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Heavy Metals Associated with the Major Constituents of Potato Tubers and Peanut Seeds

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Introduction

Informations hitherto published on heavy metals in vegetable foods have mainly been related to their gross measurements as a whole. Accordingly, there are little analytical data available, concerning the detailed distributions of heavy metals in the various parts as well as in the componential substances. Heavy metals are generally distributed unevenly in vegetable foods. Some portion of them is removed on the way of industrial or home preparation. Newly ascertained informations of the distribution would be of some help in estimating accurately the intake of heavy metals by human beings.

Uneven distribution of heavy metals in plant tissues might reasonably be explained through our consideration of the wide distribution of various compounds capable of forming complexes with heavy metals. Possible chemical forms of heavy metals in plants are to be inorganic ions, inorganic metal oxides, organic acid salts and organic complexes. Concerning the diet, it has often been suggested that the chemical form in which a certain element is present governs extensively its availability to animals. However, very little has been known of the forms in which heavy metals occur in plants. Accordingly, it would be important to elucidate the chemical forms of heavy metals in foods from both the physiological and toxicological points of view.

In this paper, a few attempts to fractionate heavy metals associated with the major constituents of potato tubers and peanut seeds are described.

Materials and Methods

Tubers of potato (*Solanum tuberosum* L.), sweet potato (*Ipomoea batatas* Lam.) and raw seeds of peanut (*Arachis hypogaea* L.) were commercially purchased. The potato tubers were well washed and peeled as usually done in home preparation. Peanut seeds were divided into seed coats, embryos and cotyledons with a knife.

The methods of fractionation of potato tubers and peanut cotyledons are summarised in Fig. 1 and Fig. 2, respectively. Each extraction procedure was repeated twice.

The isolation of spherosomes was carried out by the method of Jacks *et al.*[3]. Peanut cotyledons (50 g) were ground with mortar and pestle in 200 ml of 0.4 M sucrose. The mixture was centrifuged at $15{,}000 \times g$ for 20 min to produce a creamy band or fat pad on the surface of the supernatant liquid. The fat pad was removed with a spatula, and resuspended in 30 ml of 0.25 M sucrose. This suspension was centrifuged again at $15{,}000 \times g$ for 20 min. The washed spherosomes (fat pad) were washed again with a small volume of distilled water.

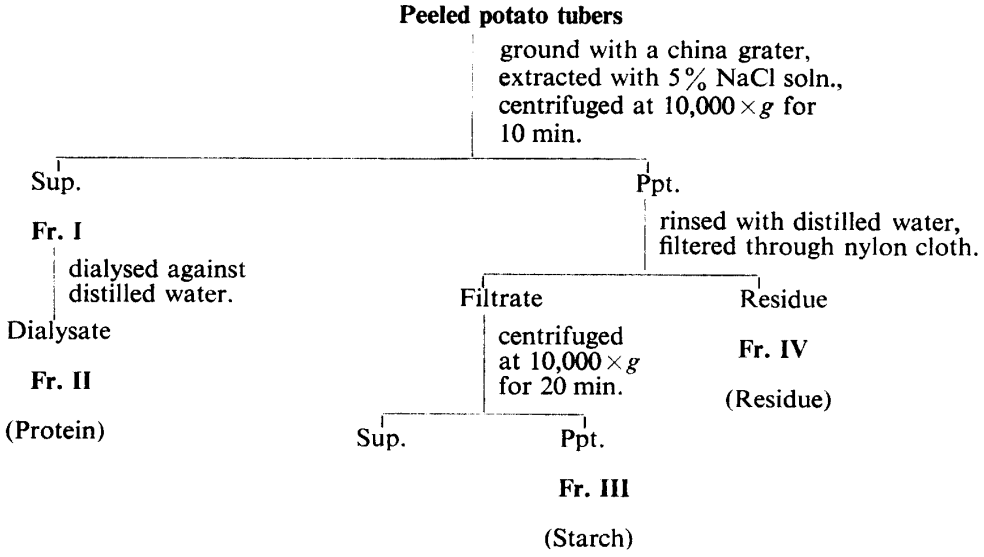

Fig. 1. Method of fractionation of potato tubers.

