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Speciation of manganese in tea leaves and tea infusions

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A speciation scheme was developed for identification of the chemical forms of manganese in tea leaves and tea infusions. The range of manganese in five different groups of tea leaves was found as 1107–2205 $\mu g\,g^{-1}$ on dry basis. It was noted that 30.0% of Mn was passed into the water that was present in the form of Mn(II), and also 2.5% of Mn was distributed in the total organic bound and was passed into the various solvents. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Tea can be seen as a popular beverage after water. The elements present in tea samples have been reported in the literature (Stage and Millin, 1975; Michie and Dixion, 1977; Tsushida and Takeo, 1977). For this reason, its nutritional significance may be explained if the chemical forms of its elements can be identified. This is called speciation (Dunemann and Schwedt, 1986). The aim of speciation analysis is often to provide information about the elements. The bioavailability of metal-containing species in food has been correlated with the extractability obtained by enzymolysis with gastric juice and intestinal juice (Walter, 1990).

The determination of metal species is a complex problem that can be specific for a particular sample. A possible approach to surmount such difficulties efficiently and rapidly is to couple various chromatographic and optical spectrometric methods (Ajlec and Stupar, 1989).

Few analytical techniques are 'species specific', i.e. respond only to a particular chemical form of an element in solution. Preliminary chemical separation may be used to isolate the metal associated with colloidal particles or with organic ligand from other forms of metal. The characteristics of some separation methods commonly applied in speciation measurements are liquid–liquid extraction, dialysis, electrophoresis, ultrafiltration, centrifugation, ion-exchange, anodic stripping voltammeter and some other techniques (De Mora and Harrison, 1985; Whitfield and Turner, 1986; Jackson and Lee, 1988; Walter, 1990; Buffi *et al.*, 1992).

Earlier researchers have used EPR spectrometry to determine manganese (II) in aqueous solution (Corsini *et al.*, 1987) and the method of co-precipitation with $Al(OH)_3$ in water samples was employed to specify Mn(II) (Mizuike, 1983). They have used also enzyme applications for manganese speciation in foodstuffs (Schwedt and Neumann, 1992).

Manganese is an essential trace element. About 2.5–7 mg manganese is suplied in the daily diet of the adult. Its principal function is as an integral constituent or activator of enzymes (Corrinne *et al.*, 1986).

In our study, an analytical scheme was proposed for the speciation of manganese in tea samples. Solvent extraction methods were used for the speciation of total organic bound Mn in the tea leaves and tea infusions. Co-precipitation and also ion exchanger resins were used for speciation of Mn (II) in tea infusions.

MATERIALS AND METHODS

Instrumentation and materials

A Philips PU 9100X Flame Atomic Absorption Spectrophotometer interfaced with a computer for data collection was used in the absorbance mode with the manufacturer's recommended air-acetylene flow rate (4.5/1.1, litre min^{-1}) and a 50 mm single slot burner. Absorbances were measured at 279.5 nm with a band-pass width of 0.5 nm, the burner height was optimised with respect to the light beam to give a maximum absorbance signal for a standard Mn solution aspirated into the flame. A Shimadzu 2000S double beam UV-VIS spectrophotometer was used for measuring absorbance and the spectrum of solvent extracts.

Turkish black tea leaves were purchased in a local market. Atomic absorption stock solution (1000 mg $litre^{-1}$ Mn in 2 N nitric acid) for manganese was obtained from Merck Chemical and a working solution of manganese (2–8 mg litre^{-1}) was prepared by appropriate dilution. Solvents, acids and all other reagents used were of analytical reagent grades. Water was de-ionised further and purified with a Milli-Q water purification system (Millipore, USA).

Ion exchanger columns, 2.00 g LAB II and 5.00 g LAB V were packed into a 7 cm × 15 mm id. polyethylene tube. Glass wool plugs were placed at both ends of the packed tube to avoid the loss of resin during operation.

Procedures: Experiment 1

Determination of total Mn in black tea leaves

A batch of 250 mg tea leaves was weighed and placed into a flask; then the sample was digested with 4 ml of pure concentrated HNO_3 and 1 ml of pure concentrated $HClO_4$ acids. The mixture was heated for 60 min until no more brown oxides of nitrogen were evolved and the solution began to give off white fumes. After cooling, the digest was transferred into a 50 ml calibrated flask and diluted to a volume of 50 ml with 2 N nitric acid, the amount of Mn was determined by Flame AAS. This procedure was repeated for five different types of Turkish tea samples.

