight species of nickel, zinc and molybdenum were more stable against thermal treatment. Nevertheless, boiling increases the proportions of ionic species and labile complexes.


*Keywords:* Pea; Trace metals; Phosphorus; Element species; Size exclusion chromatography; Inductively coupled plasma mass spectrometry

## 1. Introduction

Legumes are quite rich sources of proteins, carbohydrates and mineral elements for human and animal nutrition (Belitz & Grosch, 1992; Deshpande & Deshpande, 1991). However, the quality of diet based solely on food of plant origin is poor because of limited contents of some amino-acids (namely methionine and lysine in legume and cereal proteins, respectively) and presence of antinutritional factors, such as proteinase and amylase inhibitors, lectins, tannins, and phytic acid. Cooking and other ways of processing improve nutritional value of legumes. Even conventional procedures lead to improvement of legume protein digestibility and partly diminish antinutritional factors (Alonso, Otrúe, & Marzo, 1998; Davídek, Velíšek, & Pokorný, 1990; Habiba, 2002). However, little is known about the influence of processing on mineral element status in legumes and legume-based food.

Mineral element composition of legume seeds is distinctive when compared to other food of plant origin (Varo, Lähelmä, Nuurtamo, Saari, & Koivistoinen, 1980). The contents of molybdenum, nickel and iron in legume seeds are higher while contents of manganese are somewhat lower than those in cereal grains. Amounts of phosphorus, zinc and copper are comparable in both groups. Despite high total contents, the bioavailable contents of essential mineral elements are limited due to element interactions with dietary fibre, phytic acid or other components of legumes (Alonso, Rubio, Muzquiz, & Marzo, 2001). Absorption of mineral element from the diet is related to chemical forms and to possibilities of their transformations during food processing and food digestion. There is little knowledge about the nature of mineral element species or compounds present in food. Determination of total element concentration cannot give sufficient information for estimation of biological significance of the element in the diet (possible beneficial or toxic effects). Therefore the development of analytical procedures for element analysis has been aimed at speciation analysis (Sanz-Medel, 1998), i.e. differentiation and determination of individual species of the element (e.g. metal ions of different oxidation states, organometallic compounds, low- and high-molecular weight complexes of metals). The results of speciation analysis are now widely utilized in clinical, biological and environmental sciences. Analytical procedures for element speciation analysis in biological samples are

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mostly based on hyphenation of chromatographic or electrophoretic methods, with element-specific detection methods such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission (ICP–OES) or mass spectrometry (ICP–MS) (Łobiński & Szpunar, 1999; Szpunar, 2000).

Size exclusion chromatography (SEC), hyphenated with element-specific detectors, such as, ICP–OES (Schöppenthau, Nölte, & Dunemann 1996) and ICP–MS (Koplík, Pavelková, Cincibuchová, Mestek, Kvasnička, & Suchánek, 2002), was successfully applied for element species fractionation in extracts of legumes. The methodology, based on SEC/ICP–MS, was recently improved and accurate quantification of the element fractions, by an external calibration technique (Koplík, Borková, Mestek, Komínková, & Suchánek, 2002), was achieved. Comprehensive validation and uncertainty estimation of SEC/ICP–MS procedures using external calibration and isotope dilution, were done (Mestek, Komínková, Koplík, Borková, & Suchánek, 2002). Therefore, it is now possible to quantitatively evaluate alteration of individual element species during processing of legumes or other foods. This paper describes changes of solubility and soluble species fractions of eight elements investigated by SEC/ICP–MS in the course of soaking and boiling of peas.

## 2. Materials and methods

### 2.1. Instrumentation

All ICP–MS measurements were carried out with an ICP mass spectrometer Elan 6000 (Perkin-Elmer, Norwalk, CT, USA) equipped with cross-flow nebulizer, Scott's double pass spray chamber and Gilson 212 peristaltic pump for sample aspiration. Sample decomposition was performed in a microwave decomposition unit, UniClever (Plazmatronika-Service, Wroclaw, Poland). pH values of buffer solutions were measured with a pH 03 instrument (Labio, Prague, Czech Republic). The HPLC apparatus consisted of a Superdex 75 HR 10/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden) (optimal fractionation range 7–30 kDa), high pressure pump, Varian Inert 9012 (Varian, Walnut Creek, CA, USA), glass column Superformance 150×10 mm (Merck, Darmstadt, Germany), packed with chelating resin Chelex 100 (Merck) in $NH_4^+$ form and two Rheodyne 9125 injectors equipped by 100 and 500 μl PEEK sample loops, respectively. PEEK or PTFE capillaries (internal diameter 0.25 mm) connected all parts of the apparatus.

