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Effect of boiling and peeling on manganese content of some vegetables determined by derivative anodic stripping chronopotentiometry (dASCP)

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Abstract

The purpose of this paper was two-fold: to optimise an analytical method based on derivative anodic stripping chronopotentiometry (dASCP) for reliable trace manganese determination in different fresh vegetables and aromatic plants, and to use this technique to asses the effect of boiling and peeling processing on their manganese content. The deposition potential was −1700 mV and the deposition time 120 s; in this conditions the limits of detection 8.0 ng kg^{-1} (ppt) and the accuracy of the method, assessed using certified reference materials, was within 95.0%. Among the studied fresh vegetables, the highest content of manganese was found in vegetables with dark green leaves as chicory and spinach (respectively, 3.5 and 3.3 mg/100 g), while vegetables with light green leaves as lettuce, together with carrots, garlic and pore mushrooms had manganese levels lower than 1.0 mg/100 g. Boiling processing cause a significant decrease of manganese levels in artichokes, tomatoes, chicory, garlic, mushrooms, peeled carrots and potatoes, spinach, and string beans ($p \leqslant 0.005$, ANOVA). Fennels, lettuce, marrow, unpeeled carrots and unpeeled potatoes did not show any statistical significant changes after boiling. Also peeling significantly influenced the content of manganese of carrots and potatoes ($p \leqslant 0.01$, ANOVA) and favoured manganese loss during boiling.

Keywords: Boiling; Derivative anodic stripping chronopotentiometry; Manganese; Peeling; Vegetables

1. Introduction

Manganese is an essential trace metal for human and animals, since it is involved in many physiological processes. Particularly it plays an important role in the metabolism of proteins, carbohydrates, lipids and in the production of steroids sexual hormones, moreover is the cofactor of enzymes such as RNA synthetase, glutamine synthetase, pyruvate decarboxylase, Mn-superoxido desmutase and arginase (Wedler, 1984, 1993). Moreover manganese plays an important rule in regulating the nitrogen metabolism and the photosyntesis in plants. Eleven oxidation states of manganese are known, from −3 to +7, but the most abundant species both in mammals and plants is Mn^{2+}, followed by Mn^{3+} and Mn^{4+} (Kaim & Schwederski, 1996). Manganese at moderate concentrations, is known to be essential for the developing and functioning of neuronal activity in the brain. Dietary Mn

