Trace Elements Species Fractionation in Rye Flour and Rye (*Secale cereale* L.) Seedlings

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Abstract

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The fractionation of Cd, Cu, Mo, Ni, and Zn species in extracts of rye (cv. Fernando) seedlings (grown up in both standard and Cd^{2+}-enriched medium) and rye flour was performed by SEC/ICP–MS method. The majority of Cu, Zn, and Ni in all samples were bound in the 1–2 kDa fraction. Molybdenum occurred in all samples in the fraction of 3 kDa. During five days of cultivation in a solution of 30 μmol/l Cd^{2+}, the plants accumulated as much as 5 mg/kg fresh matter of Cd, but its soluble portion represented only 12–15%. The prevailing portion of Cd complexes was contained in the fraction of 3 kDa, while the minor part occurred in the fraction of 20 kDa. The speciation of elevated Cd in plants differs from that of other metals present at a physiological level. The metal-rich fractions of the extracts of all samples (i.e. those of 1–2 kDa) were refined by immobilised metal affinity chromatography. The isolated ligands of trace elements were peptides rich in dicarboxylic aminoacids.

Keywords: rye; metal; speciation; trace elements; MALDI-MS; ICP-MS; IMAC

In Europe, especially in its northern part, rye represents a crop of a long tradition. It has been used for bread and other foodstuff production. Its consumption for human nutrition reaches millions of tons per year. Besides of carbohydrates, proteins and some vitamins, rye is also an important source of minerals and trace elements, e.g. consumption of 100 g of rye bread (one slice) can cover as much as 30% of the recommended daily intake of zinc (Lindhauer & Dreisoerner 2003). Whole rye grain contains approx. 4–6 mg/kg of Cu, 13–45 mg/kg of Zn, 0.5 mg/kg of Mo, and 0.2–2.7 mg/kg of Ni, respectively (Souci *et al*. 2000). However, the determination of the total content of trace elements in food commodities does not provide fully relevant information for nutritional or toxicological evaluation as the effects of both essential and toxic elements contained in food depend on their chemical forms (Fraústo da Silva & Williams 2001). The element speciation analysis represents

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the way how to access information on the structure and properties of metal-binding (or metalloid-binding) compounds (Ebdon *et al*. 2001).

Plant metallothioneins (MTs) and phytochelatins (PCs) are considered as important metal-binding compounds in the plant materials (Kotrba *et al*. 1999). Whereas metallothioneins are proteins or polypeptides of molecular weight of 5–20 kDa, phytochelatins represent mostly oligopeptides whose molecular weights range approx. between 0.5 and 2 kDa. These compounds are synthesised by plants or some microorganisms if they are exposed to increased concentrations of some toxic (Cd, Pb, As, Ag) or even essential (Zn, Cu) elements. Another stress factors can also induce the production of these compounds. It is supposed that the metal-binding compounds protect the plant against toxic effects of metals (Cobbett 2000), maintain the concentrations of elements in cytoplasm at acceptable levels, provide a pool for the storage of metal ions, and affect the activities of metalloenzymes (Thumann *et al*. 1991). Other compounds such as organic acids (citric, malic, oxalic etc.), inositolphosphates, phenolic compounds, heterocycles, and amino acids are also involved in the binding of metals in plants. However, PCs belong to frequently investigated plant ligands. A number of papers concerning the analysis of PC-metal complexes have been published recently (see e.g. Kneer & Zenk 1997; Leopold & Günther 1997; Vachina *et al*. 1999, 2000; Chassaigne *et al*. 2001; Montes-Bayón *et al*. 2004). Although these papers are invaluable for the understanding of the stressed plants metabolism and the mechanisms of metals detoxification, they do not provide sufficient information on the speciation of elements (especially essential elements) in food commodities. There is a lack of data concerning the chemical structure of biological ligands of metals in mature plants and namely in their parts utilised for the production of foodstuffs. Among the recently appeared papers dealing with cereals and/or cereal based foods, some articles can be mentioned focused on the selenium speciation in wheat flour (Diaz Huerta *et al*. 2003), effect of selenate supplementation on various kinds of cereals (Stadlober *et al*. 2001), changes in the chemical form of selenium during bread production (Bryszewska *et al*. 2005), and arsenic speciation in rice (Heitkemper *et al*. 2001) and infant food (Vela & Heitkemper 2004).