### 2.2. Standards and reagents

Tris (hydroxymethyl) aminomethane (Tris), serving for preparing of the mobile phase and extractant solution, was purchased from Fluka (Neu-Ulm, Germany). Hydrochloric acid (30%) and nitric acid (65%) were both of Suprapur® grade (Merck, Darmstadt, Germany). Cobalt, copper, indium, iron, manganese, molybdenum, nickel and zinc stock solutions ($\rho = 1000$ mg/l) were obtained from Merck too. Phosphorus stock solution ($\rho = 1000$ mg/l) was prepared by dissolving an appropriate amount of ammonium dihydrogenphosphate (purity 99.999%) (Aldrich Chemical Co., Milwaukee, WS, USA) in deionized water. A set of peptide and protein standards obtained from Sigma (St. Louis, MO, USA) was used for calibration of molecular weight determination by SEC. Distilled deionized water (Milli-Q, Millipore, Bedford, MA, USA) was used for preparation of all solutions.

### 2.3. The sample

Seeds of pea (*Pisum sativum* L.) cultivar Lantra were obtained from the Central Institute for Supervising and Testing in Agriculture, Brno. The seeds were delivered 6 months after the harvest. Proximate composition of sample was 12.4% of moisture, 2.5% of ash, 19.3% of protein and 1.1% of fat. These analyses were accomplished according to standard methods (Kirk & Sawyer, 1991). Sample processing, simulating kitchen preparation of pea, consisted of soaking, followed by boiling. The original pea seeds were soaked for 12–14 h in deionized water. The mass ratio pea: water was 2:25. The soaked pea was then boiled in the same water for 45 min. In the course of boiling, the ratio 2:25 was kept constant. The original as well as the processed peas (soaked pea and boiled pea), were submitted to analyses. The soaking and boiling took place in the same 100-ml beaker, which was decontaminated before use by leaching in 10% (v/v) $HNO_3$. The total contents of phosphorus, manganese, iron, cobalt, nickel, copper, zinc, and molybdenum were determined in all three types of sample. Other sets of original and processed peas samples were extracted by 0.02 M Tris–HCl, pH = 7.5 buffer solution (see below) and contents of elements in the obtained extracts were determined too. The extracts were submitted to further analyses of element species by SEC/ICP–MS. In addition, the water remaining after soaking and boiling of peas was analysed too.

### 2.4. Preparation of sample extracts for determination of elements and speciation analysis

The original seeds were milled in a vibration mill under liquid nitrogen to obtain fineness $<0.2$ mm. Two grammes of finely powdered sample were extracted with 50 ml of 0.02 M Tris–HCl buffer solution, pH = 7.5 by 1 h of shaking in a polypropylene flask. The suspension was centrifuged and the clear supernatant was used for further analyses. The buffer solution was previously

purified by passing through the glass column packed with Chelex 100 resin. The pH value of the extraction solution was selected with respect to solubility of 11S and 7S globulins, representing the main proteins of seeds of leguminous plants (Belitz & Grosch, 1992).

Preparation of extracts of the soaked and boiled pea seeds was analogous. The procedure starts with 2 g of original uncrushed pea seeds. After soaking, or soaking and boiling the seeds were withdrawn with the aid of purified Pt wire and crushed in an agate mortar. The crushed material was then extracted by Tris–HCl buffer solution and the extract was centrifuged as described above. The water remaining after boiling was cleared of suspended matter by centrifugation and the supernatant was then submitted to the same analyses as the buffer extracts of pea.

### 2.5. Determination of total content of elements

Samples of seeds (0.5–0.8 g) or extracts (10 ml) were decomposed by pressurized microwave digestion in PTFE vessels with 6 ml (seeds) or 3 ml of $HNO_3$ (extracts) for 10 min. The decomposition took place under controlled conditions (max. microwave power 135 W per one vessel, max. pressure 4.0 MPa). The temperature of sample during decomposition reached about 200 °C. The obtained sample digests were transferred into appropriate calibrated flasks and made up to volume, i.e. 100 ml (seeds) or 50 ml (extracts). The original pea was milled before decomposition (see above) whereas, in the case of soaked and boiled pea, the whole uncrushed seeds were decomposed. The starting amounts were 2 g of original uncrushed pea. The treated (soaked or soaked and boiled) material was then decomposed. The whole amount of the treated pea, originating from 2 g of pea, had to be decomposed in three separated portions in three digestion vessels. The obtained digests were then combined. Contents of elements were calculated on the basis of sample weight of the original pea. The water remaining after pea soaking was treated before analysis as follows: individual soaked seeds were taken out from the beaker by the use of purified Pt wire and remaining water was acidified with 2 ml of 65% $HNO_3$. The beaker was then covered by a watch glass and heated for 2 h on a hot plate at 80 °C. After cooling, the contents of the beaker were transferred to a 50 ml calibrated flask. In the case of the water remaining after boiling of pea, the water was centrifuged and acidified before analysis.

The concentrations of elements were determined by ICP–MS technique with external calibration. Indium added to all samples, blanks and standard solutions served as an internal standard. Details of the analytical method were given in a previous paper (Fingerová & Koplík, 1999); operating conditions for the Elan 6000 mass spectrometer are summarized in Table 1. Analyses were performed separately for phosphorus and metallic elements. Determination of phosphorus by ICP–MS was accomplished by measurement of an oxide particle $^{47}(PO)^+$ under somewhat changed plasma conditions, as mentioned in a previous paper (Koplík et al., 2002). Accuracy of analytical results was verified by analysis of NIST SRM 1515 Apple Leaves and SRM 1570a Spinach Leaves.