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deficiency, which enhance susceptibility to epileptic functions, appears to affect seriously its homeostasis in the brain; Tanaka (1982) observed that blood manganese concentration in epileptic patients is lower than that of normal people. On the other hand, manganese acts also as toxicant because of its pro-oxidant activity: this metal that abnormally concentrates in the brain, particularly in the basal ganglia, may cause neurological disorders similar to Parkinson's disease (Zheng, 2001). The RDA (Recommended Daily Allowance) for manganese established by the Food and Drugs Administration is 2.5–5.0 mg/day for an adult, and 0.25–2.5 mg/day for a 0–14 years old child. Vegetables are known to be a good source of manganese: spinach 0.5–0.8 mg/100 g, green peas 0.3–0.8 mg/100 g, beans 0.4–0.6 mg/100 g (Pennington et al., 1995). Nutritionists recommend an increase in fruit and vegetables consumption to replace food high in fat and calories; therefore it is of great concern to assess their content of microelements as manganese, that have a biological role at low doses, and are potentially toxic at high doses. It is recognised that the microelements content of plants varies according to genetic differences (varieties and cultivars) (Dugo, La Pera, Giuffrida, Salvo, & Lo Turco, 2004), environmental factors, cultural practices, harvesting and post-harvesting procedures (Rincon, Zurera, Moreno, & Ros, 1990). It is well known that industrial processing and cooking have a considerable influence on the nutrient content at the point of consumption (Polo, Lagarda, & Farrè, 1992; Rincon et al., 1990; Somogyi, 1990). The purpose of this paper was to develop a sensitive, accurate and rapid analytical method as anodic stripping chronopotentiometry (dASCP), for reliable trace manganese determination in different vegetables and aromatic herbs, and to employ the optimised technique to asses the effect of boiling and peeling on manganese content of vegetables. The analytical techniques conventionally used for manganese analysis in food samples are both emission and absorption spectroscopy. These analytical methods request time consuming sample pre-treatments suitable for the complete destruction of the organic matrix as wet digestion, and dry ashing (Pennington et al., 1995; Tinggi, Reilly, & Patterson, 1997). Moreover the commonly used sample preparation procedures and analytical techniques that involve the use of high temperatures, as dry ashing followed by atomic absorption spectroscopy, may cause a severe loss of manganese compounds trough volatilization. Literature reports some publications about the use of anodic stripping voltammetry for manganese determination (Locatelli & Torsi, 2000; Di & Zhang, 2003) but there is a lack of available data about the use of derivative anodic stripping chronopotentiometry for manganese analysis in food samples, using a glassy carbon mercury coated working electrode. Dugo, La Pera, Lo Turco, Mavrogeni, and Alfa (2003a, 2003b) and La Pera, Lo Curto, Visco, La Torre, and Dugo (2002, 2003) had successfully employed dASCP for trace metals (Cd, Cu, Pb and Zn) analysis in different food matrices. Derivative anodic stripping chronopotentiometry is a two phase technique: a deposition step during which metal ions are reduced at constant potential (accumulation), followed by the stripping step (quantification) involving re-oxidation by a chemical oxidant, usually Hg (II). The analytical signal is the time taken for re-oxidation (transition or stripping time, τ) which is determined by measuring the area under the peak in the dt/dE vs E plot, where dt/dE represent the inverse of the time derivative of the recorded E (Town & van Leewen, 2001). The stripping time is proportional to the concentration of the metal and to the electrolysis time, nevertheless by lengthening the electro-deposition time the sensitivity of the method should be enhanced (Jagner, 1978). It was empirically proved that stripping voltammetry is more susceptible than stripping chronopotentiometry to interferences by electro-active organic molecules and by non faradic currents, that caused a severe sensitivity decrease (Town & van Leewen, 2002). Nevertheless dASCP is more suitable than SV for analysis of samples containing significant amounts of organic matter, as food samples. The proposed method does not need any laborious sample pretreatment, but a hydrochloric acid extraction. Stripping techniques based on electrolytic pre-concentration of manganese as the Mn/Hg amalgam should suffer from three problems: the low solubility of Mn in mercury, the deposition potential for Mn (II) reduction ($E_{dep} = -1700$ mV) which is close to the hydrogen reduction potential, and formation of intermetallic Cu–Mn compounds in the mercury film of the working electrode (Di & Zhang, 2003). The first problem is overcome by performing the analysis in conditions of low manganese electrolytic-cell concentration (<50 ng kg^{-1}); this is allowed by the extraordinary sensitivity of this technique which, in the optimized electrochemical conditions, achieves detection limits lower than 10 ng kg^{-1} (ppt). The evolution of hydrogen is eliminated by performing the analysis in 25 mM ammonia–ammonium chloride buffer (pH 9.5). Copper (II) interference may be avoided by adding in the electrochemical cell an excess of gallium(III) which prevented from intermetallic Mn–Cu complex formation in Hg amalgam. The accuracy of the optimised chronopotentiometric method was tested with analysing certified reference materials and was also compared with graphite furnace atomic absorption spectroscopy; the obtained results confirmed that dASCP is a valid tool for rapid and reliable Mn determination in vegetables, particularly prone for routine analysis.

2. Materials and method

2.1. Reagents and apparatus

All the reagents used were of analytical grade. Hydrochloric acid (34–37%), Hg (II) (1000 mg kg^{-1}, 1.0 N HCl), Mn (II) (1000 mg kg^{-1}, 0.5 N HNO_3), Cd (II) (1000 mg kg^{-1}, 0.5 N HNO_3), Cu (II) (1000 mg kg^{-1}, 0.5 N HNO_3), Pb (II) (1000 mg kg^{-1}, 0.5 N HNO_3), Zn (II) (1000 mg kg^{-1}, 0.5 N HNO_3) standard solutions and the 0.5 M ammonia buffer (pH 9.5) were purchased from Panreac (Barcelona, Spain). The Mn (II) standard solution was diluted with ultra-pure water to obtain a 1 and 5 mg kg^{-1} Mn (II) solutions. $Ga(NO_3)_3 \cdot 3H_2O$ (5 g, 99.9%) and absolute methanol used to clean the electrodes were purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). Nitric acid (Merk, Darmstadt, Germany) was used to digest the dried sample. The accuracy of the method was assessed using certified reference materials as cabbage (IAE 359), carrot powder (ARC/CL 4) and leaves of poplar (GBW07604). Manganese (II) analysis were carried out on a PSA ION 3 potentiometric stripping analyzer (Steroglass, S. Martino in Campo, Perugia, Italy), which was controlled by NEOTES 2.0.1 software (Steroglass) run on an IBM-compatible personal computer. The working electrode was a teflon fitted glassy carbon electrode (diameter 3 mm), coated with a thin mercury film as described in previous papers (La Pera et al., 2002, 2003); an Ag/AgCl electrode (3 M KCl), and a platinum wire were also used as the reference and the auxiliary electrode, respectively. When not in use, the electrodes were stored in ultra-pure water. The mercury film of the working electrode is removed after each analysis by polishing with absolute methanol and filter paper. A pH meter MI229 BDH equipped with glass + combination pH electrodes (BDH, Milan, Italy) was used to measure the pH values of the samples. To confirm the analytical results of the potentiometric method, the plants extracts were subjected to AAS, using a Shimadzu 800 series graphite furnace atomic absorption spectrometer, equipped with auto sampler ASC-6100 and a Mn hollow cathod lamp.