Our previous experiments proved that soluble low molecular binding compounds of Cu, Zn, and other metals in buckwheat, amaranth (Mestek *et al*. 2007a), and rape (Mestek *et al*. 2007b) are rich in dicarboxylic amino acids and do not contain PCs. In the present work an analogous approach to the element species fractionation based on size exclusion chromatography – inductively coupled plasma mass spectrometry was applied for the analysis of rye flour and tissues of rye plants cultivated hydroponically both in normal fertiliser solution and in cadmium-enriched one. The fractions of low molecular mass compounds were purified by immobilisd metal affinity chromatography (IMAC) and analysed by MALDI-MS. Moreover, amino acid compositions of these purified fractions were determined.

MATERIAL AND METHOD

Instruments. The ICP-MS measurements were done using the Elan 6000 spectrometer (Perkin-Elmer/Sciex, Norwalk, USA) equipped with Meinhard nebuliser, a cyclonic spray chamber, and Gilson 212 peristaltic pump. The sample decomposition was performed in the UniClever microwave decomposition unit (Plazmatronika-Service, Wroclaw, Poland). Acidity of the buffer solutions was measured by pH 03 instrument (Labio, Prague, Czech Republic). The HPLC apparatus used for the sample fractionation by on-line SEC/ICP-MS coupling consisted of a Varian Inert 9012 high pressure pump (Varian, Walnut Creek, USA), two Rheodyne 9010 injectors placed before and beyond the column. Both Superdex 75 HR 10/30 column (Amersham Pharmacia Biotech, Uppsala, Sweden, dimensions 300 × 10 mm, optimum fractionation range 3–70 kDa) and Fractogel EMD Bio SEC (dimensions 600 × 16 mm, optimum fractionation range 5–1000 kDa, Merck) columns were applied. Preparative scale size exclusion chromatography utilising the Fractogel column was used for the target sample fraction isolation. The apparatus consisted of a LCP 4020 high-pressure pump (Ecom, Prague, Czech Republic), an injector Rheodyne 9010 equipped with 2 ml PEEK sample loop, and Fractogel EMD Bio SEC column. The samples were freeze-dried using Alpha 1-2 LD instrument (Martin Christ, Osterode am Harz, Germany). MALDI-MS analyses were performed on Biflex IV (Bruker Daltonics, Bremen, Germany).

Reagents. Nitric acid used for the sample decomposition was of Suprapur® Grade (Merck, Darmstadt, Germany). Cd, Cu, Mo, Ni, Zn, and Rh stock solutions of 1000 mg/l (all Merck, Darmstadt, Germany) were used for the preparation of the calibration solutions and internal standard solutions. The mobile phase for the chromatographic separation and the extractant was prepared from tris(hydroxymethyl)aminomethane (Tris) (Fluka, Neu-Ulm, Germany) buffered by hydrochloric acid (Suprapur® Grade, Merck). Another materials and reagents used during the isolation of the metal binding peptides involved Chelex 100 chelating ion exchange resin (Merck), Sephadex G15 gel (Pharmacia), acetonitrile, 2,5-dihydroxybenzoic acid, trifluoracetic acid (all Fluka), and dithiothreitol (DTT) (Merck). De-ionised water (Millipore, Bedford, USA) was used for the preparation of all solutions used during the analyses. The fertiliser solution contained 8.0 g $Ca(NO_3)_2$, 2.0 g KH_2PO_4, 2.0 g KNO_3, 2.0 g $MgSO_4 \cdot 7\ H_2O$, 1.0 g KCl, and 0.1 g $FeSO_4 \cdot 7\ H_2O$ per liter (all reagents were of analytical grade and were obtained from Penta, Chrudim, Czech Republic). $CdSO_4 \cdot 4\ H_2O$ used during rye cultivation was also obtained from Penta.

Samples and sample extract preparation. Wholemeal rye flour was obtained from a retail store. Its proximate composition was 11.1% of moisture, 1.2% of ash, 7% of protein, and 1.4% of fat. The analyses having been accomplished according to the standard methods (Kirk & Sawyer 1991). Rye seeds (cv. Fernando) were obtained from Selekta a.s. (Prague, Czech Republic). Two batches of seeds were sown on filtration paper sheets and the seedlings were cultivated for seven days in the solution of the mineral fertiliser described above. Than the solution in one batch was enriched by 30 μmol/l $CdSO_4$; the samples originated from this batch will be called "Cd enriched". The second batch served as the control sample. After further five days of cultivation, the leaves were separated from the roots and both parts of plants were carefully washed with distilled water. The samples were stored at –18°C until the analyses.

