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Trace Element Determination in *Brassica oleraceae* var. *acephale* by Differential Pulse Polarography

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A fast and simple differential pulse polarographic (DPP) method for the determination of trace elements in a certain form of cabbage is described. This vegetable is commonly used as food in Turkey, especially in the Black Sea region. Using DPP polarograms of wet digested cabbage samples (leaf) in pH 2 and 4 acetate buffer Mo, Cr, Se, Pb, As and Zn quantities were determined. The best separation and determination condition for Cu and Fe was in EDTA at pH 6.5, and for Ni and Zn it was ammonia buffer at pH 9.8. The trace element quantities in digested leaf samples were as follows: Se(IV) about 40 μg/g, As(III) 83 μg/g, Cr(III) 23 μg/g, Zn(II) 60 μg/g, Mo(VI) 5 μg/g, Pb(II) 7 μg/g, Fe(III) 3 μg/g and Cu(II) 95 μg/g. Only 3 elements, Cu, Zn and Ni(II), were determined in stalk samples of the same cabbage. Their quantities were very small when compared with those from leaf samples. The Cu(II) content was 0.8 μg/g, Zn(II) was 16 μg/g and Ni(II) was only 0.03 μg/g. This method enabled the determination of heavy trace elements using an inexpensive instrument and without any separation or pre-concentration procedures.

Key Words: Cabbage, cole, trace elements, determination, differential pulse polarography.

Introduction

Trace elements play a very important role in human nutrition. Their quantities in the human body vary so much that in many instances they are essential to life, while in others they are toxic even at very low concentrations. Since they are taken mostly from the diet, their determination in food is very important. The chemical state and concentrations of trace elements in biological material are such that different techniques and methods of analysis are usually required for their determination. Using electrothermal atomic absorption spectrometry (ETAAS), Se in wheat, fruits, fish and meat has been analyzed[1]. Neutron activation techniques have high sensitivity, but they are not frequently used because of the specialized techniques, time and cost involved[2]. Other techniques such as atomic emission spectrometry with inductively coupled plasma excitation (AES-ICP)[3] and X-ray fluorescence (XRF)[4] are very expensive and do not

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offer sufficient sensitivity for accurate determination of trace elements. Electrochemical methods have the advantage that they require relatively inexpensive instrumentation, have demonstrated ability for multi-element determination[5−7] and are capable of determining elements accurately at trace and ultra trace levels[8].

The determination of trace levels of elements in biological and food matrices is usually difficult because of long and tedious procedures and risks of interference problems. The interfering ions have to be separated by preconcentration techniques such as solvent extraction, ion exchange, or hydride generation. These are all time-consuming procedures, and losses of elements are possible. It is therefore very important to accomplish the determination with minimum interference problems. With electrochemical methods, the interference problems can be solved by changing only the supporting electrolytes or the pH. Cauliflower samples have been analyzed for their trace element contents[9] using DPP; the effects of digestion on the recovery of elements have been investigated and it was shown that some elements were lost during digestion. Pb and Cd in vegetables have been determined using ETAAS[10].

Cabbage is a leafy vegetable and is part of the human diet all over the world. There are several varieties of it and a cole known as *Brassica oleraceae* var. *acephale* is one of them. This type of cole grows in the Black Sea region, Mediterranean coast and Southern Europe. It is a main and traditional food in Turkey, especially in the Black Sea region. In one work[11] using energy-dispersive X-ray fluorescence it was stated that no traces of heavy metals were found in the same cole sample but only Fe in a few sample was 0.14 ppm. With a fluorimetric method used for cabbage for a different form it was shown that the Se content was very low[12].

The aim of this work was to determine the elements in a certain type of cabbage, which is a very important vegetable because of its high consumption in the Black Sea region. For this purpose DPP was used and a new method was established. In the proposed method there is no need for sophisticated instruments or tedious separation procedures.

Experimental

Apparatus

For the polarographic measurements a PAR Model 14 A polarographic analyzer system equipped with a PAR mercury drop timer was used. A Kalousek electrolytic cell with a reference saturated calomel electrode (SCE), separated by a liquid junction, was used in a 3-electrode configuration. The counter electrode was platinum wire. The natural drop time of the mercury electrode was 2-3 s (2.2 mg/s). The polarograms were recorded with a Linseis (Selb, Germany) LY 1600 X-Y recorder. DP polarograms were recorded under the conditions of 1 s, a scan rate of 5 mV/ s and a pulse amplitude of 50 mV.

Reagents

All reagents used were of analytical reagent grade (proanalysis). The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany).