2.2. Samples

Fresh vegetables as artichoke, broccoli, carrots, celery, chicory, fennel, garlic, green peas, lettuce, marrow, onion, pore mushrooms, spinach, string beans, tomatoes and potatoes, were studied. Among the aromatic plants bay, basil, chili, origan, parsley, rosemary and sage were analyzed. All the studied fresh vegetables and aromatic plants were harvested in Sicily in the period of September–November 2003. All the fresh samples, were delivered to the laboratory in few hours after harvesting, immediately washed, dried and subjected to the extraction procedure Five samples of each type of vegetable were collected; representative samples were obtained by weighting an aliquot of 10.0 g taken from different part of each vegetable. The vegetables extracts were stored at 4 °C until the analysis. The effect of peeling of carrots and potatoes on manganese concentration in raw and boiled samples, was assessed on five groups of carrots and five of potatoes. Each group was represented by four vegetables grown in the same field and harvested in the same day. Within each group, the samples were analyzed as follows: raw-unpeeled, boiled-unpeeled, raw-peeled, boiled-peeled. Aromatic herbs were analyzed only for manganese content in raw samples.

3. Experimentals

3.1. Samples processing

For the chronopotentiometric determination of manganese, fresh vegetables were washed with ultrapure water to remove soil and dust from the surface and dried at 110 °C to constant weight in order to measure their moisture contents. To value the effect of boiling processing on manganese content, fresh vegetables were boiled in 150 mL of tap water till edible; after boiling they were drained and dried as already described. 0.5 g of raw or boiled dried vegetables were dissolved in 10.0 mL of 35% HCl at 90 °C for 40 min. To remove any solid residue, the acid extracts were filtered trough filter paper (0.45 μm). The peels of raw carrots and potatoes were subjected to the extraction procedure described to investigate the effect of peeling on manganese content. For atomic absorption spectroscopy confirmation analysis, 0.5 g of certified cabbage, carrot powder and leaves of poplar were weighted accurately into a crucible, dried in a 110 °C oven. The dried samples were then digested with 2 mL of concentrated nitric acid before dry-ashing in a muffle furnace at 550 °C for 2 h. The dry ashed samples were dissolved in 10 mL of 35% HCl and analyzed; the most sensitive line for manganese was 279.5 nm with a band pass of 0.2 nm.

3.2. Derivative anodic chronopotentiometric stripping analysis (dASCP)

After the plating of the working electrode with mercury (Dugo et al., 2003a, 2003b; La Pera et al., 2002, 2003), 10.0 μL of sample extract were put into the electrochemical cell together with 0.20 mL of 0.5 M ammonia–ammonium chloride buffer (pH 9.5), 19.0 mL of ultra-pure water, 0.1 mL of Hg (II) 1000 mg kg^{-1} and de-oxygenated for 5 min by N_2 purging. The best electrochemical conditions were optimized by fixing the final

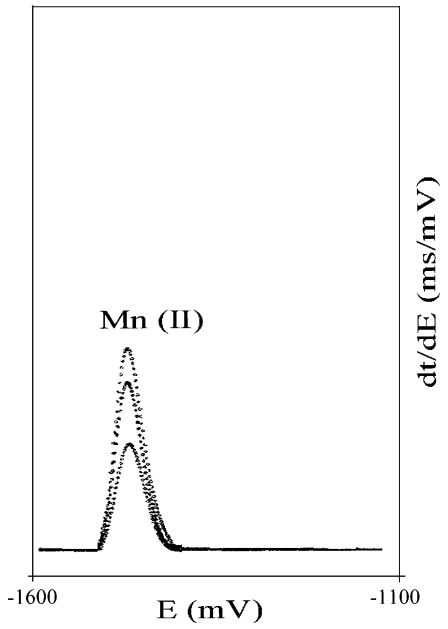

Fig. 1. Potentiometric curve of Mn (II) determined in the certified reference cabbage at $E_{dep} = -1700$ mV, $t_{dep} = 120$ in ammonia buffer (pH 9.5).

acquisition potential and the cleaning potential, respectively, at −500 and −1500 mV, and varying the deposition potential and the deposition time as afterwards described; the solution was stirred at 1000 rpm. The manganese stripping peak was registered around −1460 mV (Fig. 1). The quantitative analysis was done by the multiple-point standard additions method. Optimum precision and accuracy were obtained with the addition of two 12.5 μL aliquots of a 1.0 mg L^{-1} standard solution of Mn (II) and performing the measurements five times (Renman & Jagner, 1997). Calibration curves with a correlation coefficients >99.5% were obtained. The linear concentration range was 0–1000 μg L^{-1}. In the optimized electrochemical conditions, the manganese analysis lasted 25 min.