Extraction of tea leaves by different solvents

A bulk of 5.00 g dry tea leaves were extracted by using the Soxhlet extraction method. In successive extraction methods, the solvents were added successively into the set-up CCl_4, $CHCl_3$, ethyl acetate, diethyl ether, methyl isobutyl ketone (MIBK) and water. The same solvents were utilised also in the batch extraction method. Following this, each solvent was evaporated by the evaporator and the residue was dissolved in an acid mixture (2 ml concentrated HNO_3 and 0.5 ml concentrated $HClO_4$); then the digest was transferred into a 25 ml calibrated flask and diluted to a volume of 25 ml with 2 N nitric acid; the amount of Mn in each extract was determined by Flame AAS. This procedure was repeated for five different types of Turkish tea samples.

Extraction of tea leaves by different acid solution solvents and total polyphenol in water and acid extracts

A second bulk of 5.00 g dry tea leaves was extracted in 40 ml deionized water, 40 ml hydrochloric acid and nitric acid (2–10 N) solution for 20 min. Each extract was filtered and the solution was transferred into a 50 ml calibrated flask and diluted to a volume of 50 ml using 2 N nitric acid; the amount of Mn in each extract was determined by Flame AAS.

The Folin–Denis Spectrometric method was employed for total polyphenol in water and acid extracts using tannic acid as the basis (Horwitz, 1980). Ten millilitres of water and acid extracts were added to 100 ml volume flasks containing 75 ml deionized water. To this were added 5 ml of Folin–Denis reagent (100 g $Na_2WO_4.2H_2O$, 20 g phosphomolybdic acid, 50 ml of concentrated H_3PO_4 in 1 l deionized water), 10 ml of Na_2CO_3 solution (10%) and diluted to volume with deionized water. The solution was well mixed and determined after 30 min at 760 nm by spectrophotometry. Quantitation was carried out using calibration graphs obtained from the standard tannic acid solution (10–40 mg litre^{-1}, four standard) which were treated according to the above analytical procedure. This procedure was repeated for one Turkish tea sample.

Experiment 2

Preparation of tea infusions and determination of total Mn

Tea infusions were prepared by allowing the desired amount of Turkish tea Y1 or Y2 sample (5.00 g) to infuse with 500 ml of deionized water at 95°C for 15 min. The extract was filtrated and acidified with 2 N nitric acid and, the amount of Mn was determined by Flame AAS.

Determination of Mn(II) in tea infusions by co-precipitation

Two grams of $Al(NO_3)_3.9H_2O$ and 0.5 g NH_4Cl were added to a 100 ml tea infusion, then mixed and heated. After co-precipitation of Al^{3+} with 6 N ammonia the solution was decanted and the precipitate obtained was washed with deionized water. The precipitate then was acidified by 2 N nitric acid and eventually the amount of Mn was determined by Flame AAS.

Sorption of total cationic and anionic Mn by ion-exchange

A 10.0 ml tea infusion was percolated in the column through at a flow rate of about 3 ml/min. The column was washed with 2 N nitric acid and the amount of Mn in the eluent was determined by Flame AAS. The tea Y1 or Y2 samples were analysed in triplicate for manganese using Flame AAS.

Batch extraction of tea infusions by different solvents

Fifty millilitres of tea infusion (using tea Y1 sample) were extracted from the water extract using CCl_4, $CHCl_3$, ethyl acetate, diethyl ether and methyl isobutyl ketone (MIBK) one after another. The solvents were evaporated in an evaporator and the residue was dissolved in an acid mixture of 2 ml concentrated nitric acid and 0.5 ml concentrated $HClO_4$. Next, the digest was transferred into a 25 ml calibrated flask and diluted to a volume of 25 ml with 2 N nitric acid and the amount of Mn in each of the eluent was determined by Flame AAS. This procedure was repeated for Turkish tea sample Y1.

Quantitation of manganese in each procedure was carried out using calibration graphs obtained from the standard solution which varied in concentration (2.0–8.0 mg/l) and were prepared from manganese stock solution. The control of each procedure was eliminated from the calibration graph.