10 g of leaves, 7 g of roots (previously crushed in an agate mortar), or 2 g of flour were extracted with 50 ml of 0.02 mol/l Tris-HCl buffer solution (pH 7.5) by 1-h shaking in a polypropylene flask. Then the mixtures were centrifuged (20 000 g, 4°C, 20 min). The buffer solution was previously purified by passing through a column packed with Chelex 100 resin in NH_4^+ form.

Analytical methods

Determination of total content of elements. Solid samples (1 g of roots and leaves or 0.5 g of flour) or extracts (10 ml) were decomposed by pressurised microwave digestion in PTFE vessels with 3 ml HNO_3 for 10 minutes. The sample digests were transferred to 50 ml calibrated flasks and Rh solution (internal standard) was added to obtain final concentration of 50 μg/l. The determination of elements was done by ICP-MS technique with external calibration (details can be found e.g. in Koplík *et al*. 2002).

SEC/ICP-MS analyses. Buffer solution of 0.02 mol/l Tris-HCl (pH 7.5) served as the mobile phase, the flow rates were 0.5 ml/min and 2 ml/min for Superdex 75 column and Fractogel column, respectively. The sample extracts were injected onto the SEC column by the Rheodyne 9025 injector with 100 or 2000 μl PEEK sample loop, respectively. In the case of analytical scale chromatography, the quantification was carried out by post-column injection of the calibration solution using the second injector equipped with 500 μl sample loop. The flow of effluent was delivered to the nebuliser of ICP-MS, the duration of SEC/ICP-MS analysis was 50 min while the chromatograms consisted of 1000 steps of 3 s each (for details of the procedure see Mestek *et al*. 2002).

Isolation of metal binding compounds. The selected metal-bearing fractions of the sample extracts were isolated by preparative scale SEC (for conditions see above). The volume of each collected fraction was 6 ml; two independent separation runs were performed in order to combine both portions together. The chelating compounds were then refined by immobilised metal affinity chromatography (IMAC) technique using their adsorption on Chelex-100 in a Cu^{2+} form placed in a 1 ml PE column. The sample flow was 0.5 ml/min. The adsorbed ligands were eluted with 0.3 mol/l ammonia solution; the final volume of the eluate was 6 ml. Then 1 ml of the antioxidant solution (0.2% DTT) was added and after 20 min of incubation at 20°C, the mixture was freeze-dried. The details of the procedure were described in the previous article (Mestek *et al*. 2007a).

MALDI-MS analyses. The isolated compounds were dissolved in 0.1% trifluoracetic acid and desalted by ZipTip with fixed C_{18} reverse phase (Millipore). 2,5-dihydroxybenzoic acid was used

Table 1. Total contents (mg/kg of wet matter) of trace elements in analysed samples

Element	Flour*	Rye leaves**		Rye roots**		Rye seeds***
		Cd-enriched	control	Cd-enriched	control	
Cd	0.011 (0.001)	4.7 (0.3)	0.013 (0.003)	5.1 (0.7)	0.057 (0.020)	0.008
Cu	3.02 (0.20)	1.06 (0.06)	1.04 (0.06)	4.95 (0.97)	5.64(0.97)	2.79
Ni	0.058 (0.008)	0.30 (0.06)	0.72 (0.06)	3.58 (0.71)	2.36 (0.71)	0.16
Mo	0.59 (0.04)	0.50 (0.02)	0.47 (0.02)	0.33 (0.05)	0.36 (0.05)	0.45
Zn	24.1 (1.6)	10.5 (1.1)	10.8 (1.1)	49.3 (4.2)	52.3 (4.2)	22.1
Moisture	0.11	0.93	0.93	0.89	0.89	0.10

Mean of *six, **four or ***two determinations; values in brackets represent expanded uncertainty $k = 2$

as a matrix and the measurement was carried out in a positive mode.

Analyses of amino acids. In the case of the sample preparation for amino acids determination, the addition of antioxidant was omitted and the sample was desalted by gel filtration on Sephadex G15 column (dimension 250 × 10 mm) using water as the mobile phase (flow rate 1 ml/min). After performic acid treatment and hydrolysis with HCl, the mixture was analysed by ion exchange chromatography with ninhydrin post-column derivatisation and spectrophotometric detection.