3.3. Statistical analysis

In order to value the effect of boiling processing and peeling on manganese content in vegetables, data obtained from the chronopotentiometric analysis of the samples were evaluated statistically by analysis of variances (ANOVA).

4. Results and discussions

4.1. Effect of deposition potential and deposition time on analytical sensitivity

The effect of deposition potential and deposition time on the sensitivity of Mn (II) determination, was

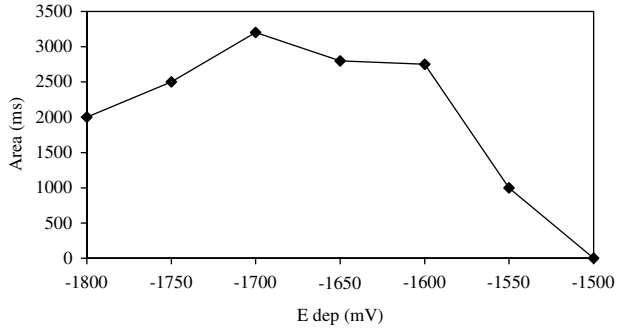

Fig. 2. Dependence of the analytical response on deposition potential at $t_{dep} = 120$ s.

valued analyzing a certified reference cabbage extracted as previously described. The effect of varying the deposition (or electrolysis) potential from −1800 to −1500 mV was studied at $t_{dep} = 120$ s. As Fig. 2 shows, the best sensitivity for Mn (II) determination by dASCP was achieved at deposition potential of −1700 mV in ammonia buffer (pH 9.5) in order to avoid H_2 evolution at the working electrode surface. Many data were found concerning the dependence of the sensitivity of the analysis on deposition time, in electro-analytical stripping techniques (Jagner, 1978; Town & van Leewen, 2001). The effect of lengthening deposition time from 30 to 600 s on sensitivity and accuracy was assessed at $E_{dep} = -1700$ mV. Limits of detection (LOD) and limits of quantification (LOQ) were calculated, respectively, by the expressions $3\sigma/S$ and $10\sigma/S$ (Pharmeuropa, 1999; Winefordner & Long, 1983): σ indicated the standard deviation of the response obtained by four measurements on the certified reference cabbage, S (ms kg μg^{-1}) was the sensitivity obtained from the slope of the calibration curves obtained at different deposition times. As Table 1 reports, the sensitivity was observed to linearly increase due to longer deposition time in the range 60–420; upon applying deposition time longer than 420 s, the sensitivity become almost independent of the deposition time, suggesting a saturation of the electrode surface (Björefors & Nyholm, 1996). The obtained data evidenced the extraordinary sensitivity of the proposed method also at short deposition time (90, 120 s) which allowed to achieve detection limits lower than 10.0 ng kg^{-1} (ppt); previous studies concerning the voltammetric determination of Mn (II) in environmental and industrial samples, reported detection limits that raged from 50 to 120 ng kg^{-1} (Abo El Maali & Abd El-Hady, 1998; Di & Zhang, 2003). The accuracy at different deposition time, was expressed as recovery percent obtained from the certified reference cabbage. The obtained results evidenced that the accuracy was independent of the deposition time, and was always in the range 94.0–101.5%.

Table 1
Dependence of sensitivity (S), limits of detection (LOD = $3\sigma/S$), limits of quantification, (LOQ = 10 σ/S) and accuracy on deposition time at $E_{dep} = -1700$ mV

t_{dep} (s)	σ (ms)	S (ms kg μg^{-1})	LOD[a] (ng kg^{-1})	LOQ[a] (ng kg^{-1})	Accuracy[a] (%)
60	505 ± 14	113,316 ± 3173	13.4 ± 0.4	44.6 ± 1.2	94.0 ± 1.5
90	440 ± 14	136,270 ± 4088	9.7 ± 0.3	32.3 ± 0.9	99.1 ± 1.2
120	490 ± 12	173,800 ± 4171	8.5 ± 0.2	28.2 ± 0.7	96.0 ± 1.1
180	700 ± 19	283,500 ± 7654	7.4 ± 0.2	24.7 ± 0.7	96.0 ± 1.0
240	730 ± 19	304,906 ± 7928	7.2 ± 0.2	23.9 ± 0.6	97.1 ± 1.1
300	640 ± 11	326,646 ± 5553	5.9 ± 0.1	19.6 ± 0.4	96.4 ± 1.2
360	510 ± 13	395,283 ± 10,672	3.9 ± 0.1	12.9 ± 0.3	100.1 ± 1.3
420	595 ± 21	637,640 ± 22,317	2.8 ± 0.1	9.3 ± 0.3	95.0 ± 1.0
480	586 ± 20	650,810 ± 22,778	2.7 ± 0.1	9.0 ± 0.3	97.0 ± 1.0
540	585 ± 20	675,663 ± 25,987	2.6 ± 0.1	8.7 ± 0.3	101.5 ± 1.6
600	585 ± 20	675,663 ± 25,987	2.6 ± 0.1	8.7 ± 0.3	101.5 ± 1.6

σ (ms) represents the standard deviation of the response obtained from four measurements performed on the certified reference cabbage.
[a] Each value was the mean of four determinations.

