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COMPARISON OF IODINE DETERMINATION IN SPINACH USING 2% CH_3COOH AND TMAH

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Abstract. Tetramethylammonium hydroxide (TMAH) is the compound most commonly applied for iodine determination in environmental samples but, at the same time, is very harmful for human health when used as an analytical reagent. For that reason, it is desirable to seek for alternative, equally rapid and easy-to-perform (but requiring less hazardous chemicals) methods of iodine analysis in environmental samples on the same detection level. The aim of the research was to assess the applicability of iodine determination in spinach after incubation of plant samples with 2% acetic acid in comparison with standard analytical procedure using solution of TMAH (tetramethylammonium hydroxide). Studies were conducted on spinach samples from two vegetation experiments carried out in pots and field including soil fertilization and foliar application of diverse iodine doses in the form of KI and KIO_3. Obtained results indicated a relatively high usefulness of sample incubation with 2% acetic acid for iodine determination in spinach plants. Still, the statistical significance of the relation (defined with the use of correlation coefficients) between iodine content determined in TMAH (*x* variable) and 2% acetic acid (*y* variable) was primarily influenced by iodine form, dose and method of its application during plant cultivation. In the pot experiment, values of correlation coefficients between tested variables were statistically significant and equal to r = 0.66. In the field study, values of correlation coefficients (between *x* and *y* variables) for plants with foliar application of KI and KIO_3 were statistically significant and equal to r = 0.99 and r = 1.00, respectively. Combined comparative analysis of data obtained in both experiments revealed that correlation between tested variables was statistically significant and its coefficient was equal to r = 0.80. Mean iodine recovery from fortified samples after incubation with 2 % acetic acid was 91.1% ±17.7% (n = 15), whereas using TMAH – 89.3% ±30.7 (n = 15).

Key words: iodine determination, TMAH, 2% acetic acid, biofortification, spinach

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INTRODUCTION

One of the issues considered in the research on functional food production is plant biofortification in elements essential for human health, including iodine [White and Broadley 2005, Blasco et al. 2008].

Iodine determination in food and environmental samples remains difficult due to chemical characteristics of the element, such as its instability in water solutions. Classic colorimetric methods of iodine assessment (using ammonium-cerium-sulfate and sodium arsenite) enable its determination on the level of few μg dm^{-3}. On the other hand, in spite of its high sensitivity, these methods are usually time-consuming and troublesome to perform. Sample preparation (including laboratory vessel preparation, dry mineralization and digestion of samples) as well as iodine analysis using these methods last a couple of days [Barker et al. 1951, Górski and Bobek 1960, Kołczak and Bobek 1964]. Apart from this procedure, other colorimetric methods of iodine determination are applied for sea water [Agrawal et al. 1999] or liquid environmental samples using FIA [Chen et al. 1991, Tanaka 1985, Kamavisdar and Patel 2002]. Alternative relatively simple analytical techniques of iodine detection in environmental samples include: ionometric [Rashed 1995, Mackowiak et al. 2005] or kinetic-catalytic methods [Tomiyasu et al. 1994]. The latter ones allow to determinate the total iodine as well as IO_3^- form occurring in plant tissues. On the other hand, an objective analysis of iodine content using the above-mentioned method is available only for low concentrations of iodine (few μg I dm^{-3} at most).

In the instrumental analysis of iodine determination the following techniques are applied: total-reflection X-ray fluorescence (TXRF) [Varga 2007], neutron activation analysis (NAA) [Shinonaga et al. 2001], ion chromatography [Dai et al. 2006], capillary electrophoresis [Huang et al. 2004], X-ray absorption near-edge structure (XANES) [Yamaguchi et al. 2006], gas chromatography-mass spectrometry (GC-MS) [Shin et al. 1996, Fuse 2003] and radioactive analysis [Ashworth et al. 2003]. In laboratory practice, spectrometric methods are also applied for iodine detection in environmental samples: AAS [Blasco et al. 2008], ICP-OES (ICP-AES) [Naozuka et al. 2003, Varga 2007], ICP-MS [Fecher et al. 1998, Glina et al. 1998], ICP-IDMS [Rädlinger and Heumann 1998] and HPLC ion chromatography coupled with ICP-MS (IC-ICP-MS) [Stärk et al. 1997]. It should be mentioned that all of the presented techniques require different procedures of sample preparation as well as optimization of measurement conditions for a particular apparatus.