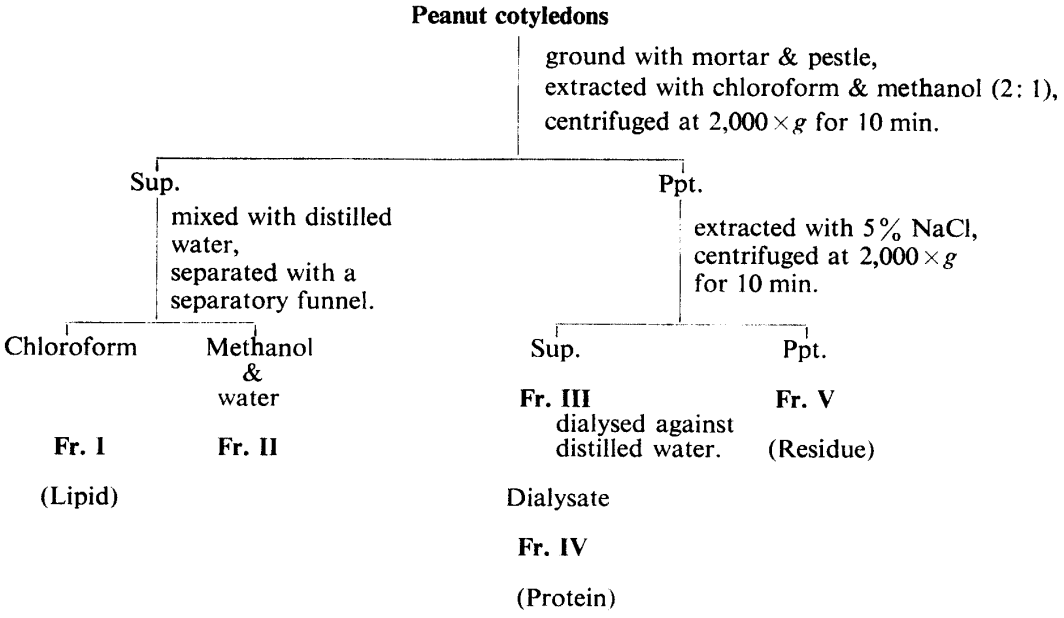

Fig. 2. Method of fractionation of peanut cotyledons.

The extraction of the interior lipid of the spherosomes was carried out as follows. The spherosomes were mixed with a mixture of chloroform (20 ml), methanol (10 ml) and water (6 ml), were shaken, and then stood for 1 h. After centrifugation at 2,000×*g* for 10 min, the lower chloroform layer containing the interior lipid was removed with a Pasteur pipette. An insoluble pellet in the methanol and water layer was separated by centrifugation at 15,000×*g* for 20 min, and then was washed with distilled water.

All the fractions were dried in a vacuum, weighed, and then were subjected to wet oxidation with nitric and perchloric acids.

Heavy metals (Zn, Mn, Cu, Fe) were determined by atomic absorption spectroscopy, using a HITACHI 170-10 TYPE atomic absorption spectrophotometer.

Results and Discussion

1) Heavy metals in potato tubers

Heavy metal contents of the peels and the peeled tubers were compared (Table 1). All the metals, particularly Fe, were highly concentrated in the peels of potato and sweet potato tubers. More than half the amount of Fe and considerable amounts of other heavy metals were removed by peeling. Bretzloff showed a calcium gradient from the outer cortex to the inner pith in his investigation of potato tubers[1]. A similar distribution would be expected in case of heavy metals.

Table 1. Heavy metal contents of potato peels and tubers

		Fresh weight g/tuber	Dry weight g/tuber	μg/tuber				ppm of dry weight			
				Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe
Potato	Peel	21	5.1	104	87	47	763	20.4	17.0	9.2	149.6
	Peeled tuber	121	21.8	408	355	140	618	18.7	16.3	6.4	28.3
Sweet potato	Peel	25	8.8	83	97	116	869	9.4	11.0	13.2	98.8
	Peeled tuber	126	45.6	214	114	137	529	4.7	2.5	3.0	11.6

Values are means of analyses of four tubers.

Stepwise fractionation by solubilization and extraction is one of the practical methods for separating heavy metals bound with various components. The selection of solvents and the extraction procedure must be carried out with considerable care, because artifacts may readily be formed by either the metals or the chelating agents. The solvent used must be free from heavy metals that may cause contamination. Hydrogen ion is an important factor affecting the dissociation of metal complexes. As H^+ concentration increases, metals begin to dissociate from the metal complexes.

Peeled potato tubers were fractionated as shown in Fig. 1. Sodium chloride solution was used as the extraction medium because it is obtainable in high purity and solubilizes globulin. Fr. I, the 5% NaCl extract, was supposed to include simple inorganic ions and various ligands (e.g., organic acids, amino acids, proteins) which were capable of binding heavy metals. Half the volume of Fr. I was dialysed against distilled water to separate high molecular complexes from low molecular complexes and free ions. The usual method of precipitating proteins with trichloroacetic acid (TCA) is not suitable for separating metal-protein complexes because TCA is quite acidic and releases metal ions from the proteins. In the dialysate (Fr. II), heavy metals bound to proteins were supposed to be dominant. The values of metal contents of Fr. II were subtracted from those of Fr. I, and the differences are expressed as the values for Fr. I–Fr. II, which represent the contents of heavy metals in the form of free ions and complexes with low molecular ligands such as amino acids and organic acids. Fr. IV was supposed to consist of unbroken cells, cell walls, membrane fragments and other insoluble materials.