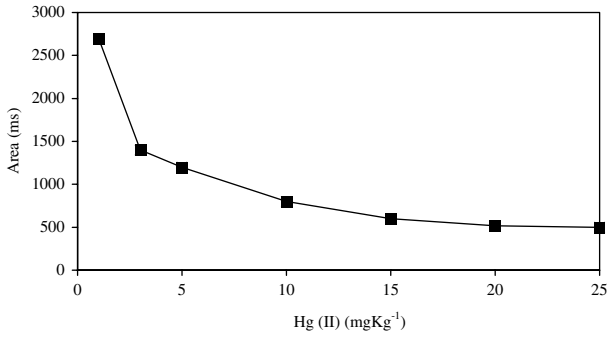

Fig. 3. Dependence of the analytical response on Hg (II) concentration at $t_{dep} = 120$ s and $E_{dep} = -1700$ mV.

4.2. Effect of Hg (II) concentration

The effect of the oxidant concentration on the analytical response of Mn (II) analysis was investigated placing into the electrochemical cell 10.0 μL of certified cabbage, 0.20 mL of 25 mM ammonia–ammonium chloride buffer (pH 9.5), 19.0 mL of ultra-pure water. The analysis were performed varying the Hg (II) concentration from 1.2 to 25.0 mg kg^{-1} at deposition potential of −1700 mV for 120 s. Fig. 3 provides evidence that the analytical response decreased as the Hg (II) concentration increases in the studied range; for further analysis a Hg (II) cell concentration of 1.2 mg kg^{-1} was used.

4.3. Performances characteristics of the procedure

The performances of the proposed method were determined in the optimized electrochemical conditions: deposition time of 120 s, deposition potential of −1700 mV final acquisition potential of −500 mV and Hg (II) cell concentration of 1.2 mg kg^{-1}. The reliability of the proposed method was assessed performing spike and recovery test on certified reference cabbage, carrot, people leaves, subjected to the hydrochloric acid extraction procedure described earlier. The obtained recoveries that ranged from 95.5% to 97.0% (Table 2), confirmed that no significant manganese loss occurred neither during the extraction procedure nor the stripping step. The precision and reproducibility of the analytical method were evaluated by executing the extraction procedure three times both on the certified reference cabbage and on a not certified cabbage sample, and quantifying manganese (II) four times in each extract. The instrument precision was indicated as the r.s.d. % for each extract ($n = 4$) and ranged from 2.6 to 2.9 for the certified cabbage and from 2.3 to 2.7 for the not certified sample; the method reproducibility was represented by the total r.s.d. % ($n = 3 \times 4$) for all the extracts and was lower than 1% both for the certified and the not certified samples (Table 3).

4.4. Interference study

Various metal cations as Cd (II), Cu (II), Ga (III), Pb (II), and Zn (II) accumulates on the mercury film of the working electrode at −1700 mV (La Pera et al., 2002, 2003), therefore they might interfere with Mn (II) analysis. In order to evaluate the selectivity of the proposed method, the influence of these cations on the analytical response was examined spiking at different levels with Cd (II), Cu (II), Ga (III), Pb (II), and Zn (II) an ultrapure water solution containing 3.0 μg L^{-1} Mn (II) and 2.5 mM ammonia buffer (pH 9.5). Particularly, the copper interference was also studied in the presence of an excess of Ga (III). The interference study provided evidence that 10^5 fold (by weight) of Cd (II), Ga (III), Pb (II) and Zn (II) did not significantly influence the potential peak area. As Fig. 4 shows, only Cu (II) remarkably influenced the analytical response: the peak area decreased to 50% of the initial value when the Cu(II) to Mn (II) ratio was 50, and was almost suppressed when the Cu (II) concentration reached 10,000 times that of Mn (II). The Cu (II) interference with the analysis of Mn (II) can be eliminated by the addition of an excess

Table 2
Spike-and-recovery test on certified reference materials (cabbage, carrot, leaves of poplar) and on lettuce

	Mn(II)	Added	Expected	Found	Mean recovery (%)
Cabbage	3.20	0	3.20	3.10 ± 0.08	
	3.20	3.00	6.20	5.9 ± 0.16	96.0 ± 1.0
	3.20	10.00	13.20	12.8 ± 0.34	
Carrot	0.49	0	0.49	0.48 ± 0.01	
	0.49	0.50	0.99	0.95 ± 0.03	95.5 ± 1.0
	0.49	1.00	1.49	1.44 ± 0.04	
Leaves of poplar	4.50	0	4.50	4.21 ± 0.17	
	4.50	5.00	9.50	9.22 ± 0.26	97.0 ± 1.5
	4.50	10.00	14.50	14.28 ± 0.40	

Each value (mg/100 g d.w.) is the mean of three determinations. The analysis were performed in 25 mM ammonia buffer (pH 9.5), $E_{dep} = -1700$ mV, $t_{dep} = 120$ s.