The main advantage of spectrometric techniques (ICP-OES, ICP-MS) is the possibility of iodine determination in wide range of its concentration. Thus, it facilitates iodine analysis in plant samples characterized by either low or high content of this element (i.e. in plants treated with soil fertilization or foliar application of iodine) using the same analytical method. One of the easiest and most popular techniques of sample preparation is based on the alkaline extraction with tetramethylammonium hydroxide solution (TMAH) [Fecher et al. 1998, Rädlinger and Heumann 1998]. This method, included in the project of European Norm [prEN 15111- R2-P5-F01, 2006], was a point of reference to the research presented in the current study. TMAH is a toxic compound posing a serious risk to human health when using as an analytical reagent. For that rea-

son, it is desirable to seek for alternative, equally rapid and easy-to-perform (but requiring application of the less hazardous chemicals) methods of iodine determination in environmental samples on the same detection level.

In Polish studies on fertilization requirements of plants, sample extraction with 2% acetic acid is commonly used for quick determination of soluble forms of macro elements in plants [Dzida 2004, Kowalska 2004, Lis-Krzyścin 2006, Nowosielski 1974].

The aim of this work was to evaluate the applicability of iodine determination in spinach leaves after incubation with 2% acetic acid using ICP-OES spectrometer as an alternative sample preparation method to the standard analytical procedure using TMAH.

MATERIAL AND METHODS

Plant culture. Studies were carried on leaves of spinach (*Spinacia oleracea* L.) cultivated in field and pots in Experimental Site of the University of Agriculture in Cracow. The subject of this research included differentiated soil fertilization and foliar application of iodine in the form of KI and KIO_3. Prior to spinach sowing, NPK fertilization was performed to supplement nutrient content to the optimal level for spinach cultivation on the basis of soil chemical analyses [Sady 2006].

In years 2006–2007 spinach 'Spinaker F_1' was cultivated in containers placed in an open field under a shade-providing fabric. In the experiment, which was conducted according to the split-plot method, soil fertilization and foliar application of iodine in the form of KI and KIO_3 were applied. Soil fertilization with KI and KIO_3 was carried out before seed sowing to the level of 15 mg I dm^{-3} soil. Foliar application of KI and KIO_3 was performed in a four-leaf stage using 0.2% iodine solution (concentration per pure ingredient) in a dose of 400 ml m^{-2} (approximately 0.8 g I m^{-2}).

In 2007 spinach 'Spiros F_1' was cultivated in field experiment designed according to the split-plot method. The study included two foliar applications of iodine in the form of potassium iodide (KI) and potassium iodate (KIO_3) in the following concentrations (per pure ingredient): 0, 0.05%, 0.1%, 0.2% and 0.3% in a dose of 50 ml m^{-2} (500 dm^3 ha^{-1}). Total amount of iodine introduced with two applications in each combination was approximately: 0, 0.05, 0.1, 0.2 and 0.3 g of iodine per m^2, respectively.

Plant material analysis. Shredded fresh spinach leaves were dried at 70°C and ground in a laboratory mill (Fritsch Pulversette 14) using 0.5 mm sieve. Iodine content was determined in dry material after incubation with 25% TMAH or 2% acetic acid by ICP-OES using high-resolution spectrometer Prodigy Teledyne Leeman Labs USA. Measurements were performed using 206.163 nm spectra line, SeaSpray nebulizer and cyclonic knockout chamber. The optimized analytical values were 1.3 kW for RF power, 35 psi for nebulizer pressure of argon, 0.9 dm^3 min^{-1} for auxiliary argon gas flow, 1.4 ml min^{-1} for sample uptake rate. The default value of 18 dm^3 min^{-1} was used for argon coolant gas flow rate. Each sample was analyzed twice in three replicates for 15 s in axial mode.