### 2.6. Size exclusion chromatography–inductively coupled plasma mass spectrometry

The chromatographic apparatus is shown in Fig. 1. Buffer solution of 0.02 M Tris–HCl (pH = 7.5) served as the mobile phase and the flow rate was 0.5 ml/min. Traces of metals were on-line removed from the mobile phase by passing through a glass column packed with Chelex 100 resin in the $NH_4^+$ form. The pea extracts or water samples were injected to the SEC column by the first Rheodyne 9025 injector with 100 μl PEEK sample loop. The second injector, equipped with a 500 μl sample loop, was inserted between the SEC column and the ICP–MS and it served for the post-column injection of calibration standard solutions. The flow of effluent (0.5 ml/min) was by T piece joined together with a flow of internal standard solution (50 μg/l of In). The mixed flow (approx. 1.3 ml/min) was delivered by peristaltic pump to the cross-flow nebulizer of the ICP–MS. Duration of SEC/ICP–MS analysis was 50 min and chromatograms consisted of 1000 points of 3 s each. The analysis of each sample consisted of two separate runs: the first run served for the metal analysis whereas the second run served for the analysis of phosphorus. Main operating conditions of the ICP–MS used for SEC/ICP–MS analyses are summarized in Table 1. In the course of the first 10 min after injection of sample to the Superdex column, when no sample fraction had been eluted from the column yet, three standard solutions of elements were consecutively injected to the flow of mobile phase using the second injector. Injections of two standard solutions were repeated after the 42nd min when all sample fractions of extract had left the column. In the case of metal determinations, the concentrations of elements in the first standard solution were: 1 μg/l Co, 25 μg/l Cu, 100 μg/l Fe, 25 μg/l Mn, 10 μg/l Mo, 10 μg/l Ni and 100 μg/l Zn. Concentrations in the second and the third standard solutions were 2 and 4 times higher, respectively. Concentrations of phosphorus in three standards for separate phosphorus analyses were 10, 20 and 50 mg/l, respectively. The 500 μl sample loop was used for standards injection to obtain broader peaks, as similar as possible to chromatographic peaks of sample fractions. After smoothing of SEC/ICP–MS data using a Golay-Savitzky digital filter, the obtained peaks of analytes were integrated and second order calibration curves (peak areas vs. analyte mass) were fitted. Details

Table 1
Main operating conditions of ICP–MS Elan 6000

| Parameter | Total content | SEC/ICP–MS |
|---|---|---|
| R.f. power | 1000 W | |
| Ion lens voltage | AutoLens mode optimized for maximum signal of Be, Co and In | |
| Nebulizer Ar flow | 0.75–0.80 l/min for the metals determination<br>0.95 – 1.00 l/min for the phosphorus determination (daily optimised) | |
| Measurement mode | Peak hopping | |
| Measured isotopes | Determination of metals:<br>$^{55}$Mn, $^{57}$Fe, $^{58}$Ni (corrected for $^{58}$Fe), $^{59}$Co, $^{65}$Cu, $^{95}$Mo, $^{66}$Zn, $^{115}$In<br>Phosphorus determination:<br>$^{47}$PO, $^{115}$In | |
| Dwell time | 50 ms | Metals: 29 ms for $^{59}$Co, 21 ms for others<br>Phosphorus: 47 ms |
| Sweeps/replicate | 10 for metal, 15 for phosphorus analysis<br>15 for phosphorus analysis | Metals: 15<br>Phosphorus: 30 |
| Acquisition time for one replicate | 4.8 s for metal analysis<br>1.6 s for phosphorus analysis | 3.0 s |
| No. of replicates | 10 | 1000 |
| Total acquisition time | 48 s for metal analysis<br>16 s for phosphorus analysis | 50 min |
| Solution uptake | 1.3 ml/min | 0.5 ml/min of column effluent +<br>0.8 ml/min of internal standard solution |

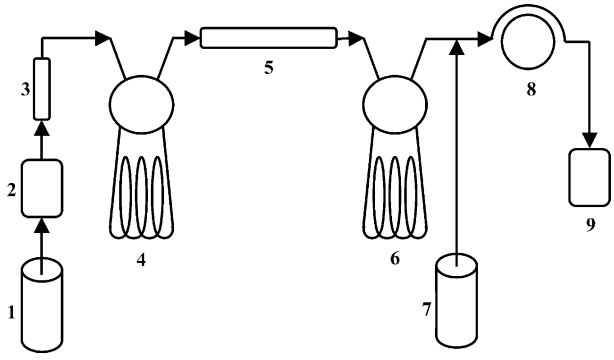


Fig. 1. Scheme of SEC/ICP–MS apparatus; 1—mobile phase reservoir, 2—high pressure pump, 3—Chelex 100 column filled, 4—first injector with 100 μl sample loop (injection of extract), 5—Superdex 75 HR column, 6—second injector with 500 μl sample loop (injection of standards), 7—reservoir of internal standard solution, 8—peristaltic pump, 9—ELAN 6000 ICP–MS.

of this procedure together with some basic validation parameters, were given previously (Mestek et al., 2002). Between two consecutive measurements, the SEC column was cleaned by injection of 0.002 M EDTA solution and washing by mobile phase for 1 h. During this procedure, the adsorbed alkaline earth metals and transition metals, disengaged from weak complexes, were removed.