RESULTS AND DISCUSSION

Trace elements extraction and fractionation

Table 1 shows total contents of Cd, Cu, Ni, Mo, and Zn in the analysed samples of rye flour, the individual parts of cultivated rye seedlings, and rye seeds serving for sowing. The contents of Cu, Ni, Mo, and Zn found in rye flour and seeds agree well with the published data (Souci *et al*. 2000). As to the contents of Cu, Zn, and Mo in the seedling parts (leaves and roots), the cadmium-enriched samples were of similar composition as the corresponding control samples. On the other hand, more Ni was retained in the roots of cadmium-enriched plants resulting in a drop of Ni content in the leaves of the Cd-enriched plants in comparison with the control sample. Trace elements present in the leaves and roots originated from the seeds and the fertiliser solution as well. The content of Cd in Cd-enriched plants exceeded more than 100 times that in the control samples. Cadmium distribution between the leaves and roots was uniform. It is obvious that during a quite short period of the exposition to cadmium, the rye plant accumulated a large amount of it. Table 2 summarises the amounts and proportions of the elements soluble in 0.02 mol/l Tris-HCl (pH 7.5) buffer solution. A majority of Cd accumulated in the plant material was transferred into the insoluble form, while only a minor part of Cd (12–15%) remained in soluble forms (both in the leaves and roots). The high level of Cd did not affect the solubility of other elements except

Table 2. Total contents of elements in extracts (buffer 0.02 mol/l Tris. pH 7.5)

Element	Flour	Rye leaves		Rye roots	
		Cd-enriched	control	Cd-enriched	control
Cd	0.005 (45%)	0.7 (15%)	0.003 (23%)	0.6 (12%)	0.003 (5%)
Cu	1.54 (51%)	0.53 (50%)	0.70 (68%)	1.73 (35%)	1.51 (27%)
Ni	0.027 (46%)	0.15 (54%)	0.66 (90%)	0.46 (13%)	0.52 (22%)
Mo	0.38 (65%)	0.25 (49%)	0.24 (50%)	0.12 (36%)	0.16 (44%)
Zn	8.4 (35%)	6.3 (60%)	6.4 (59%)	8.1 (16%)	9.6 (18%)

Results (mean of two) are given in mg/kg of wet matter. The relative value is related to the total content (see Table 1)

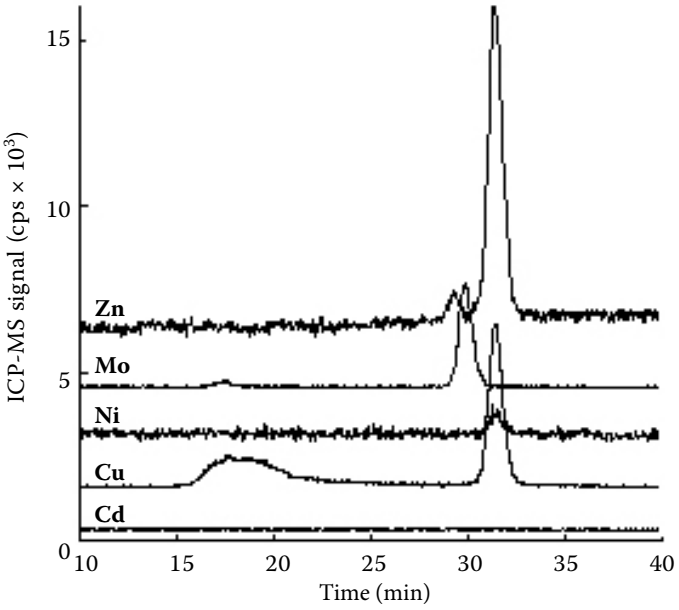

Figure 1. SEC profiles of elements in extract of rye flour

for Cu and Ni in the leaves: there their solubility decreased.

The extracts of the plant materials and rye flour were submitted to on-line SEC/ICP-MS analyses. The distribution of the elements among individual chromatographic fractions was not the same for all the types of samples analysed (Figures 1–3). Nevertheless, the chromatograms exhibit some regularity. In the extract of the control sample leaves, all Ni and Zn and a majority of Cu were concentrated in the low-molecular weight fraction (1–2 kDa, t_R = 32–33 min). Molybdenum was bound in the fraction of a somewhat higher apparent molecular weight (approx. 3 kDa, t_R = 30–31 min). A minor part of Cu was also bound in the medium-molecular weight region (20 kDa, t_R= 23 min).

The fractionation of these elements in the Cd-enriched leaves was similar to that in the control sample. Only a part of Zn accompanied Cd, which was bound in the compounds of apparent molecular weight of approx. 3 kDa (t_R = 31 min) representing the major Cd fraction. A minor part of Cd content occurred in the medium-molecular weight region of 20 kDa (t_R = 23 min).