Table 3
Instrumental precision and reproducibility for the determination of Mn (II) by dASCP in vegetables[a]

		Mn (II) (mg/100 g d.w.)	Precision (RSD %, $n = 4$)
Certified cabbage	1st extract	3.12 ± 0.09	2.9
	2nd extract	3.13 ± 0.08	2.6
	3rd extract	3.16 ± 0.09	2.8
Total mean ± SD		3.14 ± 0.02	
Reproducibility (total rsd %)		0.6	
Not certified cabbage	1st extract	1.74 ± 0.04	2.3
	2nd extract	1.75 ± 0.04	2.5
	3rd extract	1.77 ± 0.05	2.7
Total mean ± SD		1.75 ± 0.01	
Reproducibility (total rsd %)		0.9	

[a] Precision and reproducibility were assessed using certified and not certified cabbage samples.

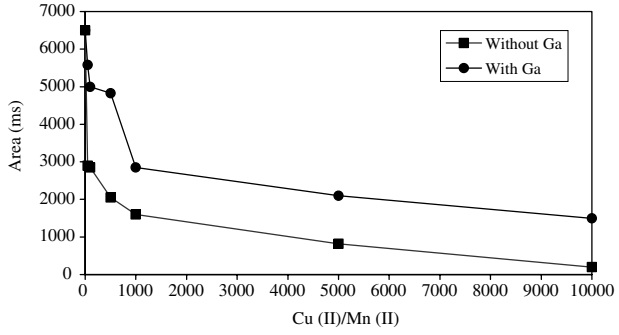

Fig. 4. Influence of Cu (II) on the analytical response of Mn (II) dASCP determination with and without an excess of Ga (III), at $t_{dep} = 120$ s and $E_{dep} = -1700$ mV.

of Ga (III) to the sample solution, since the Ga–Cu intermetallic compounds is very stable in the mercury amalgam (Jagner, 1978). In this condition the tolerance limit of Cu (II) can be increased up to 1000-fold (by weight) in the presence of an excess of Ga (III). Previous studies demonstrated that, in order to prevent copper interferences, the addition of Ga (III) is useful also for the chronopotentiometric determination of Zn (II) in food samples, at a mercury film electrode (La Pera et al., 2002).

4.5. Comparison analysis by GFAAS

Certified cabbage, carrot powder and leaves of poplar, subjected to the dry ashing procedure described earlier, were analyzed by graphite furnace atomic absorption spectroscopy (GFAAS); the obtained results were compared with those obtained by the proposed chronopotentiometric method (Table 4). Mn (II) concentrations determined by dASCP in hydrochloric acid extracts were in good agreement with GFAAS measurements in dry ashed samples. Particularly, the comparison of those analytical methods provided evidence that a lower Mn (II) loss occurred by dASCP, therefore it was used to analyze a large number of vegetable samples and to assess the effect of boiling and peeling on the concentration of Mn (II).

4.6. Analysis of vegetables

Results of moisture and manganese content in raw and boiled vegetables are given in Table 5. To prevent possible variations due to the moisture content of vegetables (76–96%) and aromatic herbs (73–94%), the manganese concentration in raw and boiled samples was referred to dry matter. Among the studied fresh vegetables, the highest mean content of manganese was found in dark green leafy species as chicory and spinach (respectively, 3.5 and 3.3 mg/100 g) followed by broccoli

Table 4
Confirmation analysis by GF AAS

Certified vegetable	Mn certified	Mn GFAAS	Mn dASCP
Cabbage	3.20	3.02 ± 0.10	3.10 ± 0.08
Carrot	0.49	0.41 ± 0.06	0.48 ± 0.01
Leaves of poplar	4.50	3.98 ± 0.25	4.21 ± 0.17

Each value (mg/100 g) was the mean of three analysis.

