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Effect of technological processing and maturity stage of seeds on the content and speciation of phosphorus and trace elements in peas

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Abstract

Changes of speciation of phosphorus, manganese, iron, cobalt, nickel, copper, zinc, and molybdenum in pea seeds, occurring as a result of blanching and boiling of green pea, were investigated. Element speciation in green pea was compared with that of mature pea. Speciation analyses were accomplished by size exclusion chromatography – inductively coupled plasma mass spectrometry. Total contents of elements, the respective soluble portions and portions passing a SEC column, were ascertained and mass balances were done. No notable differences between fresh and blanched green peas were found. During boiling of pea, most of the elements were partly leached into water. Excepting nickel and cobalt, the compounds remaining in the boiled peas became less soluble. Soluble high molecular mass element species of zinc, copper, manganese and iron, present in the blanched green peas were markedly diminished by boiling. Low molecular mass species of nickel, cobalt, zinc, and molybdenum were more stable against thermal treatment. The mature pea accumulated higher amounts of elements than did green pea. New soluble high or medium molecular mass fractions of phosphorus, cobalt and copper were formed during ripening of pea seeds. These fractions were not present in green peas. The age of mature peas and the manner of their storage were observed to affect the speciation of copper.

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

1. Introduction

Original compounds of mineral elements occurring in foodstuffs are still hardly known. Speciation analysis of elements can give some quantitative and qualitative information about various chemical forms of elements present in a sample. Elements speciation data are essential for estimation of actual biological effects of elements. Description of trace element species in food and the development of appropriate analytical methodology were recently studied regarding the following topics:

- occurrence of individual mercury species (Harrington & Catterick, 1997; Chiou, Jiang, & Danadurai, 2001; Rai, Maher, & Kirkowa, 2002; Snell, Stewart, Sturgeon, & Frech, 2000; Ubillús, Alegría, Barberá, Farré, & Lagarda, 2000) and arsenic species (Ackley, B'Hymer, Sutton, & Caruso, 1999; Beauchemin, Bednas, Berman, McLaren, Siu, & Sturgeon, 1988; Goessler, Maher, Irgolic, Kuehnelt, Schlagenhaufen, & Kaise, 1997; Larsen, Pritzl, & Hansen, 1993; Larsen, 1995; Lawrence, Michalik, Tam, & Conacher, 1986; Veléz, Ybáñez, & Montoro, 1996; Villa-Lojo, Alonso-Rodríguez, López-Mahía, Muniategui-Lorenzo, & Prada-Rodríguez, 2002) in fish and sea-food;
- occurrence of selenium compounds in food supplements, based on selenised yeasts (Ip et al., 2000; Mounicou, McSheeny, Szpunar, Potin-Gautier, & Łobiński, 2002; Wrobel, Wrobel, & Caruso, 2002) or in some plants distinguished by higher selenium contents, such as garlic, onion and nuts (Ip et al., 2000; Kannamkumarath, Wrobel, Wrobel, Vonderheide, & Caruso, 2002; McSheeny et al., 2000);
- studies of structure and determinations of metallothioneines in animal tissues (Ferrarello, Ruiz Encinar, Centineo, García Alonso, Fernández de la Campa,

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& Sanz-Medel, 2002; Łobiński, Chassaigne, & Szpunar, 1998; Nischwitz, Michalke, & Kettrup, 2003; Prange & Schaumlöffel, 2002).

Owing to bioaccumulation or supplementation, the concentrations of elements of interest in the respective samples may be relatively high. Moreover, the low molecular mass species of mercury, arsenic and selenium as well as metallothioneins are mostly quite stable. In spite of these favourable circumstances, the speciation analysis of these samples is difficult and necessitates an application of sophisticated methods, such as combination of several separation steps (e.g., size exclusion, ion-exchange and reversed phase chromatography or capillary electrophoresis) hyphenated to both element-specific detection (inductively coupled plasma mass spectrometry, ICP-MS) and molecular identification (electrospray-ionization mass spectrometry, ESI-MS) techniques (Szpunar, 2000).

On the other hand, only a few papers have been devoted to element speciation in the nutritionally most important food, such as flours (Schöppenthau, Nölte, & Dunemann, 1996), vegetables (Thiele & Schwedt, 1985), and milk (Leiterer, Truckenbrodt, & Franke, 2001). Element concentrations in these commodities are mostly low; therefore, speciation analysis is extremely difficult.