## 3. Results and discussion

Table 2 summarizes the data concerning element quantities (total, extractable and recovered by SEC) in original and processed peas, as well as in the water remaining after pea soaking and pea boiling. The contents in all samples (including water), given in μg/g, are expressed as microgrammes of the element relating to one gramme of the original pea sample. Element contents in our original pea sample are in accordance with the published levels of elements in pea (Alonso, Grant, Dewey, & Marzo, 2000; Gundersen, Bechmann, Behrens, & Stürup, 2000; Varo et al., 1980) and can be considered as normal. A considerable part of the total element content (more than 75% in the case of Mn, Co, Ni, Cu, Zn, Mo, and P) is extracted from the original pea into Tris–HCl buffer solution. Iron, characterized by lower extractability, is an exception. Thus substantial amounts of elements, represented by soluble species, are accessible for further investigation by chromatographic analysis.

Most soluble species of transition metals present in legume seeds are represented by more or less stable complexes of these elements with organic ligands such as proteins and peptides (Koplík, Pavelková et al., 2002). These compounds are eluted from the SEC column in the range of dead and total volume and, therefore, their molecular weights can be estimated. On the other hand, alkali metals and minor amounts of transition element species are adsorbed on the gel support of the SEC column and must be liberated by the EDTA cleaning procedure. Amounts of elements not recovered by SEC and adsorbed are ascertained by mass balance. As explained earlier (Koplík, Borková et al., 2002), the minor portion of transition metals retained on the SEC column can be considered as ionic and labile element species.

Effects of soaking and boiling of pea on total contents of elements can be seen in Table 2. The total contents of elements did not change during soaking. Only a few percent of nickel and iron contents dissolved water. Compared with the original pea, the extractabilities of phosphorus and manganese from soaked pea were

Table 2
Total contents of elements in original and processed pea sample

| | P | Mn | Fe | Co | Ni | Cu | Zn | Mo |
|---|---|---|---|---|---|---|---|---|
| *Original pea* | | | | | | | | |
| Total content (μg/g) (*a*) | 3600 *(100)* | 9.30 *(0.25)* | 55.1 *(1.5)* | 0.079 *(0.003)* | 1.07 *(0.05)* | 6.33 *(0.15)* | 20.7 *(0.6)* | 1.98 *(0.05)* |
| Extractable portion (μg/g) (*b*) | 2800 *(150)* | 7.63 *(0.35)* | 30.8 *(1.5)* | 0.060 *(0.003)* | 1.09 *(0.05)* | 4.78 *(0.20)* | 18.1 *(1.0)* | 1.84 *(0.10)* |
| Extractable portion (%, related to *a*) | 78 | 82 | 56 | 76 | 102 | 76 | 87 | 93 |
| Portion passing SEC column (μg/g) | 2120 *(150)* | 6.68 *(0.50)* | 25.8 *(1.5)* | 0.047 *(0.005)* | 0.87 *(0.06)* | 4.97 *(0.35)* | 19.3 *(1.4)* | 1.57 *(0.10)* |
| Portion passing column (%, related to *b*) | 76 | 88 | 84 | 78 | 80 | 104 | 107 | 85 |
| *Soaked pea* | | | | | | | | |
| Total content [μg/g] (*c*) | 3430 *(120)* | 9.33 *(0.30)* | 49.6 *(1.8)* | 0.060 *(0.003)* | 0.98 *(0.08)* | 5.87 *(0.20)* | 20.9 *(0.6)* | 1.99 *(0.05)* |
| Total content (%, related to *a*) | 95 | 100 | 90 | 76 | 92 | 93 | 101 | 100 |
| Extractable portion (μg/g) (*d*) | 2280 *(150)* | 5.36 *(0.03)* | 27.3 *(2.0)* | 0.050 *(0.003)* | 0.94 *(0.08)* | 4.18 *(0.20)* | 17.4 *(1.0)* | 1.69 *(0.10)* |
| Extractable portion (%, related to *c*) | 66 | 57 | 55 | 83 | 96 | 71 | 83 | 85 |
| Portion passing SEC column (μg/g) | 1970 *(150)* | 4.03 *(0.03)* | 23.8 *(1.5)* | 0.043 *(0.005)* | 0.74 *(0.06)* | 4.17 *(0.30)* | 17.5 *(1.3)* | 1.44 *(0.10)* |
| Portion passing column (%, related to *d*) | 86 | 75 | 87 | 86 | 79 | 100 | 100 | 85 |
| *Water after pea soaking* | | | | | | | | |
| Total content (μg/g) | 80 *(10)* | 0.02 *(0.01)* | 2.03 *(0.10)* | 0.001 *(0.001)* | 0.04 *(0.01)* | 0.09 *(0.02)* | 0.2 *(0.05)* | 0.01 *(0.005)* |
| Total content (%, related to *a*) | 2 | $<1$ | 4 | 1 | 4 | 1 | 1 | $<1$ |
| *Boiled pea* | | | | | | | | |
| Total content (μg/g) (*e*) | 2100 *(120)* | 8.31 *(0.30)* | 33.2 *(2.0)* | 0.020 *(0.004)* | 0.33 *(0.03)* | 3.20 *(0.20)* | 14.2 *(0.80)* | 0.66 *(0.04)* |
| Total content (%, related to *a*) | 58 | 89 | 60 | 25 | 31 | 51 | 69 | 33 |
| Extractable portion (μg/g) (*f*) | 500 *(50)* | 0.29 *(0.03)* | 4.46 *(0.50)* | 0.019 *(0.003)* | 0.32 *(0.03)* | 1.10 *(0.10)* | 3.08 *(0.30)* | 0.40 *(0.03)* |
| Extractable portion (%, related to *e*) | 24 | 3 | 13 | 95 | 97 | 34 | 22 | 61 |
| Portion passing SEC column [μg/g] | 390 *(40)* | 0.11 *(0.02)* | 1.47 *(0.30)* | 0.017 *(0.003)* | 0.28 *(0.03)* | 1.11 *(0.10)* | 3.05 *(0.20)* | 0.36 *(0.03)* |
| Portion passing column (%, related to *f*) | 78 | 38 | 33 | 89 | 88 | 101 | 99 | 90 |
| *Water after pea boiling* | | | | | | | | |
| Total content (μg/g) (*g*) | 1550 *(50)* | 0.72 *(0.05)* | 15.0 *(0.5)* | 0.060 *(0.003)* | 0.78 *(0.05)* | 3.49 *(0.15)* | 8.01 *(0.4)* | 1.36 *(0.05)* |
| Total content (%, related to *a*) | 43 | 8 | 27 | 76 | 73 | 55 | 39 | 69 |
| Portion passing SEC column (μg/g) | 1070 *(100)* | 0.25 *(0.05)* | 11.6 *(0.7)* | 0.053 *(0.005)* | 0.70 *(0.05)* | 3.69 *(0.25)* | 6.96 *(0.50)* | 1.33 *(0.10)* |
| Portion passing column (%, related to *g*) | 69 | 35 | 77 | 88 | 90 | 106 | 87 | 98 |