Table 5
Concentration of Mn (II) (mg /100 g d.w.) in fresh vegetables determined by dASCP

Vegetables	Moisture (%)	Mn-raw	Mn-boiled
Artichoke (*Cynara edunculus*)	94.0 ± 0.5	2.1 ± 0.3	1.3 ± 0.2
Broccoli (*Brassica oleracea*)	90.3 ± 0.7	2.8 ± 0.3	3.4 ± 0.3
Carrot (*Daucus carota*)-peeled	92.0 ± 0.6	0.6 ± 0.2	0.3 ± 0.1
Carrot (*Daucus carota*)-unpeeled	92.2 ± 0.7	0.9 ± 0.2	0.8 ± 0.3
Celery (*Apium graveolens*)	89.0 ± 0.2	1.2 ± 0.5	0.8 ± 0.5
Chicory (*Chicorium intybus*)	94.0 ± 1.0	3.3 ± 0.5	2.5 ± 0.5
Fennel (*Phoeniculum vulgare*)	96.1 ± 0.8	1.6 ± 0.3	1.1 ± 0.2
Garlic (*Allium sativum*)	80.5 ± 1.1	0.7 ± 0.2	0.6 ± 0.2
Lettuce (*Lactuca sativa*)	95.2 ± 0.8	0.5 ± 0.2	0.3 ± 0.2
Marrow (*Cucurbita pepo*)	96.0 ± 1.1	2.5 ± 0.5	2.4 ± 0.5
Onion (*Allium cepa*)	96.4 ± 0.5	1.3 ± 0.4	0.8 ± 0.3
Peas (*Pisum sativum*)	76.0 ± 1.3	1.3 ± 0.4	1.1 ± 0.4
Pore mushrooms (*Boletus eadulis*)	94.0 ± 1.0	0.8 ± 0.2	0.6 ± 0.2
Potato (*Solanum tuberosum*)-peeled	80.0 ± 1.6	0.8 ± 0.3	0.4 ± 0.2
Potato (*Solanum tuberosum*)-unpeeled	81.1 ± 1.1	1.5 ± 0.2	1.2 ± 0.2
Spinach (*Spinacia oleracea*)	95.0 ± 0.3	3.5 ± 1.3	2.1 ± 1.0
String beans (*Phaseolus vulgaris*)	93.0 ± 0.6	2.8 ± 0.6	2.5 ± 0.5
Tomato (*Solanum lycopersicum*)	95.3 ± 1.5	2.2 ± 0.4	1.5 ± 0.4
Aromatic plants			
Basil (*Ocimum basilicum*)	94.0 ± 1.0	2.0 ± 0.6	–
Bay (*Laurus nobilis*)	92.0 ± 0.2	1.9 ± 0.5	–
Chilli (*Capsicum annum*)	89.0 ± 0.5	1.2 ± 0.4	–
Origan (*Origanum vulgare*)	87.5 ± 0.6	0.2 ± 0.1	–
Parsley (*Petroselinum sativum*)	89.3 ± 0.5	2.1 ± 0.9	–
Rosemary (*Rosmarinus officinalis*)	73.0 ± 1.1	0.7 ± 0.3	–
Sage (*Salvia officinalis*)	67.2 ± 0.8	0.5 ± 0.2	–

Five samples for each type of vegetable were analysed. Each analysis was repeated three times in the conditions described in the paper.

and string beans (2.8 mg/100 g); lettuce, carrots garlic and pore mushrooms had manganese levels lower than 1.0 mg/100 g. Among the studied aromatic plants, basil, bay and parsley presented the highest levels of Mn ($\geqslant$2.0 mg/100 g), while origan and sage the lowest ($\leqslant$0.5 mg/100 g). In almost all the studied plants, with the exception of broccoli, there was a loss of soluble Mn in cooking water; the statistical significance of those changes was assessed by the analysis of variances (ANOVA). The samples were grouped according to the levels of significance referred to the modifications of manganese content in boiled vegetables respect to the same raw samples. Artichokes and tomatoes presented the most significant decrease in manganese content after boiling ($p \leqslant 0.0001$), followed by chicory, garlic, mushrooms, peeled carrots, peeled potatoes, spinach, and string beans ($p \leqslant 0.005$). Fennels, lettuce, marrow, unpeeled carrots and unpeeled potatoes did not show a statistical significant changes in Mn levels after boiling ($p > 0.05$). Particularly, a statistical not significant increase of Mn content was observed in boiled broccoli respect to the same raw samples. The modifications in the trace manganese content were also related with the peeling of the vegetables. Particularly, after peeling a significant decrease of Mn concentration was found in raw carrots and potatoes ($p \leqslant 0.01$) respect to the same raw unpeeled samples (Fig. 5). This might be associated with the presence of the metal in the peel – respectively,

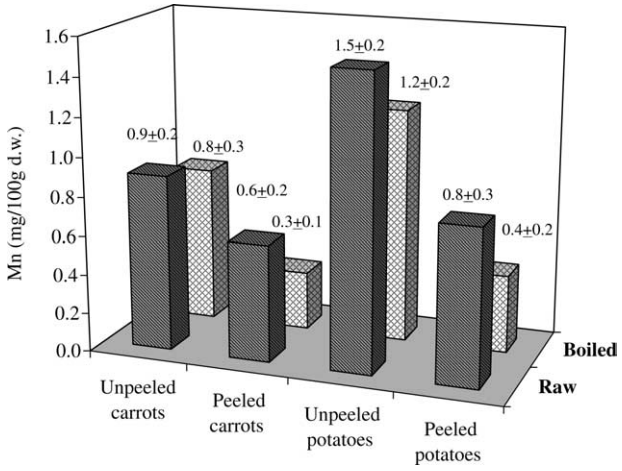

Fig. 5. Influence of peeling on manganese levels in raw and boiled potatoes and carrots.