In recent works, we applied methodology based on online hyphenation of size exclusion chromatography and inductively coupled plasma mass spectrometry (SEC/ICP-MS) for mapping of occurrence of trace elements and phosphorus in individual chromatographic fractions of several kinds of legumes (Koplík, Borková, Mestek, Komínková, & Suchánek, 2002a, 2002b; Mestek, Komínková, Koplík, Borková, & Suchánek, 2002). Mature legume seeds represent a valuable inexpensive source of proteins for human nutrition. Moreover, they are good sources of some minerals, vitamins and dietary fiber (Deshpande & Deshpande, 1991). With respect to the complexity of chemical composition of legumes, including various metal chelating compounds, the speciation of elements in legumes is a challenging analytical task. We also found significant alterations in trace elements and phosphorus speciation during cooking of pea (Koplík, Mestek, Komínková, Borková, & Suchánek, 2004). In the present study, we have focussed attention on element species alterations related to maturity of peas (green vs. fully mature peas) and processing of green peas, these being commercially widely available as blanched and frozen or canned vegetables (Jackson & Shinn, 1979) and being preferred by consumers more than dry legumes.

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 by a pH 03 instrument (Labio, Prague, Czech Republic). The HPLC apparatus for SEC/ICP-MS analysis consisted of a high pressure pump, Varian Inert 9012 (Varian, Walnut Creek, CA, USA); a glass column Superformance 150×10 mm (Merck, Darmstadt, Germany) packed with chelating resin, Chelex 100 (Merck) in NH_4^+ form; the first Rheodyne 9125 injector equipped with a 200 μl PEEK sample loop, a Superdex 75 HR 10/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden) (dimensions 300×10 mm, optimum fractionation range 3–70 kDa); the second Rheodyne 9125 injector equipped with a 500 μl PEEK sample loop; peristaltic pump and ICP-MS. PEEK or PTFE capillaries (internal diameter 0.25 mm) connected to 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 also obtained from Merck. Phosphorus stock solution ($\rho = 1000$ mg/l) was prepared by dissolving of an appropriate amount of ammonium dihydrogenphosphate (purity 99.999%) (Aldrich Chemical Co., Milwaukee, WS, USA) in deionised water. A set of peptide and protein standards, obtained from Sigma (St. Louis, MO, USA), was used for calibration of molecular mass determination by SEC. Distilled deionised water (Milli-Q, Millipore, Bedford, MA, USA) was used for preparation of all solutions.

2.3. The sample

Green pea seeds (*Pisum sativum* L., cultivar GOTIK) were obtained from green pods gathered at the experimental farm of the Central Institute for Supervising and Testing in Agriculture, located in Sedlec (village 20 km north of Prague) on 27th June 2002. Proximate composition of original green pea seeds was 75.0% of moisture, 0.9% of ash, 5.1% of protein and 0.1% of fat. These analyses were accomplished according to standard methods (Kirk & Sawyer, 1991). The seeds were divided into two portions. The first one was immediately frozen (sample GP) whereas the second one was blanched and frozen (sample BP). Blanching was accom-

plished by a 1 min treatment at 90 °C with distilled deionised water, followed by straining. The BP sample was further boiled to simulate the kitchen preparation: 6 g of BP were boiled in 20 ml of deionised water for 20 min. In further text the resulting sample is abbreviated as BBP. A sample of the mature pea seeds (MP) of the same cultivar was harvested at the same field on 2nd August 2002. Proximate composition of this sample was 17.8% of moisture, 3.0% of ash, 20.3% of protein and 1.3% of fat. The mature peas were milled in a vibration mill under liquid nitrogen to obtain a fineness <0.2 mm.

The total contents of phosphorus, manganese, iron, cobalt, nickel, copper, zinc, and molybdenum were determined in all types of sample. In addition, the water remaining after boiling of BP was also analysed. Other sets of original and processed peas samples were extracted with 0.02 M Tris–HCl, pH 7.5, buffer solution (see below) and contents of elements in the obtained extracts were also determined. The extracts were submitted to further analyses of element species by SEC/ICP-MS.

2.4. Sample preparation and analyses

All analyses were performed according to procedures previously described in detail (Koplík et al., 2002a, 2002b, 2004). Sample extracts were prepared by 1 h shaking of 2 g of powdered MP or 6 g of GP, BP or BBP (previously crushed in an agate mortar) with 50 ml of 0.02 M Tris–HCl buffer solution (pH 7.5) in a polypropylene flask. After centrifugation the extracts were analysed by SEC/ICP-MS. 200 μl of sample extract were injected into the SEC column by the first Rheodyne 9025 injector placed before the column. The flow rate of mobile phase of the same composition as extractant was 0.5 ml/min. The second injector, equipped with a 500 μm sample loop, was inserted between the SEC column and the ICP-MS. This injector served for the post-column injection of calibration standard solutions. The standards were injected during the dead time of the column. The flow of effluent was by T piece joined together with a flow of internal standard solution (50 μg/l, In) and the mixed flow (≈1.3 ml/min) was delivered by peristaltic pump to the cross-flow nebulizer of ICP-MS. Resulting element-selective chromatograms were mathematically treated and the amounts of elements in individual chromatographic fractions were calculated. Relative molecular masses of individual element species fractions, detected by ICP-MS, were estimated from the respective retention times on the basis of SEC column calibration.