From the experimental study described above, the analytical scheme shown in Fig. 1 was devised.

RESULTS AND DISCUSSION

Experiment 1: Mn speciation in black tea leaves

After the batch extraction of tea leaves with water and various solvents, Mn levels found in each solvent were determined as shown in Table 1.

As seen in Table 1, 30.0% Mn was passed into the water extracts, while the total ratio of Mn passing into all other solvents was 2.5%. This was in agreement with Michie and Dixion (1975), who had previously reported that 34% Mn was passed into water extracts.

It was further noted above that in tea samples, the Mn content of aqueous extracts was higher than that found in the organic extracts, which indicated that Mn was present primarily in an inorganic form.

When the dipole moments of the solvents were taken into account, it was proposed that the Mn in the CCl_4 or $CHCl_3$ extracts was uncharged and bonded to Mn via either organic or covalent bound (Florance and Batley, 1980). On the other hand, the Mn in the other solvents were charged organic compounds. It has been reported recently that the two tea components: alkaloids (such as caffeine, theobromine) and pigments (like chlorophyll) were passed into the CCl_4 or $CHCl_3$ extracts (Rasiah and Senerath, 1987).

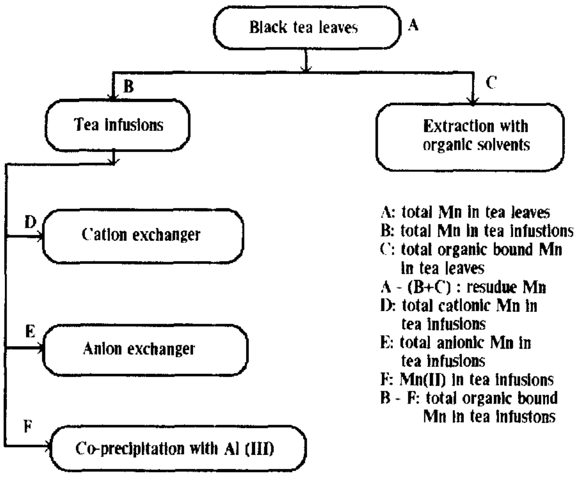

Fig. 1. Speciation scheme of manganese in tea samples.

The UV/VIS spectra of all solvents in Table 1 are shown in Fig. 2. It was noted that the CCl_4 (Fig. 2, (b)3), CHCl3 (Fig. 2, (b)2), ethyl acetate (Fig. 2, (b)4) and diethyl ether (Fig. 2, (b)5) extract spectra appeared to be similar to the chlorophyll spectrum, shown in Fig. 2(a). However, the spectra of MIBK (Fig. 2, 1 at 300–320 nm) and water (Fig. 2, (b)6) extracts were different from other solvent extracts.

Different tea leaves were extracted by various solvents in successive extractions, in accordance with the increase in the dipole moments of the solvents and the Mn levels found in each solvent are shown in Table 2.

When the batch and successive extractions were compared (Tables 1 and 2) it was noted that the Mn level was approximately the same for CCl_4 and water. Large amounts of organic-bound Mn were passed into CCl_4. It can be proposed that this structure was included in the pigments. On the other hand, Mn levels in the other solvents were decreased and a small amount of organic-bound Mn was passed into other solvents.

To determine the form of the remaining 67.5% of Mn which did not pass into water or other organic solvents, the tea leaves were extracted with water, HCl and nitric acid solutions at different concentrations and, the amount of Mn passing into each extract is shown in Fig. 3.

As can be seen from Fig. 3, although the nitric acid solution caused the organic structure, i.e. the amount of total polyphenols, to be soluble there was no increase in Mn level. As a result of this, it can be stated that the remaining Mn was present in an inorganic form, e.g. as Mn-oxides.

Experiment 2: Mn speciation in tea infusions

In Fig. 1, the proposed speciation scheme was shown, which allows partitioning of the total manganese concentration into five fractions, namely: residue, total cationic and anionic, Mn(II) and organic-bound Mn. When a 575 mg/l Al solution was added to a 2.0 mg/l Mn(II) solution, the co-precipitation recovery was found to be 97.7%.