Data are given as averages of eight results (original pea) or two results (other samples). Values in parentheses represent expanded uncertainties ($k = 2$).

slightly decreased. The extractable contents of other elements were not markedly changed. Except for manganese, the soaking obviously has no effect on the amounts of elements recovered by SEC of pea extract. It can be concluded that the soaking did not change the stability of the original element species. Further analyses by SEC/ICP–MS confirmed almost identical element species in soaked and original peas. On the other hand considerable alterations of element contents occur as a result of pea boiling. Most of the cobalt, nickel and molybdenum contents (approx. 80%) and a half of the copper content are dissolved. Lower amounts of the element are liberated to water in the cases of phosphorus, zinc (approx. 40%), iron (27%) and manganese ($<10\%$) during pea boiling. In recent experiments (Habiba, 2002) only about 20% of the phosphorus was lost as a result of 40 min cooking of pea. The amounts of soluble (i.e. extractable to Tris–HCl) compounds of manganese, copper, iron, zinc, molybdenum and phosphorus in the boiled pea were decreased compared to values in the original pea. In the case of manganese, the boiling makes this element in pea almost completely insoluble. This could be caused by denaturation of original manganese-containing biomolecules or liberation of the metal ions and formation of insoluble inorganic compounds such as $MnO_2$. On the other hand, the percentage of extractable cobalt in the boiled pea was slightly higher than that in the original pea. High extractability of nickel is not affected by pea boiling. The amount of iron recovered by SEC of pea extract is considerably decreased as a result of boiling. Iron and manganese compounds present in original pea are probably decomposed.

Transformations of soluble species of elements during soaking and boiling of pea are obvious from Fig. 2, which shows element-specific chromatograms of individual elements obtained by analysis of original and processed peas and the water remaining after pea boiling. A summary of results, including retention times of peaks, the corre-