Selective analysis was accomplished in the case of phosphorus compounds by capillary isotachophoresis. Contents of *ortho*-phosphate and phytate in selected SEC fractions were determined. Details of analytical methodology are given elsewhere (Koplík et al., 2002a, 2002b).

Total contents of elements in all pea and extract samples were determined by the ICP-MS technique with external calibration after decomposition of material by HNO_3 in the microwave decomposition unit (Koplík, Čurdová, & Suchánek, 1998).

3. Results and discussion

Elements contents in original green pea (GP), processed green pea (BP and BBP) and mature pea (MP), expressed on a dry weight basis, are shown in Table 1. Contents of P, Mn, Fe, Cu and Zn in our green peas are about one third to one half lower than average results published by Finnish authors (Varo, Lähelmä, Nuurtamo, Saari, & Koivistoinen, 1980). On the other hand, cobalt and molybdenum contents are approximately four and two times higher, respectively. These differences can be attributed to different soil and climate conditions and different cultivars. Our green pea sample is higher in phosphorus than any pea sample from a Spanish study (Periago et al., 1996). Generally speaking, the contents of mineral elements in green pea are within the normal range reported recently (Gundersen, Bechmann, Behrens, & Stürup, 2000).

Laboratory processing of green peas, simulating industrial vegetable processing (blanching) and final kitchen processing (boiling), leads to some losses of elements. Blanching caused negligible losses whereas boiling results in considerable decrease of element contents. The elements leach into water during boiling. Based on analyses of water remaining after the boiling of pea, the mass balances of all elements were calculated. As supposed, the sum of the element contents in BBP and in remaining water gives the content of GP. Losses of cobalt, nickel, copper, zinc and molybdenum contents of pea, caused by boiling, represent approximately 50% of the original amount. This is in accordance with our finding that these elements are present in legumes, mostly, as soluble compounds. On the other hand, the losses of elements whose solubility (of their compounds) is limited (i.e., iron and manganese, see Koplík et al., 2002a, 2002b) were less than 15%. Phosphorus losses in the course of green pea boiling are approximately 25%. Mature peas are abundant in most elements: during one month of final seed ripening, the contents of phosphorus and cobalt increased by approximately 60% and those of iron, nickel, copper, zinc and molybdenum by approximately 15% compared to levels in the green pea.

Extractabilities of elements from peas to aqueous buffer solution show that soluble cobalt, nickel, copper, zinc and molybdenum exceed 75% of total contents in both green and mature peas. Moreover, soluble nickel and molybdenum approach 100% of total contents. On the other hand, only one third of iron and manganese present in green pea is soluble, but extractabilities of