Heavy metal contents of the fractions are shown in Table 2. In both potato and sweet potato tubers, the major proportions of Mn, Cu and Zn were localized in the soluble low molecular fraction (Fr. I–Fr. II). Proteinaceous fraction (Fr. II) contained the heavy metals in high con-

Table 2. Heavy metal contents of potato tuber fractions

		Dry weight g	μg/tuber				ppm of dry weight			
			Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe
Potato	Fr. I–Fr. II	—	310	311	149	228	—	—	—	—
	Fr. II	1.0	44	15	23	188	43.6	15.4	23.2	188.2
	Fr. III	12.0	13	2	0	46	1.1	0.2	0	3.9
	Fr. IV	4.4	58	16	7	144	13.1	16.1	1.6	32.7
Sweet Potato	Fr. I–Fr. II	—	125	75	96	121	—	—	—	—
	Fr. II	1.3	56	18	27	244	43.1	13.8	20.8	187.9
	Fr. III	21.5	4	4	0	52	0.2	0.2	0	2.4
	Fr. IV	13.7	85	11	4	112	6.2	0.8	0.3	8.2

Potato tubers were fractionated as shown in Fig. 2.
Values are means of analyses of four entire peeled tubers.

centrations. On the other hand, only a small proportion of the heavy metals was distributed in the starch fraction (Fr. III), although starch is the major component of potatoes.

Microdistribution of heavy metals in peanut seeds is shown in Table 3. Although most of the heavy metals were located in the cotyledons, Fe and Cu were highly concentrated in the seed coats. The embryos were found to contain Zn and Mn in the highest concentration.

Table 3. Microdistribution of heavy metals in peanut seeds

	Fresh weight mg/grain	Dry weight mg/grain	μg/grain				ppm of dry weight			
			Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe
Embryo	19.4	18.6	0.9	0.4	0.2	0.9	46.2	22.0	10.3	46.6
Cotyledon	764.4	745.1	14.0	12.9	5.8	12.2	18.8	17.3	7.8	16.4
Seed coat	21.5	18.9	0.3	0.2	1.1	4.2	14.7	8.8	58.2	224.3

The dissected cotyledons were fractionated as shown in Fig. 2. The chloroform fraction (Fr. I) and the methanol and water fraction (Fr. II) were supposed to contain the lipid component and the impurities, respectively. The Fr. I was freed of chloroform by evaporation at 45°C in a vacuum, was weighed, then subjected to assay of heavy metals. Half the volume of the 5% NaCl extract (Fr. III) was dialysed against distilled water. The dialysate was supposed to be a proteinaceous fraction (Fr. IV). The residue of 5% NaCl extraction (Fr. V) was supposed to consist of unbroken cells, cell walls, membrane fragments and insoluble components other than these. Each fraction was dried, weighed, then heavy metals were determined. The results were shown in Table 4. Only a small proportion of the heavy metals was distributed in the lipid fraction (Fr. I in Table 4), which is the major component of peanut cotyledons. Relatively low contents of the heavy metals of the lipid fraction indicate that lipid has only a weak affinity for heavy metals. The proteinaceous fraction (Fr. IV in Table 4) contained the heavy metals in the highest concentration. Copper showed a characteristic distribution; the major portion of Cu was localized in the proteinaceous fraction. Only a small proportion of heavy metals was distributed in the soluble components of low molecular weight (Fr. III–Fr. IV in Table 4), while potato tubers contained higher proportions of heavy metals in the low molecular fraction (Fr. I–Fr. II in Table 2). Such a

Table 4. Heavy metal contents of peanut cotyledon fractions

	Dry weight mg	μg/Cotyledon				ppm of dry weight			
		Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe
Fr. I	276	0.6	0	0.4	1.7	2.2	0	1.4	6.2
Fr. II	17	0.1	0	0	0.1	5.9	0	0	5.9
Fr. III–IV	—	0.7	1.2	0.3	1.1	—	—	—	—
Fr. IV	109	7.0	5.4	4.4	3.6	64.2	49.5	40.4	33.0
Fr. V	247	6.6	6.2	1.0	7.5	26.7	25.1	4.0	30.4

difference in distribution might be attributed to the differences in moisture and in protein contents between peanut cotyledons and potato tubers. About half of Zn, Mn and Fe were left unextracted in the insoluble residue (Fr. V). Heavy metal-phytin complexes are supposed to be contained in this fraction. Isolation and characterization of heavy metal-phytin complexes would be a further problem.