Stock standard solutions (0.1 M) of Se(IV), As(III), Zn, Cr(III), Pb, Cu, Fe(III) and Ni(II) were prepared with triply distilled water from their nitrate and sulfate salts. Selenite stock solution was prepared from SeO_2 by dissolving it in hot water. Stock Fe(III) and $NaAsO_2$ solutions were prepared using HCl. As the supporting electrolyte 1.0 M acetate-acetic acid buffer was used and its pH value was changed using

either NaOH or HCl. When needed EDTA was also added. Dilute solutions were prepared before every use to avoid solution degradation.

Procedure

Sampling and digestion

The Brassica type cole, which is commonly used in the Black Sea region as the main food, was collected in samples from the city of Giresun, located on the Black Sea coast. It has long and separate green-blue leaves. They were first separated as leaves and stalks, cut into small pieces after washing and then dried in an oven at about 105 °C until constant weight. About 10 g of it was wet digested in a long-necked 100 mL flask with an acid mixture HNO_3:$HClO_4$ (7:1). First 10 mL of this acid mixture was added and it was left overnight with a glass funnel covering the mouth of the flask. The next day the flask was heated over a flame by turning the flask until all nitrogen oxide fumes were given off. When the digestive sample turned dark brown, 10 mL of $HNO_3$was added after cooling the sample to prevent an explosion. The flask was heated once more until about 2 mL of solution remained and then 10 mL of the acid mixture and 0.5 mL of H_2SO_4 were added and the resulting mixture was heated. Digestion was completed when the solution was clear and approximately 1 mL of solution remained. Finally 2 mL of HCl was added and heated for about 10 min to convert all the Se(VI) to Se(IV). The digested sample was cooled to room temperature, the funnel was rinsed into the flask with water and the contents were transferred into a 10 mL calibrated Teflon flask, making up to the mark with distilled water. The same amounts of acids, after vaporization, had no impurity peak when a polarogram was obtained under the same conditions.

Polarographic determination

A total of 10 mL electrolyte was de-aerated by a stream of nitrogen gas (99.999%) for about 5 min. Polarograms were taken by scanning the potential in the negative direction from 0.0 to –1.5 V, depending on pH, at a scan rate of 5 mV/s. The peak potentials of Se(IV), Fe(III), Cu, Cr(III), Zn, Ni and Pb, which are commonly found in foodstuffs, were determined at different electrolytes in the presence and absence of EDTA. The polarogram of the digested sample was taken under various conditions and the trace elements in the sample were determined by standard additions.

Results and Discussion

The Brassica-type cole plant was digested after separating the leaves and stalks, and then both of them were analyzed separately. The polarograms of samples were taken at different pH values and with different electrolytes to prove the existence of ions and validate their quantities.

Trace elements in leaf samples

As given in the digestion procedure, about 10 g of sample was digested and after evaporation of acids it was diluted into 10 mL. The polarogram was taken after the addition of 0.1-0.5 mL of this sample solution into the polarographic cell, which contained 10 mL of supporting electrolyte. The volume of the sample depends on the concentration of element under consideration. A polarogram of leaves at pH 2 in acetate buffer is given in Figure 1 as an example. The current observed at about zero potentials may belong both to copper and partly to iron; they cannot be obtained separately at this pH. As can be seen there is a broad peak

between -0.2 and -0.36 V, which belongs to the reduction peak of Mo(VI). There are peaks at -0.55, -0.73, -0.90 and -1.09 V. According to our preliminary studies in the same medium the peak at -0.55 V belongs to Se(IV), the peak at -0.73 V to As(III), the peak at -0.90 V to Cr(III) and the peak at -1.09 V to zinc. Their existence has been proven by standard additions and by taking polarograms in different electrolytes. From the polarograms taken in 0.1 M HAc-Ac^- solution at pH 2, the quantities of these elements were calculated with the standard addition method. The content of Se(IV) was 40 ± 3 μg/g, As(III) was 83 ± 7 μg/g, Cr(III) was 23 ± 3 μg/g and Zn was 60 ± 5 μg/g (the results are given per gram of dry sample).