Table 1
Total contents of elements in original and processed pea sample

	P	Mn	Fe	Co	Ni	Cu	Zn	Mo
Green pea								
Total content (μg/g) (*a*)	4800 (120)	12.0 (0.3)	66.4 (1.8)	0.045 (0.004)	2.67 (0.08)	8.74 (0.20)	45.8 (1.0)	2.58 (0.07)
Extractable portion (μg/g) (*b*)	2980 (160)	3.6 (0.1)	21.9 (1.5)	0.041 (0.003)	2.83 (0.09)	7.46 (0.30)	34.8 (1.8)	2.65 (0.10)
Extractable portion (%, related to *a*)	62	30	33	91	106	85	76	103
Portion passing SEC column (μg/g)	3040 (160)	0.8 (0.05)	14.1 (1.0)	0.041 (0.004)	2.43 (0.08)	7.60 (0.40)	34.1 (2.0)	2.51 (0.15)
Portion passing column (%, related to *b*)	102	22	64	100	86	102	98	95
Blanched pea								
Total content (μg/g) (*c*)	4660 (130)	12.0 (0.3)	70.5 (2.5)	0.044 (0.004)	2.70 (0.10)	8.36 (0.25)	48.0 (1.0)	2.71 (0.08)
Total content (%, related to *a*)	97	100	106	97	101	96	105	105
Extractable portion (μg/g) (*d*)	2870 (160)	6.26 (0.2)	21.6 (1.5)	0.040 (0.004)	2.64 (0.12)	6.63 (0.30)	37.5 (1.2)	2.74 (0.10)
Extractable portion (%, related to *c*)	62	52	31	90	95	79	78	101
Portion passing SEC column (μg/g)	2980 (180)	0.55 (0.05)	8.3 (0.6)	0.041 (0.004)	2.80 (0.14)	7.08 (0.35)	36.8 (1.2)	2.73 (0.10)
Portion passing column (%, related to *d*)	104	9	38	103	106	106	98	100
Boiled blanched pea								
Total content (μg/g) (*e*)	3660 (100)	10.3 (0.3)	57.7 (2.3)	0.024 (0.003)	1.35 (0.06)	4.04 (0.20)	26.3 (0.8)	1.23 (0.05)
Total content (%, related to *a*)	76	86	87	53	51	46	57	48
Extractable portion (μg/g) (*f*)	1260 (80)	1.1 (0.1)	17.0 (0.5)	0.019 (0.003)	1.35 (0.07)	2.73 (0.15)	12.4 (0.5)	0.99 (0.04)
Extractable portion (%, related to *e*)	34	11	29	79	100	68	47	80
Portion passing SEC column (μg/g)	1270 (80)	<0.02	2.7 (0.1)	0.018 (0.003)	1.22 (0.07)	2.70 (0.10)	10.8 (0.5)	0.87 (0.04)
Portion passing column (%, related to *f*)	101	0	16	95	90	99	87	88
Mature pea								
Total content (μg/g) (*g*)	7850 (210)	12.2 (0.30)	76.1 (2.0)	0.074 (0.005)	3.11 (0.09)	10.6 (0.20)	52.8 (1.2)	3.01 (0.08)
Total content (%, related to *a*)	163	102	115	164	116	121	115	117
Extractable portion (μg/g) (*h*)	5100 (170)	9.1 (0.30)	44.6 (1.5)	0.062 (0.004)	2.73 (0.10)	8.41 (0.20)	43.3 (1.0)	3.01 (0.10)
Extractable portion (%, related to *g*)	65	75	59	84	88	79	82	100
Portion passing SEC column (μg/g)	4450 (180)	0.6 (0.1)	39.2 (1.5)	0.054 (0.004)	2.44 (0.10)	8.58 (0.25)	38.9 (1.0)	2.82 (0.15)
Portion passing column (%, related to *h*)	87	7	88	87	89	102	90	94

Contents of elements are on dry weight basis. Data are given as averages of eight results (peas) or two results (extracts). Values in parentheses represent expanded uncertainties ($k = 2$).

compounds of these elements are increased during seed ripening, thus reaching 59% and 75% in mature pea for iron and manganese compounds, respectively. Solubility of phosphorus compounds is intermediate and is slightly increased during ripening.

Chemical status of elements in green peas is partly altered by pea processing. Extractabilities of phosphorus, cobalt, nickel, copper, zinc and molybdenum compounds are not affected by blanching, representing only short thermal treatment intended for inactivation of enzymes. On the other hand, the boiling of green peas substantially decreases extractabilities of most elements. Only nickel extractability remains at 100%. Decrease of extractable amounts of elements is caused by two factors. The first is denaturation of the original metallobiomolecules, making some element species insoluble. This is typical for elements bound mostly to proteins. The second is decrease of total element content of seeds during boiling as a result of leaching of soluble element compounds into water.

Element species extracted from raw peas (GP and MP) and processed green peas (BP and BBP) were submitted to species fractionation and quantification by SEC/ICP-MS. Element specific chromatograms of these samples are shown in Figs. 1 and 2. The sum of the element quantities in individual fractions passing the SEC column was ascertained for each sample and compared to the element's content in each sample extract. Recoveries of individual elements during SEC were calculated as well. In GP and BP samples, the recoveries approach 100% for most elements except iron and manganese. Soluble compounds of phosphorus,