Heavy metals and ligands which are present separately *in situ* might have an opportunity to come into contact with one another to produce artifacts during the extraction procedure. One way of avoiding such artifacts, obtaining more information about heavy metal complexes, would be the separation of the subcellular organelles prior to extraction.

Peanut spherosomes were reported by Jacks *et al.*[3] to be particles about 1.0 to 2.0 μ in diameter, bounded by a limiting membrane and were composed of 98% total lipids, 0.77% phospho lipid and 1.27% protein by dry weight. Peanut spherosomes were isolated according to their method, and then divided into the limiting membrane and the interior lipid, by extraction with a mixture of chloroform, methanol and water. Heavy metal contents of each fraction are shown in Table 5. The limiting membrane fraction was found to contain the heavy metals in high concen-

Table 5. Microdistribution of heavy metals in peanut spherosomes

	Dry weight g	μg/g sphrosome				ppm of dry weight			
		Zn	Mn	Cu	Fe	Zn	Mn	Cu	Fe
Membrane	0.032	1.53	0.12	0.56	3.71	47.9	3.9	17.6	116.2
Lipid	0.968	1.35	0.19	0.29	3.14	1.4	0.2	0.3	4.5
Whole	1.000	2.88	0.31	0.85	6.85	2.9	0.3	0.8	6.9

trations. Presumably these heavy metals combine with proteins in the membrane because the membrane was reported to include most of the proteins associated with the spherosome[3]. The interior lipid was found to contain the heavy metals in relatively low concentrations. As a whole, the isolated spherosomes contained the heavy metals in relatively low concentrations, although the spherosomes constitute the major portion of the interiors of peanut cotyledon cells. The low contents of heavy metals in the spherosomal fraction agree with the result that the lipid fraction contained only a small amount of heavy metals as shown in Table 4. The agreement would rule out an artifact that might be caused by releasing the heavy metals originally bound to the lipid from the ligand during the extraction procedure.

Another attempt was made to isolate the interior lipid mechanically by means of ultrasonic disintegration of the spherosomes followed by repeated freezing and thawing. The isolated oily substance contained only a small amount of heavy metals (Table 6).

Table 6. Heavy metal contents in mechanically separated oil

ppm of dry weight			
Zn	Mn	Cu	Fe
1.3	0.1	0	2.8

Relatively low contents of heavy metals in both the starch fraction of potato tubers and the lipid fraction of peanut cotyledons indicate that starch as well as lipid has only a weak affinity for heavy metals.

Highly concentrated heavy metal occurrence in the proteinaceous fractions in both cases of potato tubers (Table 2) and peanut cotyledons (Table 4) indicates that proteins might be the major ligands that form complexes with heavy metals in plants. Dieckert and Rozacky isolated Mn containing protein from peanut seeds[2]. Isolation and characterization of metal-protein complexes from plant materials would be a further important problem.

Summary

Distribution and chemical forms of heavy metals (Zn, Mn, Cu, Fe) in potato tubers and peanut cotyledons were investigated by the method of stepwise fractionation with different solvents.

1) All the metals, particularly Fe, were highly concentrated in the peeles in both potato and sweet potato tubers. More than half the amount of Fe and considerable amounts of other heavy metals were removed by peeling.

2) In potato tubers, the major proportions of heavy metals were noted to be located in the soluble low molecular fraction. The proteinaceous fraction contained heavy metals in high concentrations. On the other hand, only small proportions of heavy metals were distributed in the starch fraction, the major component of potato tubers.

3) In peanut seeds, most proportions of hevay metals were located in the cotyledons. The embryos were found to contain Zn and Mn in the highest concentration, while Fe and Cu were highly concentrated in the seed coats.

4) In peanut cotyledons, only small proportions of heavy metals were distributed in the lipid fraction, the major component of peanut cotyledons. The soluble component of low molecular weight contained only small proportions of heavy metals. The proteinaceous fraction contained heavy metals in the highest concentration The major portion of Cu was localized in the proteinaceous fraction.

5) In the isolated spherosomes, the major interiors of the cell of peanut cotyledon, heavy metals were localized in the limiting membranes in high concentrations. On the other hand, the interior lipid contained heavy metals in relatively low concentrations.

6) It is presumed that starch as well as lipid has only a weak affinity for hevay metals, and proteins are the major ligands that form complexes with heavy metals in plants.

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