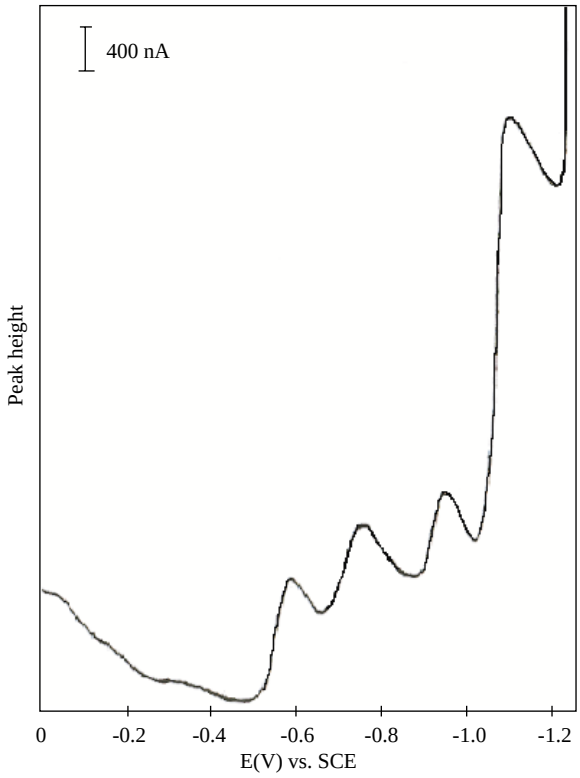

Figure 1. DPP polarogram of cabbage leaf sample: 10 mL 1 M acetic acid, acetate solution, pH 2, +0.2 mL digested sample.

The polarogram of the same leaf sample taken at pH 4 in the same electrolyte is given in Figure 2. There is a broad peak at about –0.12 V, which belongs to Fe and Cu, but they cannot be separated at these low pH values. According to our previous studies[9], the best medium for their determination was EDTA at pH values higher than 6. This medium has been used for the separation and determination of Cu and Fe in cabbage samples. There were 2 very small peaks at pH 4 (Figure 2); the first, at –0.38 V, belongs to Mo(VI), and the second, at -0.42 V, to Pb. Because of the large Zn peak the sensitivity had to be low and thus these peaks were too small to determine under this current condition. (They were determined under higher sensitivity). The peak at –0.74 V belongs to Se, the peak as a shoulder at –0.83 V to As, the peak at –0.92 V to Cr and the peak at –1.05 V to Zn. The quantities of Mo(VI), Pb, Se(IV), Cr(III) and Zn were determined in this medium by the standard addition method. The quantities found were 5 ± 1 μg/g for Mo(VI), 41 ± 3 μg/g for Se(IV), 20 ± 3 μg/g for Cr and 54 ± 5 μg/g for Zn. Because of its small peak as a shoulder at about –0.83 V, As(III) could not be determined accurately at pH 4.

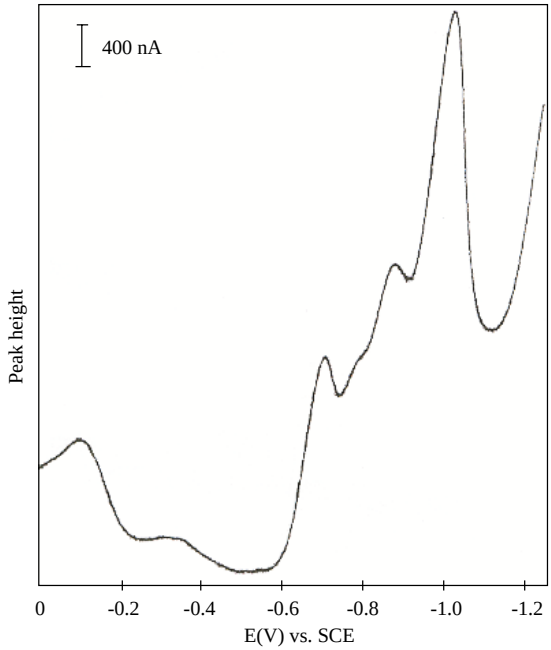

Figure 2. DPP polarogram of cabbage leaf sample: 10 mL 1 M acetate buffer, pH 4, + 0.2 mL digested sample.

Determination of Cr(III) was possible both in pH 2 and 4 medium since they could be observed as separate peaks without interference (Figures 1 and 2) As shown in Figure 3 the Cr peak in pH 4 medium was observed at –0.92 V; although the peak is not as sharp as in pH 2 its quantity has been determined from this peak. The results obtained for both conditions (pH 2 and 4) were quite similar, while it was 22 ± 3 μg/g at pH 2, and 20 ± 4 μg/g at pH 4.