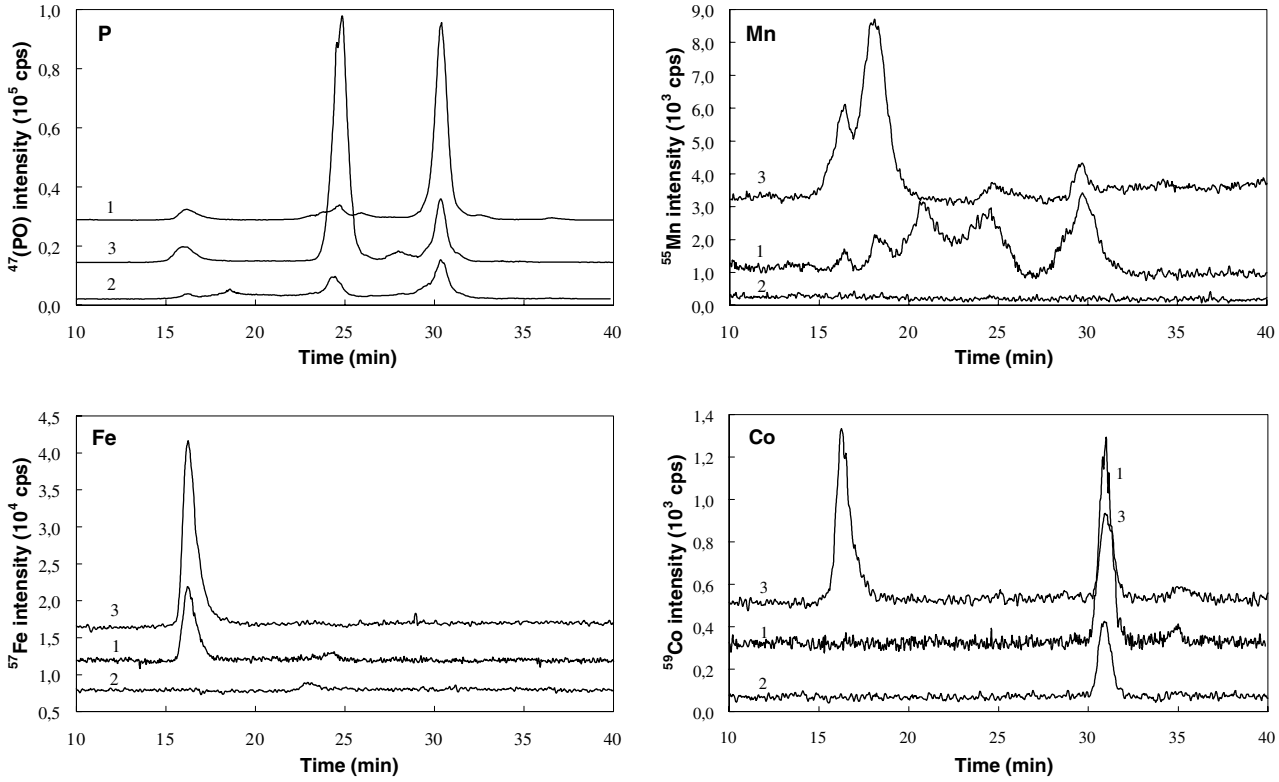

Fig. 1. Examples of chromatograms of P, Mn, Fe and Co compounds in extracts of green and mature peas: 1 – GP; 2 – BBP; 3 – MP.

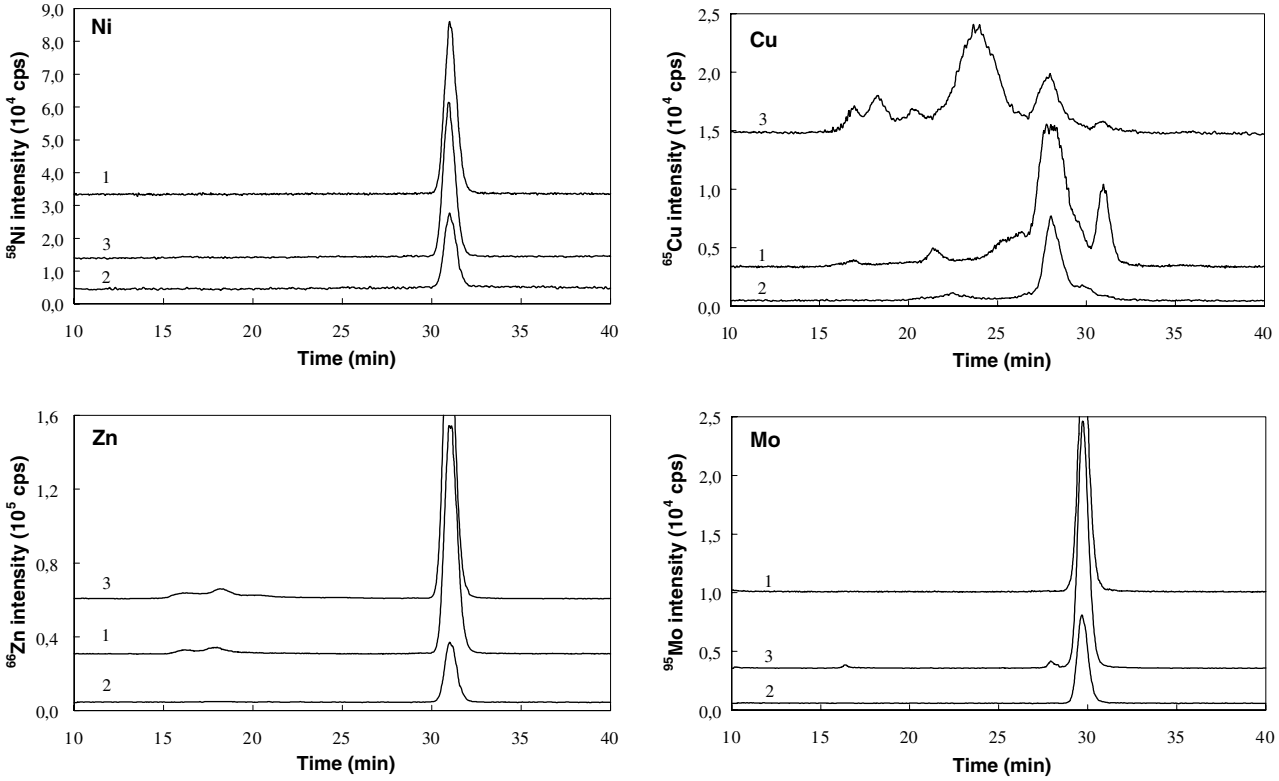

Fig. 2. Examples of chromatograms of Ni, Cu, Zn and Mo compounds in extracts of green and mature peas: 1 – GP; 2 – BBP; 3 – MP.