Table 1. Mn levels measured in five different tea samples using various solvent extracts

Solvents	Dipole moment (Debbie)	Range ($\mu g\,g^{-1}$)	Average ($\mu g\,g^{-1}$)	Efficiency (%)
CCl_4	0.00	3.2–14.7	10.5	0.6
$CHCl_3$	1.01	5.5–12.5	8.8	0.5
Diethyl ether	1.54	5.1–7.4	6.1	0.3
MIBK	1.60	10.1–19.2	4.5	0.8
Ethyl acetate	1.78	3.2–7.1	4.9	0.3
Water	1.87	377.0–637.0	562.0	30.0
Total Mn in tea leaves		1107.0–2205.0	1871.0	

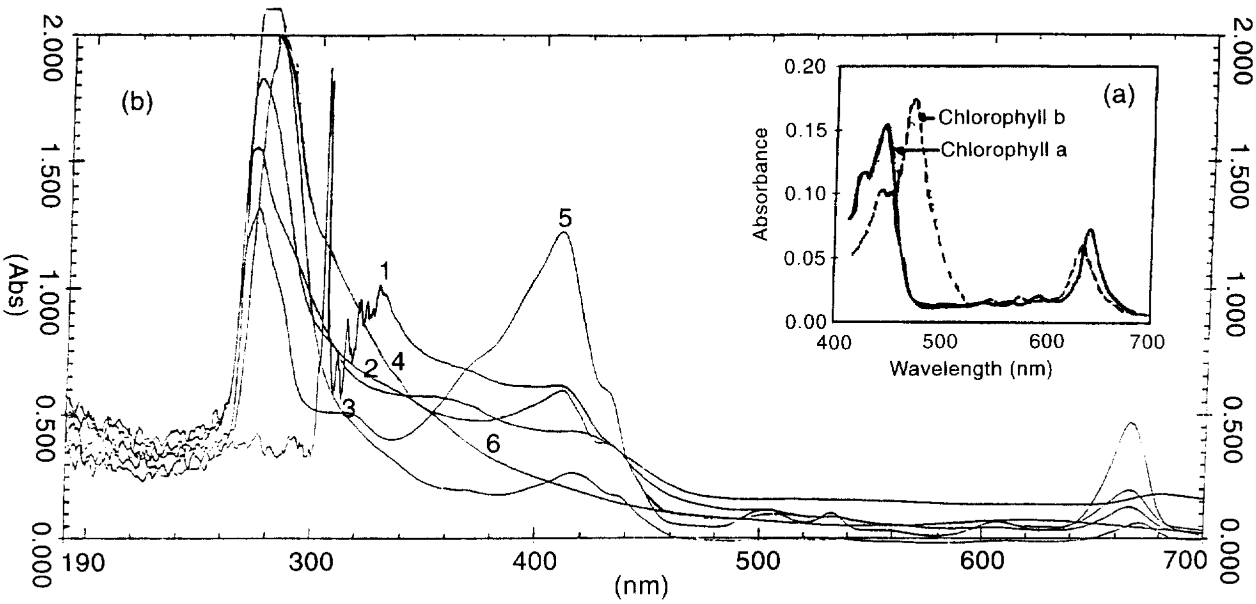

Fig. 2. UV/VIS spectra of the various solvent extracts. (a) spectra of chlorophyll a and b (Devlin, 1969); (b) 1: MIBK; 2: $CHCl_3$; 3: CCl_4; 4: ethyl acetate; 5: diethyl ether; 6: water.

Table 2. Mn levels found in each solvent extract obtained from five different tea samples

Solvents	Range ($\mu g\,g^{-1}$)	Average ($\mu g\,g^{-1}$)	Efficiency (%)
CCl_4	3.8–14.0	11.9	0.6
$CHCl_3$	2.1–3.2	2.8	0.1
Diethyl ether	1.2–1.3	1.2	0.1
MIBK	1.6–1.7	1.6	0.1
Ethyl acetate	1.0–1.1	1.0	0.1
Water	411.0–630.0	551.1	29.5
Total Mn in tea leaves	1107.0–2205.0	1871.0	