0.10 ± 0.07 mg/100 g ($n = 5$) in the carrots peel and 0.40 ± 0.1 mg/100 g ($n = 5$) in the potatoes peel, so that its removal could explain the lower Mn levels in the peeled samples. Moreover, the obtained data confirmed that the peel prevented mineral loss during the boiling processing, since peeled carrots and potatoes showed greater decreases of the manganese concentration during boiling in comparison with the same unpeeled samples.

Table 6
Nutrient density and percentages of recommended daily allowance (RDA) per 100 g of vegetable

Vegetable	$ND\%_{raw}$	$ND\%_{boiled}$	$RDA\%_{raw}$	$RDA\%_{boiled}$
Artichoke	3111 ± 444	1926 ± 321	42 ± 6	26 ± 4
Broccoli	5090 ± 565	6181 ± 562	56 ± 6	68 ± 6
Carrot-peeled	727 ± 242	363 ± 121	12 ± 4	6 ± 2
Carrot-unpeeled	1090 ± 218	969 ± 323	18 ± 4	16 ± 5
Celery	2400 ± 960	1600 ± 800	24 ± 10	16 ± 8
Chicory	13,200 ± 1886	10,000 ± 2000	66 ± 9	50 ± 10
Garlic	682 ± 195	585 ± 195	14 ± 4	10 ± 3
Lettuce	1053 ± 421	632 ± 421	10 ± 4	6 ± 4
Marrow	9090 ± 1818	8273 ± 1655	50 ± 10	48 ± 10
Onion	2000 ± 667	1231 ± 410	26 ± 9	16 ± 5
Peas	684 ± 214	589 ± 196	26 ± 8	22 ± 7
Pore mushrooms	1454 ± 363	1091 ± 364	16 ± 4	12 ± 3
Potato-peeled	376 ± 188	188 ± 94	16 ± 5	8 ± 4
Potato-unpeeled	706 ± 91	565 ± 94	30 ± 4	24 ± 4
Spinach	4516 ± 1673	3613 ± 1806	70 ± 26	56 ± 28
String beans	6588 ± 1401	5647 ± 1129	56 ± 11	50 ± 10
Tomato	4631 ± 842	3157 ± 831	44 ± 9	30 ± 11

5. Nutritional assessment of the modifications in Mn content of boiled vegetables

To assess weather the statistically significant changes found in the manganese levels during the boiling of vegetables have any nutritional importance, nutrient density values (ND) and percentages of recommended daily allowance, were calculated and shown in Table 6. Particularly, the nutrient density represent the nutrient calories benefit ratio (NCBR) and, as Renner, Schaafsma, and Scott (1989) reported, can be calculated by the following expression:

$$ND\% = [(N_p/E_p) \cdot (E_r/RDA)] \cdot 100,$$

where N_p is the manganese content in the vegetable expressed in mg in 100 g of product, E_p (kcal) is the energy supplied by the vegetable, RDA (mg) is the recommended daily intake for manganese and E_r (kcal) the recommended energy daily requirement. E_r data were considered for raw and boiled vegetables, reported by Souci, Fachmann, and Kraut (1994), E_r and RDA were considered for an adult and were, respectively, 5 mg/day and 2000 kcal/day (NRC, 1989). The nutrient density percentages were much higher than 100% for all the studied raw and boiled samples, confirming that a sufficient consumption (100 g) of vegetables provide a high intake of manganese respect to the low caloric supply of those aliments (Renner et al., 1989). Of all the studied raw and boiled vegetables, spinach, chicory, broccoli, marrow and string beans provided the highest manganese RDAs percentages (>50%), whereas lettuce the lowest (<10%) followed by carrots, garlic pore mushrooms and peeled potatoes (<20%). As a consequence of the already observed loss of manganese, the reported percentages of RDA values provide evidence that boiling and peeling involve a remarkable decrease of manganese percentages particularly for artichoke, peeled carrots and peeled potatoes.

6. Conclusion

Derivative anodic stripping chronopotentiometry (dASCP) enabled to determine trace manganese levels in a large number of vegetables and to value the effects of boiling and peeling on the content of this micronutrient in the studied samples. Moreover, due to its low costs and its high accuracy and sensitivity, dASCP can be considered as an attractive and valid alternative to atomic absorption spectroscopy for trace and ultra-trace manganese analysis in food matrices.

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