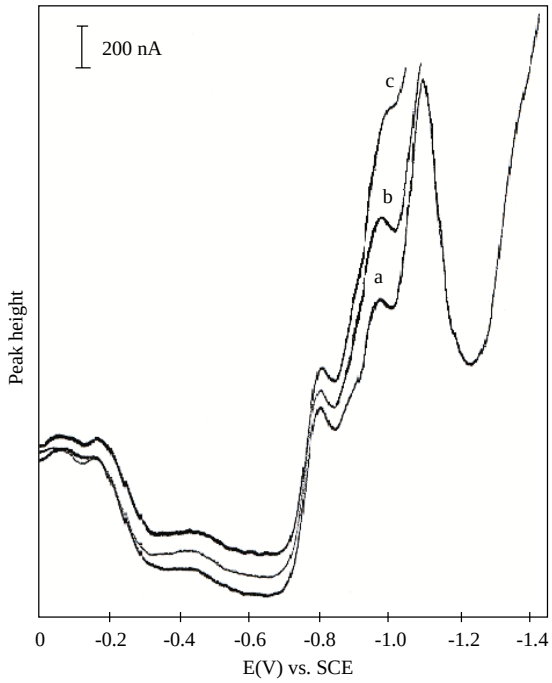

Figure 3. Determination of Cr in cabbage leave sample: (a) 10 mL 1 M acetate buffer, pH 4, + 0.2 mL sample, (b) a + 0.05 mL 10^{-3} M Cr(III), (c) (b) + 0.05 mL 10^{-3}M Cr(III).

As mentioned above, Mo(VI) and Pb peaks in Figure 2 were small, since the polarogram had to be taken at lower sensitivities. It was possible to determine them using higher sensitivities and larger sample volumes. After digestion the sample contained white precipitate because of oversaturation of some salts, $KClO_4$ and $PbSO_4$. Since sulfuric acid was used during digestion, some of the Pb ion was in the form of $PbSO_4$ precipitate in the sample solution. Thus, the sample solution was first heated up to dissolve the precipitate of $PbSO_4$ and then the Pb peak could be observed and determined. As can be seen, care has to be taken when H_2SO_4 is used for digestion. For the determination of Pb, polarograms were taken under 2 conditions and consistency was observed between them. Pb content found in pH 4 buffer with a peak at –0.40 V was 7 μg/g and at pH 6 with a peak at –0.46 V was 8 μg/g.

The determination of Fe and Cu was performed in EDTA solution at pH 6.5. At lower pH values and without EDTA, Fe and Cu peaks are near to 0 V, even at positive potentials, and thus they cannot be separated and determined. At about pH 4, it is possible to determine Cu but in this case Fe is still about 0 V. The best medium according to our previous work [9] and to our preliminary studies in the same sample medium was found as pH 6.5 in EDTA. The polarogram of leaf sample for the determination of Fe(III) taken in this medium is given in Figure 4. To obtain a peak for Fe, the sensitivity had to be 8 times larger than that needed for Cu. Because of the high sensitivity the standard additions had to be made with 10-fold smaller concentrations of Fe than usual. The Cu peak was off the scale since it was much larger than the Fe peak. Determination of Cu was performed in the same medium, but at lower sensitivity, as given in Figure 5. Calculations showed that while Cu content was 95 ± 5 μg/g Fe content was only 3 μg/g.

The results obtained for trace element quantities in leaf samples are summarized in the Table.

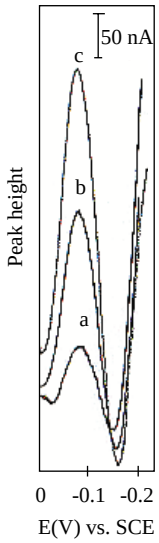

Figure 4. Determination of Fe in cabbage leaf sample: (a) 10 mL sodium acetate, pH 6.5 + 2 mL 0.1 M EDTA + 0.2 mL sample, (b) (a) + 0.02 mL 10^{-3} M Fe(III), (c) (b) + 0.02 mL 10^{-3}M Fe(III).

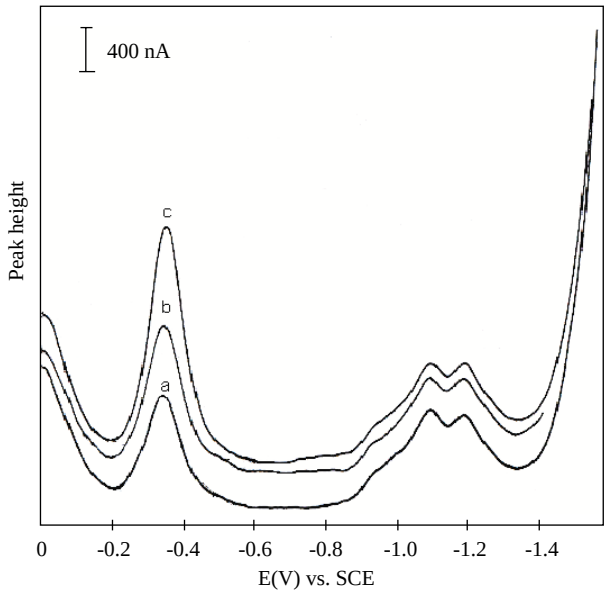

Figure 5. Determination of Cu in cabbage leaf sample: (a) 10 mL sodium acetate, pH 6.5, 1.0 mL 0.1 M EDTA + 0.2 mL sample (b) (a) + 0.1 mL 10^{-3}M Cu(II) (c) (b) + 0.1 mL 10^{-3}M Cu(II).