As concerns the root extracts of both samples, all Mo was bound in the fractions of apparent molecular weight of approx. 3 kDa (t_R = 30 min) and the majority of Cu and Zn were bound in

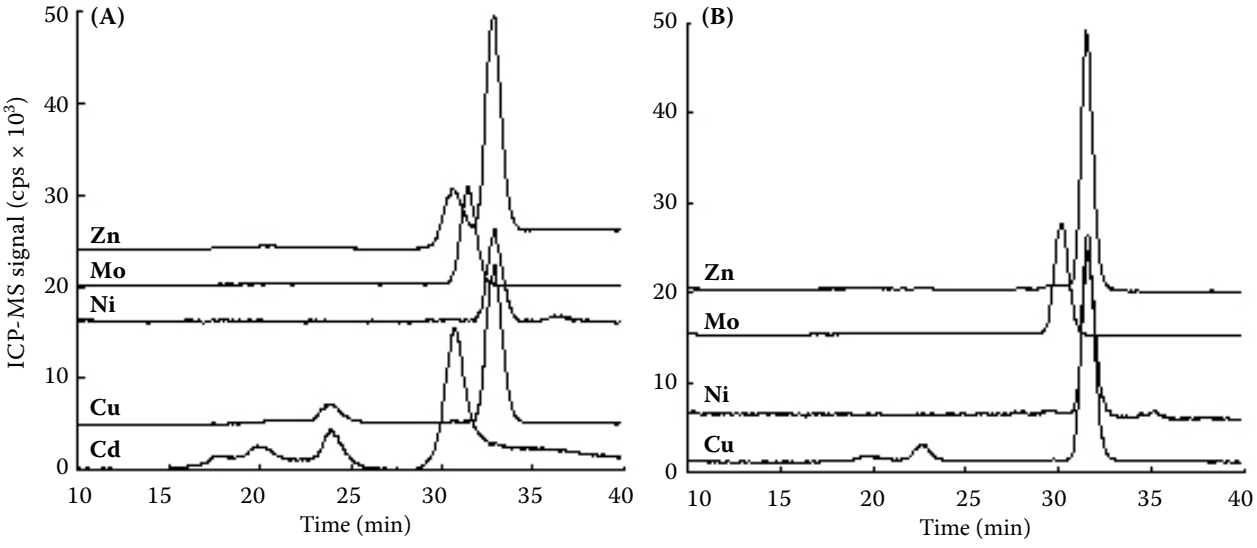

Figure 2. SEC profiles of elements in extract of rye leaves (A) Cd-enriched, (B) control

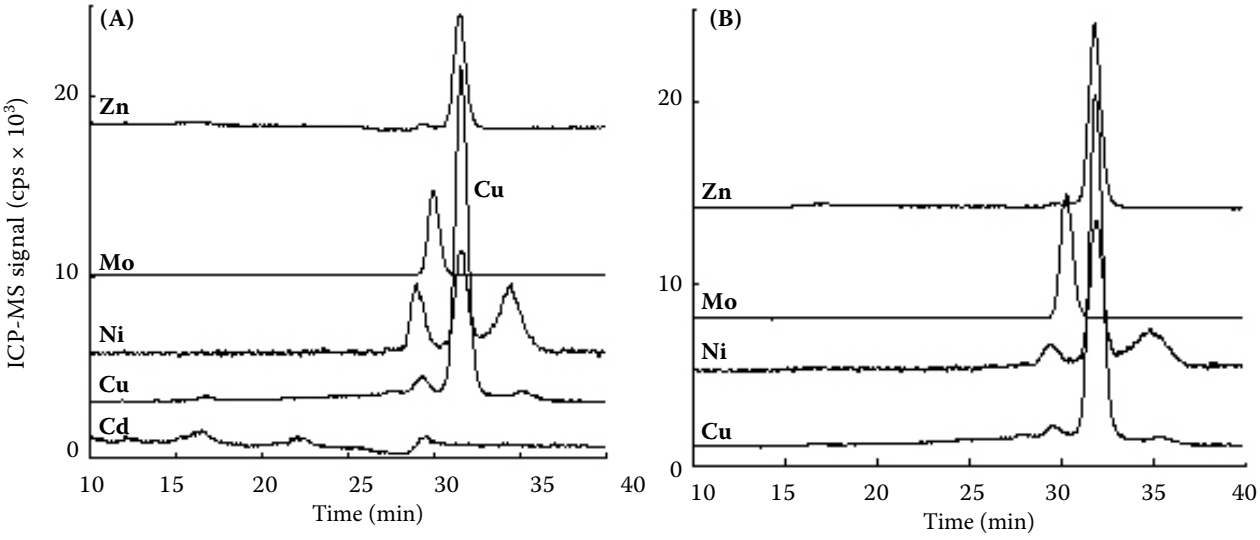

Figure 3. SEC profiles of elements in extract of rye roots (A) Cd-enriched, (B) control