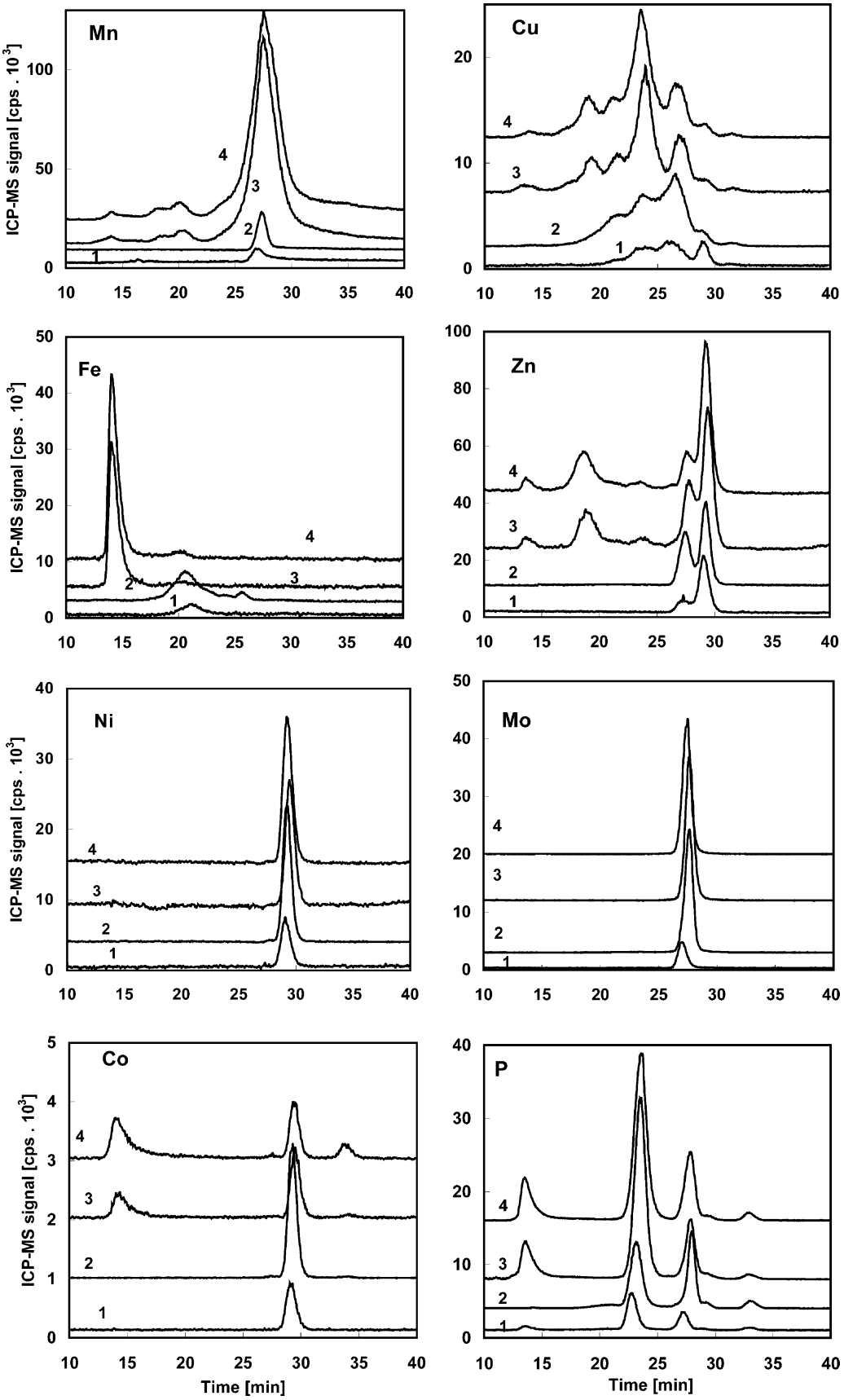


Fig. 2. Examples of chromatograms of Mn, Fe, Ni, Co, Cu, Zn, Mo and P compounds in extracts of pea and in the water remaining after pea boiling; 1—extract of boiled pea, 2—water after boiling of pea, 3—extract of soaked pea, 4—extract of original pea.

sponding apparent relative molecular weights of detected species and element quantities bound to individual species, is given in Table 3.

Most of the phosphorus in extract of original pea is bound to the medium molecular weight (6 kDa) fraction, while minor amounts are bound to the low molecular weight (1 kDa) and high molecular weight (170 kDa) compounds. Extract of the soaked pea shows practically the same pattern of phosphorus compounds. In the course of boiling, the phosphorus compounds are partially leached to water. Water, after boiling, contains approx. 40% of the original phosphorus. The proportion among individual fractions is changed: high molecular weight phosphorus compounds are decreased and low molecular weight are somewhat increased. The cause can be seen in denaturation of phosphoproteins, making them insoluble and partial hydrolysis of phosphorus containing molecules, resulting in low molecular weight species. Phosphorus compounds remaining in the boiled pea are mostly insoluble. The species in buffer extract, representing the minor soluble part of the phosphorus compounds, seems to be the same as those in the water after boiling.

Table 3
Summary of analyses of element species in original and processed peas by SEC/ICP–MS method