Table. Trace element quantities in cabbage leaf and stalk samples[a].

Medium	Se	As	Cr	Zn	Mo	Pb	Fe	Cu	Ni
Acetate Buffer* (pH 2)	40 ± 3	83 ± 7	22 ± 3	60 ± 5	-	-	-	-	-
Acetate Buffer* (pH 4)	41 ± 3	-	20 ± 3	54 ± 5	5 ± 1	7 ± 1	-	-	-
Acetate Buffer + EDTA* (pH 6.5)	-	-	-	-	-	8 ± 1	3 ± 1	95 ± 5	-
Acetate Buffer** (pH 4)	-	-	-	17 ± 3	-	-	-	-	-
Ammonia Buffer** (pH 9.8)	-	-	-	16 ± 2	-	-	-	0.8 ± 0.1	0.03 ± 0.01

[a] μgg^{-1}, $x \pm t\ x\ s\sqrt{}\ n$, t: confidence interval, 90% ($n = 4$), *leaf, ** stalk

Trace elements in stalk samples

The stalks of the cabbage were analyzed separately. About 10 g of the stalks was digested as given in the digestion procedure. The polarogram taken with a 0.4 mL sample at pH 4 had a peak only at -1.03 V, which belongs to Zn. Zn content was determined with standard additions and it was 17 ± 2 μg/g. Since there was also a small current at pH 4 where Cu existed, the pH was adjusted to 6.5 in the presence of EDTA, and Cu content was determined. As expected, the quantity of Cu was very small and thus it could be determined only by using a 1 mL sample and by using high sensitivity. It was 0.8 μg/g by standard additions.

According to our preliminary work [13], Ni content can easily be determined in NH_3buffer at about pH 9.8. The polarogram of the cabbage stalk sample taken in this medium showed 2 peaks, a very small peak at about –1.1 V and a large peak of Zn at –1.34 V, which was off the scale. For the determination of Ni the sample solution had to be increased (1 mL instead of 0.1 mL). Ni content was so small that its peak

could only be observed at the highest possible sensitivity of the instrument. Thus, the standard additions of Ni had to be made using 10^{-4} M solution, instead of the usual 10^{-3} M. The Ni content was 0.03 μg/g.

It was possible to determine the quantity of zinc from 2 different polarograms, the first in acetate buffer at pH 4 and the second in ammonia buffer at pH 9.8. The results were very similar, namely 17 $\pm$ 3 μg/g at pH 4 and 16 $\pm$ 2 μg/g at pH 9.8. The results are summarized in the Table together with the results obtained for the leaf samples for comparison. The trace element concentrations in stalk samples are quite low when compared with the leaves. The accuracy of the results was confirmed by measuring the element quantities in different conditions and with a synthetic sample containing similar quantities of ions present in cabbage. Their quantities in the synthetic sample were found with relative errors changing between +5% and -12% , depending on the concentration.

Conclusion

The present differential pulse polarographic method, which was used for the first time for the determination of heavy elements in cabbage, enabled the direct determination of their concentration without any separation or pre-concentration techniques. Electroanalytical methods are quite simple, the sensitivity and selectivity is high, and the instruments used are inexpensive. It is possible to determine the concentration of at least 5 to 6 elements simultaneously. With the proposed method it was possible to determine Cu, Fe, Pb, As, Cr, Ni and Zn very accurately, without any separation or extraction procedure. The results for digested leaf samples were Se(IV) about 40 μg/g, As(III) 83 μg/g, Cr(III) 23 μg/g, Zn(II) 60 μg/g, Mo(VI) 5 μg/g, Pb(II) 7 μg/g, Fe(III) 3 μg/g and Cu(II) 95 μg/g. The accuracy of the results was confirmed by measuring the element quantities in different conditions and with a synthetic sample containing similar quantities of ions present in cabbage with a relative error changing between + 5% and -12%, depending on the concentration. This method can be applied to biological samples containing complex mixtures of metal ions.

Acknowledgments

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