the 1–2 kDa fraction (t_R = 32 min). Only minor amounts of Cu and Zn were bound in the fraction of apparent molecular weight of approx. 3–5 kDa (t_R = 29 min). The fractionation of Ni species in the extracts of roots differed from that in the leaves extracts. Besides a fraction of apparent molecular weight of approx 1–2 kDa (t_R = 32 min), fractions of higher (3–5 kDa, t_R = 29 min) and lower apparent molecular weighs (< 1 kDa, t_R = 34–35 min) were also detected. ICP-MS signal of Cd in the extract of Cd-enriched roots was weak; a bulk of soluble Cd was present in ionic species and/or labile complexes. However, two fractions of apparent molecular weights of 3 kDa (t_R = 29 min) and 20 kDa (t_R = 22 min) could be recognised.

In the flour extract, all Ni and a majority of Cu and Zn were bound in a low-molecular weight region of 1–2 kDa (t_R = 32 min), only a minor part of Zn species could be found in the fraction of apparent molecular weight of approx. 3–5 kDa (t_R = 29 min), and a minor part of Cu was contained in a wide zone corresponding to apparent molecular weight of 50–100 kDa (t_R = 15–20 min). The fractionation of Mo species did not differ from that in the seedlings extracts; all Mo was concentrated in the fraction of apparent molecular weight of 3 kDa (t_R = 30 min).

The total contents of elements passing the chromatographic column were ascertained as well (Table 3). The element recovery of the chromatographic analysis indicates the percentage of stable complexes of the particular elements, i.e. complexes which are not affected by extraction and by chromatographic analysis. The portion of an element eluted in a volume highly exceeding the total volume of the column corresponds to free

Table 3. The proportion of elements passing SEC column

Element	Flour	Rye leaves		Rye roots	
		Cd-enriched	control	Cd-enriched	control
Cd	n.d.	0.55 (76%)	n.d.	0.15 (25%)	n.d.
Cu	1.43 (93%)	0.49 (92%)	0.51 (73%)	0.85 (49%)	1.17 (78%)
Ni	0.023 (85%)	0.04 (29%)	0.05 (8%)	0.36 (79%)	0.24 (47%)
Mo	0.35 (91%)	0.21 (86%)	0.17 (73%)	0.11 (90%)	0.16 (100%)
Zn	3.8 (45%)	3.6 (58%)	1.4 (22%)	1.4 (17%)	0.55 (6%)

Results (mean of two) are given in mg/kg of wet matter. The relative value is related to the extractable content (Table 2); n.d. – not detected

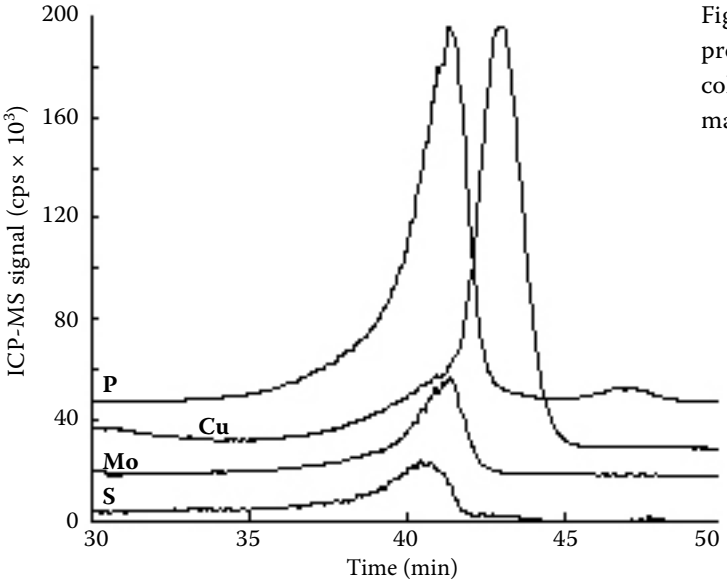

Figure 4. Detail of low-molecular region of SEC profiles of elements in extract of rye flour, Fractogel column. Chromatograms of Ni and Zn copy chromatogram of Cu

metal ions and/or labile complexes. These species are retarded on the column via non-size exclusion effects such as adsorption or ion-exchange (Koplík *et al.* 2002).