| Element | $t_R$ (min) | Apparent $M_r$ (kDa) | Element contents in individual species (μg/g) | | | |
|---|---|---|---|---|---|---|
| | | | Original pea | Soaked pea | Boiled pea | Water after boiling |
| P | 13.7 | 170[a] | 300 | 270 | 20 | 10 |
| | 23.5 | 6 | 1320 | 1250 | 250 | 570 |
| | 28.1 | 1.1[a] | 450 | 410 | 110 | 440 |
| | 33.1 | <0.5[a] | 50 | 40 | 10 | 50 |
| Mn | 14.2 | 140[a] | 0.07 | 0.06 | 0 | 0 |
| | 17–22 | 9–53 | 0.35 | 0.23 | 0 | 0 |
| | 27.7 | 1.2[a] | 6.27 | 3.74 | 0.11 | 0.25 |
| Fe | 14.0 | 150[a] | 23.7 | 21.3 | 0 | 0 |
| | 20.8 | 14 | 2.1 | 2.5 | 1.47 | 10.3 |
| | 25.8 | 2.5[a] | 0 | 0 | 0 | 1.29 |
| Co | 14.1 | 146[a] | 0.025 | 0.014 | 0 | 0 |
| | 29.4 | 0.7[a] | 0.018 | 0.028 | 0.017 | 0.052 |
| | 34.0 | <0.5[a] | 0.004 | 0.001 | 0 | 0.001 |
| Ni | 29.3 | 0.8[a] | 0.87 | 0.74 | 0.28 | 0.70 |
| Cu | 14.0 | 150[a] | 0.10 | 0.10 | 0 | 0.02 |
| | 16–22.5 | 8–75 | 0 | 0 | 0 | 0.90 |
| | 19–25 | 3–26 | 0 | 0 | 0.41 | 0 |
| | 19.3 | 24 | 0.76 | 0.60 | 0 | 0 |
| | 21.5 | 11 | 0.47 | 0.47 | 0 | 0 |
| | 23.8 | 5 | 2.57 | 2.03 | 0 | 0.98 |
| | 26.8 | 1.7[a] | 0.92 | 0.78 | 0.50 | 1.55 |
| | 29.3 | 0.7[a] | 0.11 | 0.14 | 0.19 | 0.20 |
| | 31.5 | <0.5[a] | 0.05 | 0.05 | 0.01 | 0.04 |
| Zn | 13.9 | 156[a] | 0.95 | 0.94 | 0 | 0 |
| | 18.9 | 27 | 4.95 | 4.59 | 0 | 0 |
| | 23.9 | 5 | 3.28 | 1.35 | 0 | 0 |
| | 27.6 | 1.3[a] | 2.69 | 1.71 | 0.32 | 1.81 |
| | 29.3 | 0.7[a] | 7.43 | 8.90 | 2.73 | 5.15 |
| Mo | 27.6 | 1.3[a] | 1.57 | 1.44 | 0.36 | 1.33 |

[a] Estimation is out of optimum fractionation range of the column (3–70 kDa) given by producer.

Manganese compounds present in pea undergo considerable changes during pea processing. Soaking surprisingly leads to reduction of manganese extractability and stability of soluble manganese species. The chromatographic profile of manganese shows a major peak in the low molecular weight region (1 kDa), a minor broad zone corresponding to a wide interval of molecular weight (9–53 kDa) and one minor peak in the high molecular weight region (140 kDa). A similar pattern occurs for the soaked sample. High molecular and medium molecular weight species completely disappear from the extract of the boiled pea. Only traces of manganese are found in the low molecular weight region, both in the boiled pea extract and water, after pea boiling.

Compared with other elements, iron shows the lowest extractability from the original pea. Most of the extractable iron in original and soaked peas (approx. 78%) is bound to high molecular weight (150 kDa) substances, while a minor part (approx. 8%) is bound to the medium molecular weight fraction (14 kDa). Uncomplexed ferric and ferrous ions, which were not eluted by SEC at all, represent the rest (14%). Boiling of pea leads to considerable change of iron status. About one quarter of the iron content appears in the water after boiling, where it is mostly bound to the medium molecular weight fraction. A minor part of the iron in water after boiling was found in the low molecular weight region (2 kDa). In the boiled pea, most of the iron remains insoluble (iron extractability decreases to 13%). Moreover, only one third of the iron extractable from the boiled pea is recoverable by SEC. Thermal treatment made most of the iron compounds unstable with respect to a small shift of pH so that the extraction by Tris–HCl buffer resulted in splitting of original molecules and liberation of metal ions. SEC/ICP–MS analysis of buffer extract of the boiled pea revealed that high molecular weight iron compounds (probably metalloproteins) were decreased and traces of iron were bound to the medium molecular weight fraction. Decreased solubility of iron can be ascribed to change of iron valency. Oxidation of ferrous iron to ferric iron, by oxygen, is followed by ferric ion hydrolysis to form insoluble ferric hydroxide. A recent study (Quinteros, Farré, & Lagarda, 2001) found that, in the fraction of soluble iron from legumes, ferric iron prevails over ferrous. The amount of ferrous iron in bean and lentil is decreased as a result of oxidation during cooking.

Approx. 40% of cobalt present in extract of the original pea is bound to the high molecular weight (140 kDa) fraction and approx. 35% is contained in two low

molecular weight species (0.7 and <0.5 kDa). The rest is not recoverable by SEC. During soaking of pea some redistribution of the cobalt occurs: most of cobalt is transferred to low molecular weight species (0.7 kDa). During pea boiling, most of the cobalt (approx. 75%) is transferred to water. Cobalt remaining in the boiled pea is almost completely soluble in Tris–HCl buffer and is bound only to the low molecular weight (0.7 kDa) fraction. The same cobalt species was found in water after boiling.