cobalt, nickel, copper, zinc and molybdenum extracted from BBP are mostly SEC-recoverable. Recoveries approach 90%. Only recovery of copper remains approximately at 100% for all samples. High recoveries of phosphorus and most metals (approximately 90%) were also ascertained in the case of MP extract. Low recoveries of iron and manganese were dramatically reduced during green pea processing. Recovery of manganese compounds was also decreased as a result of pea ripening. Incomplete SEC recovery of element shows that some portion of the soluble element compounds represented by ionic species or labile complexes is decomposed during chromatography (see Koplík et al., 2002a, 2002b).

Table 2 summarises the results of SEC/ICP-MS analyses of peas extracts. Retention time, apparent relative molecular mass and element quantity are given for each individual element species fraction. The respective element specific chromatograms are shown in Figs. 1 and 2.

The phosphorus elution profile of GP extract contains a dominant peak in the low molecular mass region (<0.5 kDa) and minor peaks in medium (3–4 kDa) and high (>100 kDa) molecular mass regions, respectively. Distributions of phosphorus species fractions in MP extract are different: the main fraction, representing approximately two thirds of the phosphorus, was found in the medium molecular mass region; one quarter was found in the low molecular mass region and minor fraction was found in the high molecular mass region. Processing of green pea leads to some changes. The high molecular mass phosphorus fraction is diminished in

Table 2
Summary of analyses of element species in peas by SEC/ICP-MS method

Element	t_R (min)	Apparent M_r (kDa)	Element contents in individual species (μg/g)			
			GP	BP	BBP	MP
P	16.3	>100	320	70	50	340
	18.6	75	–	180	140	–
	24.8	4.4	270	400	370	2910
	26.4	2.7	50	310	–	–
	28.0	1.0	–	–	–	120
	30.4	<0.5	2400	2020	710	1080
Mn	16.4	>100	0.03	0.02	–	0.1
	18.1	96	0.07	–	–	0.4
	20.8	28	0.2	0.03	–	–
	24.6	4.9	0.2	0.2	–	0.04
	29.7	0.5	0.3	0.3	–	0.06
Fe	16.3	>100	11.5	7.0	–	39.2
	22.5	13	–	–	2.7	–
	24.4	5.3	2.6	1.3	–	–
Co	16.3	>100	–	–	–	0.037
	31.0	<0.5	0.038	0.037	0.018	0.014
	35.0	<0.5	0.003	0.004	–	0.003
Ni	31.0	<0.5	2.43	2.80	1.22	2.44
Cu	17.0	>100	0.1	–	–	0.54
	18.3	87	–	–	–	0.91
	20.2	37	–	–	–	0.48
	21.5	20	0.34	–	–	–
	22.5	13	–	<0.05	0.15	–
	23.8	7.0	–	–	–	4.86
	28.0	1.0	5.95	5.89	2.55	1.58
	31.0	<0.5	1.21	1.19	–	0.21
Zn	16.3	>100	2.0	–	–	1.4
	18.3	87	3.1	–	–	2.6
	20.2	37	–	–	–	0.6
	24.7	4.6	–	–	–	0.3
	31.0	<0.5	29.0	36.8	10.8	34.0
Mo	16.3	>100	–	–	–	0.01
	27.9	1.1	–	–	–	0.02
	29.7	0.5	2.51	2.73	0.87	2.79

Contents of elements are on dry weight basis.

BBP, probably as a result of denaturation of phosphorylated proteins.