The portion of stable complexes of Cd in the extract of Cd-enriched leaves highly exceeded those in the extracts of Cd-enriched roots (76% vs. 25%, respectively). In the case the of leaves, the elevated level of Cd was accompanied also by an increased amount of stable complexes of Cu and Ni. This was probably caused by a higher production of ligands in the cells of Cd-exposed plants.

The proportions of stable complexes of Cu, Mo and Ni in the rye flour extract were very high (85–93%) and exceeded those in the extract of seedlings tissues. The proportion of Zn stable complexes reached only 45%, however, and it also exceeded those in the seedlings tissues.

The flour extract was submitted to further investigation by on-line preparative scale SEC/ICP-MS hyphenation using Fractogel column. During these experiments, sulphur and phosphorus were also monitored as the constituents of the possible ligands of trace elements (cysteine-rich peptides

Table 4 Relative contents of amino acids* (% mol/mol) in refined low-molecular weight fraction of sample extracts

Amino acid	Rye flour P-S subsfraction	Rye flour metal subfraction	Rye leaves		Rye roots	
			Cd-enriched	control	Cd-enriched	control
Cys	13	6			4	
CM Cys**					10	5
Asp + Asn	19	38	15	17	17	16
Thr		4		4	5	4
Ser	4		12	9	10	11
Glu + Gln	24	23	14	17	19	17
Gly	16	8	20	16	15	15
Ala	5	4	7	8	7	6
Val				4	4	4
Leu					5	
Lys			6	6	16	4
Pro				5	5	4

* the contents of other not reported amino acids were below 4%; **S-carboxymethylcysteine

and phytates). The column was found to be able to separatethe species of phosphorus and sulphur and those of copper, nickel, and zinc present in the low-molecular weight region, however, this separation was poor (Figure 4).

Characterisation of the binding partners of metals

The low molecular weight fractions of the extracts of all samples analysed were isolated using preparative scale SEC and, after purification by immobilised metal affinity chromatography (IMAC) on Chelex 100 resin in a Cu^{2+} form (Mestek *et al.* 2007a, b), were submitted to further analyses. Only the fractions rich in Cu, Ni, and Zn, but not the Cd-containing fractions, were analysed in the seedlings extracts. In the rye flour extract, two sub-fractions were collected and subsequently proceeded with: the sub-fraction rich in S and P and that rich in the metals (Figure 4). The chelating compounds isolated from each sample were analysed for amino acids composition and some of them also by MALDI-MS.

Table 4 summarises the amino acid compositions of the refined fractions of low-molecular chelating compounds isolated from the individual samples. The ligands isolated from the metal sub-fraction of flour were rich in Asx and Glx, while the content of Cys was low (possibly as a consequence of the poor chromatographic resolution from the S-P sub-fraction). Besides Asx, the S-P sub-fraction involved high portions of Cys, Glx and Gly. Its amino acid composition was close to that of PCs. The ligands isolated from both extracts of leaves (Cd-enriched and control) were very similar. The dominant components were Asx, Glx, and Gly again, however, other amino acids (Ser, Ala and Lys) were also present. The ligands isolated from the root extracts exhibited the most complex composition: in addition to the above mentioned amino acids, a less frequent amino acid carboxymethylcysteine was found in the roots. Carboxymethylcysteine content was increased in