Nickel compounds present in original and processed peas are completely extractable. Good solubility of this element is a characteristic feature of various legume seeds, such as bean, soybean (Koplík, Pavelková et al., 2002) and lentil seeds (Koplík, Borková et al., 2002). After boiling, the nickel content in pea decreases approximately to one third of the original value. Almost all nickel (80–90%) present in original and processed peas and in the water after boiling is recoverable by SEC and it is bound solely to the low molecular weight (0.8 kDa) fraction. The same species was detected as a unique form of nickel in the water after pea boiling.

Copper chemistry in pea is very complex. Only one quarter of the copper present in the original pea is insoluble. Soluble species are completely recoverable by SEC. SEC/ICP–MS analysis of original pea extract revealed seven species ranging in molecular weight from 0.5 to 150 kDa. The main copper peak in the elution profile, corresponding to molecular weight 5 kDa, is overlapped with the main peak of phosphorus compounds. This fraction contains approx. 50% of the soluble copper. Another three species, the molecular weights of which are 1.7, 24 and 11 kDa, account for 19, 16 and 10% of copper, respectively. The soaked pea extract showed very similar chromatographic profile. Boiling considerably changed copper status in pea. Approx. 50% of the copper dissolved in water and the copper remaining in the boiled pea was mostly insoluble (extractability decreases to 34%). The shape of the copper elution profile was changed after boiling. Some sharp peaks were made more diffusive so that two broad zones appeared in extract of the boiled pea and in the water after boiling. Moreover, some peaks all disappeared. The 1.7 kDa fraction became the main copper species in the boiled pea and water after boiling.

Soluble compounds of zinc, representing 88% of the total content in original pea, were completely recoverable by SEC. Two low molecular weight species (0.7 and 1.3 kDa) contained approx. 40 and 15% of the zinc, respectively. Other species were found in the medium molecular weight (27 and 5 kDa) and high molecular weight (156 kDa) regions. The 1.3 kDa zinc peak was overlapped with the main peaks of manganese and molybdenum and minor peak of phosphorus. Soaking of pea led to transfer of some zinc from the 5 and 1.3 kDa species to 0.7 kDa species. In the course of pea boiling approx. 40% of the zinc dissolves in water. The solubility of zinc in the boiled pea is limited (extractable portion is only 22%). Zinc-specific elution profiles of boiled pea extract and water after the pea boiling contained only two low molecular weight species (0.7 and 1.3 kDa). Other zinc species disappeared.

Molybdenum compounds present in original and soaked peas were almost completely extractable. Molybdenum was eluted in a single peak in the low molecular weight region (1.3 kDa), overlapped with the minor peak of phosphorus. Boiling of pea liberated most of the molybdenum into water. Residual molybdenum in the boiled pea is of limited solubility (the extractability decreased approx. to 60%). A single molybdenum species detected in the boiled pea and water after boiling seemed to be identical to that in the original sample.

## 4. Conclusion

Individual elements show diverse behaviour as a result of pea cooking. Before experimenting could be anticipated that thermal treatment would cause denaturation and precipitation of metalloproteins and decomposition of original metallobiomolecules. But the extent of element species alteration varied with the nature of the element and stability of the element species. Main effects of pea boiling on phosphorus and trace elements can be characterized as follows:

1. The losses of total contents of most elements, as a result of leaching into water, are considerable and represent one quarter (Co) to two thirds (Zn) of the original amounts. Loss of manganese is very low (about one tenth) because of low solubility of the transformed manganese species.
2. Solubility of all elements except nickel and cobalt, is reduced considerably (Mn, Fe, Cu, Zn) or partlys (Mo). Proportions of free metal ions and labile species not recovered by SEC are increased (e.g. iron).
3. Soluble high molecular element species are removed completely (Mn, Fe, Co, Cu, Zn) or almost completely (P).
4. Soluble low molecular weight species of cobalt, nickel, zinc and molybdenum are stable against thermal treatment and do not undergo any observable change.
5. Original medium molecular weight species of copper are made partly insoluble. The remaining soluble copper partly undergoes species transformation, resulting in a relative increase of the low molecular weight fraction. Any copper ion evolved from the original species is bound to another (low molecular weight) ligand and so both raw and boiled peas do not contain any uncomplexed copper.

These effects are not easy to interpret. Changes of chemical status of elements in thermally processed peas might influence some chemical reactions of food or feed components coming into contact with processed peas. Catalytic effect of uncomplexed ferric and ferrous ions, evolved from original iron species during boiling, on oxidation of lipids or ascorbic acid, can be assumed. Copper ions represent another efficient catalyst of oxidation but, even in the boiled peas, all copper remains either insoluble or bound into stable chelate.

It is very difficult to interpret the above data from a nutritional point of view. There is no simple relation between chemical speciation of elements, partly presented in this article, and the bioavailability of elements. Compounds of chemical elements present in processed food undergo considerable further changes during digestion. With respect to many other factors affecting availability of elements, biological in vivo studies are needed to obtain reliable data. On the other hand, knowledge of chemical forms of elements in digested food would be of interest. It can be expected that some methods of element speciation analysis will be utilized in such research.

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