Probably, phytate is the main phosphorus species in legume seeds (Reddy, Sathe, Pierson, & Salunkhe, 1989) and proportions of phytate and *ortho*-phosphate are changing during seed ripening. Therefore, the chromatographic fractions containing phosphorus were submitted to isotachophoretic analyses of these compounds. Three fractions (high, medium and low molecular mass) were collected from repeated injections of GP and MP extracts. Contents of both species in individual fractions were recalculated to obtain contents of phosphate and phytate phosphorus and these values were compared to total phosphorus contents. These results are given in Table 3. Phytate prevails over phosphate in medium molecular mass fractions (3–4 kDa) of both samples. During ripening of peas, more phosphorus appears in the medium molecular mass fraction, which becomes the dominant phosphorus fraction in mature seeds. At the same time, the ratio phytate/phosphate is further increased. In the low molecular mass fraction of GP, which represents the majority of soluble phosphorus compounds, phosphate slightly prevails over phytate. In the minor part, high molecular mass fractions of GP and MP phosphate also prevail over phytate. Although the molecular mass of phytic acid alone is only 0.66 kDa, its detection in 3–4 kDa and >100 kDa regions is not erroneous because, in neutral or slightly alkaline solutions, the anions of phytic acid can be conjugated to positively charged functional groups of peptide and protein molecules. The mass balance of phosphorus showed that the phytic acid accounts for 73% of total recoverable phosphorus in mature pea extract and 35% in green pea extract. This increase of soluble phytate phosphorus content is in accordance with reported changes of phytic acid content during legume seed ripening. Honke, Kozlowska, Vidal-Valverde, Frias, and Górecki (1998) found that phytic acid content in peas is approximately four times increased in the course of final maturation, taking 14–50 days, depending on actual weather conditions. The sum of determined contents of phosphate and phytate phosphorus shown in Table 3 does not give the total phosphorus content obtained by SEC/ICP-MS because the samples could contain some other phosphorus species, such as less phosphorylated inositols or sugar phosphates.

Compounds of manganese present in pea are mostly very unstable. Only a limited part of manganese can be extracted from peas. Boiling of GP further decreases the extractability. Only traces of manganese compounds are recoverable by SEC, representing 22% and 7% of manganese extractable from GP and MP, respectively. No manganese appeared during SEC/ICP-MS analysis of BBP extract. Moreover the shapes of manganese elution profiles are hardly reproducible. One can deduce, from these and former results (Koplík et al., 2002a, 2002b; Schöppenthau et al., 1996), that most manganese compounds present in legumes are decomposed during sample preparation and analysis.

Iron species were predominantly found in the high molecular mass region (>100 kDa) in both GP and MP. Traces of iron extractable from GP occur also in the medium molecular mass region (approximately 5 kDa). However, about one third of soluble iron from GP is not recovered by SEC. Ripening of pea seeds leads to increase of extractability of iron compounds and to conversion of originally labile species to more stable compounds, probably proteins, such as ferritin, which was found to bind both iron and phosphorus in pea seeds (Wade et al., 1993). Boiling of blanched green pea diminishes original high molecular iron species and traces of iron are transferred to form a new medium molecular mass (13 kDa) species. Most of the iron compounds are made insoluble by boiling. Moreover, the majority of iron, which remains extractable from BBP, is not recovered by SEC/ICP-MS. Thermal treatment leads to decomposition of iron compounds of limited stability, probably to form ferric or ferrous ions. We formerly found a similar effect of thermal treatment on iron compounds as a result of cooking of fully mature peas (Koplík et al., 2004).

Both cobalt and nickel are highly or even completely extractable from peas and at the same time they are highly recoverable elements by SEC/ICP-MS. The cobalt specific chromatographic profile of GP extract consists of two peaks, both in the low molecular mass

Table 3
Distribution of *ortho*-phosphate and phytate phosphorus in SEC fractions of phosphorus compounds in extracts of green (GP) and mature (MP) peas

Fraction	GP			MP		
	P content (μg/g)	Percentage of P present as		P content (μg/g)	Percentage of P present as	
		Phosphate	Phytate		Phosphate	Phytate
High molecular mass (>100 kDa)	320	41	29	340	32	25
Medium molecular mass (3–4 kDa)	320	22	70	2910	3	92
Low molecular mass (⩽1 kDa)	2400	46	31	1200	26	39
Sum of fractions	3040	43	35	4450	11	73

Contents of phosphorus are on dry weight basis.

region (<0.5 kDa). This pattern is conserved in BP extract too. Major low molecular mass species partially persist, even in BBP. The decrease of the cobalt peak is caused by reduction of total cobalt content during boiling and some reduction of cobalt extractability. During pea ripening, high molecular mass cobalt species are formed and become dominant, as is obvious from analysis of the MP extract. Nickel is eluted in a single peak falling into the low molecular mass region (<0.5 kDa) in case of all pea extracts. Neither processing nor ripening seem to affect the identity of detected nickel species in original pea.