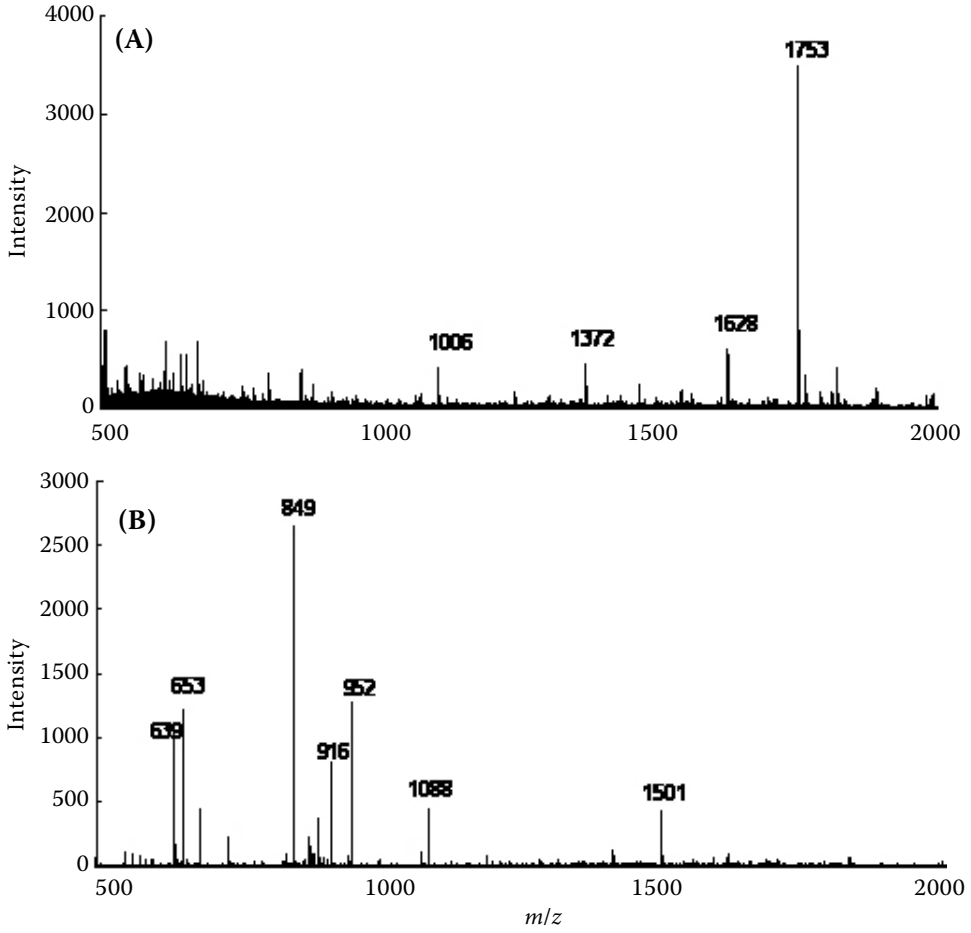

Figure 5. MALDI MS spectra ($M\text{-}H^+$) of low-molecular refined weight fraction of rye flour (A) and Cd-contaminated rye leaves (B)

the cadmium-enriched sample. However, Asx, Glx, and Gly were the main constituents. Dicarboxylic amino acids prevailed in the ligands isolated from all types of samples, however, it is obvious that composition of the ligands was modified during the transport from the roots to the seeds. This was also confirmed by MALDI-MS. Examples of the spectra of the ligands isolated from low-molecular weight fractions of rye flour (metal-rich sub-fraction) and Cd-enriched rye leaves are shown in Figure 5. The spectrum of the rye flour sample contained a dominant peak corresponding to molecular weight of 1752 Da and several minor peaks of compounds of molecular weights 1627, 1371, and 1005 Da. On the other hand, the spectrum of the ligands isolated from rye leaves contained compounds of lower molecular weights ranging between 638 and 1500 Da.

CONCLUSIONS

The element species fractionation in an extract of rye flour showed quite a similar pattern as did the analysis of the extracts of amaranth, buckwheat, and soybean flours, legume seeds, and rapeseeds (Koplík *et al*. 2002; Mestek *et al*. 2002, 2007a, b). Particularly, dominant low-molecular weight fractions of Cu and Zn (1–2 kDa) and Mo (3 kDa) were typical for most of these samples.

As the rye plant tissues (roots and leaves) are concerned, some similarity of the trace element species pattern with that of rye flour is obvious. The cultivation of rye plants in an artificially Cd-contaminated solution resulted in the accumulation of approx. 5 mg/kg (wet weight basis) of Cd in both roots and leaves as compared with < 0.1 mg/kg in the control samples. The majority of the accumulated Cd remained insoluble in the alkaline (pH 7.5) buffer. While most of the soluble Cd in Cd-enriched roots was represented by ionic species and/or labile Cd complexes, cadmium in Cd-enriched leaves was mostly bound in stable chelates of the apparent *M* of 3 kDa. In spite of a negligible effect of Cd accumulation on the chromatographic profiles of other trace elements, some quantitative differences could be recognised. The patterns of stable chelates of Zn and Ni in both leaves and roots extracts were substantially increased in the Cd-enriched samples as compared to control ones.

The ligands of trace elements isolated from metal-rich low-molecular weight (1–2 kDa) sub-fraction of rye flour by IMAC technique were peptides rich in Gly and dicarboxylic amino acids. The molecular weight of any of the components of this sample, as measured by MALDI-MS, did not correspond to phytochelatins (PCs). Therefore, PCs probably did not participate in metal binding in the soluble parts of mature seeds. However, the amino acid composition of the adjacent chromatographic sub-fraction resembled to that of PCs. As the metal-rich sub-fraction was free of phosphorus, the presence of phytic acid-metal compounds in low-molecular soluble fraction of rye flour is out of question.

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