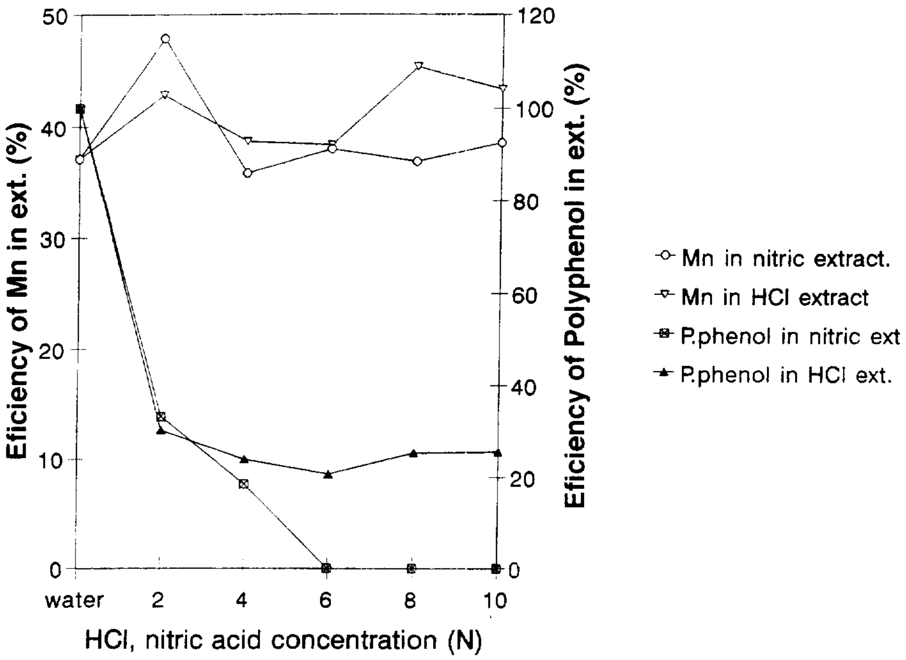

Fig. 3. Extraction of tea leaves with water, hydrochloride and nitric acid solution.

Table 3. Determination of total Mn and Mn species in tea infusions ($n = 3$)

Species	Tea Y1 sample Mn ($\mu g\,g^{-1}$, on dry basis)	Tea Y2 sample Mn ($\mu g\,g^{-1}$, on dry basis)
Total Mn	421.0 ± 8.9	366.0 ± 7.3
Mn(II)	406.3 ± 4.2	328.0 ± 3.0
Total cationic Mn	400.1 ± 4.2	329.1 ± 3.5
Total anionic Mn	—	—
Total organic-bound Mn	14.7 ± 9.8	38.0 ± 8.2
Total Mn in tea leaves	1107.0	1871.0

Table 4. The Mn levels found in each extract of tea sample Y1 after batch extraction from tea infusion

Solvents	Mn ($\mu g\,g^{-1}$, on dry basis)
CCl_4	2.4
$CHCl_3$	1.4
Diethyl ether	3.6
MIBK	6.7
Ethyl acetate	10.0
Total Mn in each extract	23.8

According to the results of this analysis, the Mn forms found in the two tea samples, Y1 and Y2, can be seen collectively in Table 3.

As it can be seen from Table 3, no anionic Mn was absorbed in the tea infusions. The Mn(II), and the total cationic Mn levels were similar and the *t*-test indicated that the differences were not significant at a 95% confidence level thus, we can say that the total cationic Mn was present in the form of Mn(II).

The total organically bound Mn in tea Y1 infusions can be calculated from Fig. 1 or various solvents can be used for the extraction of total organically bound Mn from tea infusions. The results are given in Table 4.

As can be seen above, the value of total Mn, i.e. 23.8 $\mu g\,g^{-1}$, in each extract was in the range of the total organic-bound Mn that was found in Table 3. In the literature, it was reported that theaflavin was passed into MIBK from tea infusion (Fernando and Plambeck, 1988).

In summary, a total of 30.0% Mn was passed into the tea infusions of which 95% was in the form of Mn(II) and the remaining 5.0% in the form of total organic-bound Mn. The use of co-precipitation with Al(III) was useful for determination of Mn(II) in tea infusions. Manganese is rather poorly absorbed from the small intestine by a mechanism similar to the absorption of iron (Corrinne *et al.*, 1986). It might be suggested that the bioavailability of manganese in tea infusions is lower.

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