Copper is a well extractable element, even in the case of BBP, and it is characterised by complete recovery in the course of SEC/ICP-MS analysis. In the copper elution profile of GP extracts, two peaks in the low molecular mass region (approximately 1 kDa and <0.5 kDa, respectively) account for 94% of total recoverable copper. Minor peaks were also found in the medium (20 kDa) and high molecular mass regions (>100 kDa). Both mentioned low molecular mass species persist after blanching of green pea, but medium and high molecular species are diminished by GP processing. Copper extractable from BBP is mostly bound in the 1 kDa fraction; some copper is transferred to a new fraction of relative molecular mass 13 kDa. Ripening of pea seeds is accompanied by a slight increase of total copper content. Several new copper species, ranging from medium to high molecular mass region, appear in the MP extract. The medium molecular mass (7 kDa) fraction of copper becomes dominant and the amount of copper bound to both low molecular mass fractions (1 and <0.5 kDa) decreases. Moreover, some copper is bound to 37, 87 and >100 kDa fractions.

In spite of other elements, the chromatographic profile of copper in mature pea is markedly changed during sample storage (see Fig. 3). After as short time as one month of seed storage, some shifts of peak position and magnitude are observable. Longer pea storage (eight months) induces some decrease of molecular masses of main copper fractions and relocation of more copper to a low molecular mass (<0.5 kDa) fraction. If the MP sample is stored as powder, the chromatogram of copper compounds is dramatically changed, even during one month. Almost all copper is relocated to the low molecular mass (<0.5 kDa) fraction. It seems that this fraction has a large binding capacity for metal ions liberated from original cupric proteins after chemical changes induced by breaking up the seed structure. It is important to note that this low molecular mass fraction binds (in pea seeds) most of the zinc, all nickel and a great deal of cobalt as well.

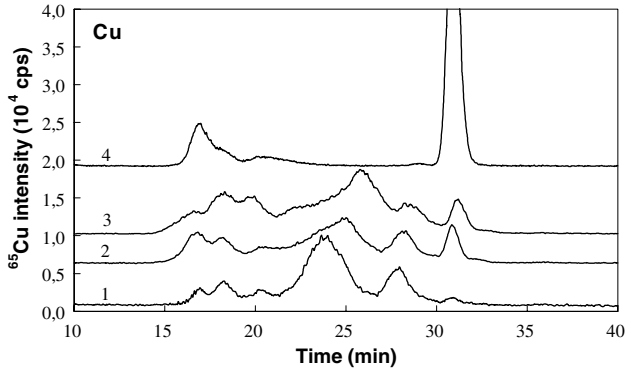

Fig. 3. Effect of age on Cu speciation in mature pea sample: 1 – fresh; 2 – one month after the harvest; 3 – eight month after the harvest; 4 – stored in form of powder.

Soluble zinc compounds present in GP and MP have practically the same chromatographic profiles: more than 80% of recoverable zinc is bound to low molecular mass (<0.5 kDa) fraction. Traces of zinc are detected in the high molecular mass region too. Processing of green pea diminishes all high molecular mass zinc species. In spite of complete SEC/ICP-MS recovery of zinc from GP, approximately 10% of extractable zinc from BBP and MP is not recovered. This reflects the occurrence of some zinc as labile complexes or uncomplexed metal ions.

Molybdenum compounds are highly or even completely extractable from all pea samples. Practically all molybdenum from all peas samples is eluted in a single peak in the low molecular mass region (0.5 kDa), which can be resolved from adjacent main peaks of zinc, nickel and cobalt compounds.

4. Conclusion

The chemical state of mineral elements in pea seeds is changed as a result of technological processing and ripening. It can be characterised as follows:

(1) Soluble species of most elements present in green peas are more or less stable at pH 7.5 and are not decomposed during size exclusion chromatographic separation. Iron and manganese species are exceptions: SEC does not recover one third of the iron compounds and most of the manganese compounds. All of the cobalt, nickel and molybdenum and a great deal of the phosphorus and zinc extractable from green peas are bound in the low molecular mass fraction. Iron forms high molecular mass species. Some high molecular mass species were detected for phosphorus and zinc as well.

(2) The blanching of green pea practically does not change either total amount or chemical speciation of elements but boiling does. High molecular mass species of all elements are diminished. Considerable portion of iron and manganese compounds are made insoluble or transformed to labile complexes or ionic species. Extractabilities of all other elements, except nickel, are also decreased. However, the low molecular mass species of

cobalt, nickel, zinc and molybdenum mostly persist and can be considered as stable against thermal treatment.

(3) During final ripening of pea seeds, the contents of elements, except manganese, are increased. A considerable amount of phosphorus is accumulated. This is accompanied by increased phytic acid content in mature pea extract, which is preferably bound to the medium molecular mass fraction. Moreover, some soluble high molecular mass species of cobalt, and manganese, and medium molecular mass species of copper are formed. Amounts of iron and copper bound to the high molecular mass fraction increases.

(4) Speciation of copper is changed during mature pea storage. If the pea is stored as meal or powder, copper is readily relocated from original species to more stable low molecular mass species.

Whatever detailed description of element species fraction changes, the nutritional consequences of these results cannot be easily estimated. Further information about chemical identity and stability of element species in food is needed.

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