Sample incubation with TMAH. Sample preparation was performed according to the project of the European Norm (prEN 15111- R2-P5-F01, 2006) for ICP-MS. The

following procedure was applied: 0.5 g of grounded air-dried sample was set in 25 ml polypropylene tubes. 5 ml of redestilled water as well as 1 ml of 25% TMAH (Fluka no. 87729) were added, tubes were tightly closed and incubated for 3 hours in 90°C. Immediately after the end of incubation hot samples in tubes were mixed by vortex and centrifuged at 8000 g for 15 min. After centrifugation, supernatants were filtered through quality filter discs to 25 ml volumetric flasks. Sediments in tubes were rinsed with 5 ml of redistilled water, mixed by vortex and centrifuged again at 8000 g for 15 min. After centrifugations, supernatants were combined with the previous ones. Filter discs were rinsed with redistilled water to reach the final volume of 25 ml.

Sample incubation with 2% acetic acid. The experimental procedure of sample preparation with 2% acetic acid was performed according to the following procedure: 0.5 g of grounded air-dried sample was set in 25 ml polypropylene tubes. 5 ml of 2% acetic acid was added; tubes were tightly closed and incubated for 3 hours in 90°C. Immediately after the end of incubation hot samples in tubes were mixed by vortex and centrifuged at 8000 g for 15 min. After centrifugation, supernatants were filtered through quality filter discs to 25 ml volumetric flasks. Sediments in tubes were rinsed with 5 ml of 2% acetic acid, mixed by vortex and centrifuged again at 8000 g for 15 min. After centrifugations supernatants were combined with the previous ones. Filter discs were rinsed with 2% acetic acid to reach the final volume of 25 ml.

In order to verify the correctness of iodine determination after incubation with TMAH or 2% acetic acid, sample fortification was applied in the amount of 2 mg I per 1 dm^3 of solution measured on ICP-OES spectrometer. Due to small amount of available plant material, study on iodine recovery was performed on 15 samples (after incubation with TMAH and 2% acetic acid) from diverse combinations – control plants as well as plant biofortified with this element through soil fertilization and foliar nutrition.

Statistical calculations. Statistical calculations of obtained results were performed with the use of Statistica 8.0 PL program for $P < 0.05$. Mean iodine content, correlation coefficients as well as regression equations were calculated for the relation between iodine concentration in spinach after incubation with TMAH (x variable) and the content of this element determined using 2% acetic acid (y variable).

RESULTS AND DISCUSSION

Studies presented in this work were preceded by preliminary trials of iodine determination using colorimetric method with ammonium-cerium-sulfate and sodium arsenite [Barker et al. 1951, Górski and Bobek 1960, Kołczak and Bobek 1964]. Appropriate sample dilution directly after mineralization was, however, a major difficulty when applying this method. As an effect, it was frequently necessary to re-prepare samples which was time-consuming and produced differentiated results of iodine determination in the same samples (detailed data not presented).

Works were also taken up on adapting the standard method of plant sample extraction with 2% acetic acid in spinach. This procedure consists of 30-minute extraction (shaking) of 0.5–1 g sample of air-dried plant material with 100 ml of 2% acetic acid in room temperature [Nowosielski 1974]. Still, this method of sample preparation did not

prove successful as iodine content in the extracts was below the limits of its detection using ICP-OES spectrometer (detailed data not presented). In such a case, sample incubation with 2% acetic acid according to the method presented in the Materials and Methods chapter was carried out in order to determine iodine content in spinach plants. In the available literature on methods and analytical techniques of iodine determination (widely presented in the Introduction) no information can be found concerning the possibility of iodine determination (as well as other trace elements) in that extraction medium using ICP-OES technique. Thus, an objective discussion of the presented results with other studies is impossible. It is worth to mention that 2% solution of acetic acid can be used as background electrolyte for iodine analysis with capillary electrophoresis [Michalke 1999].

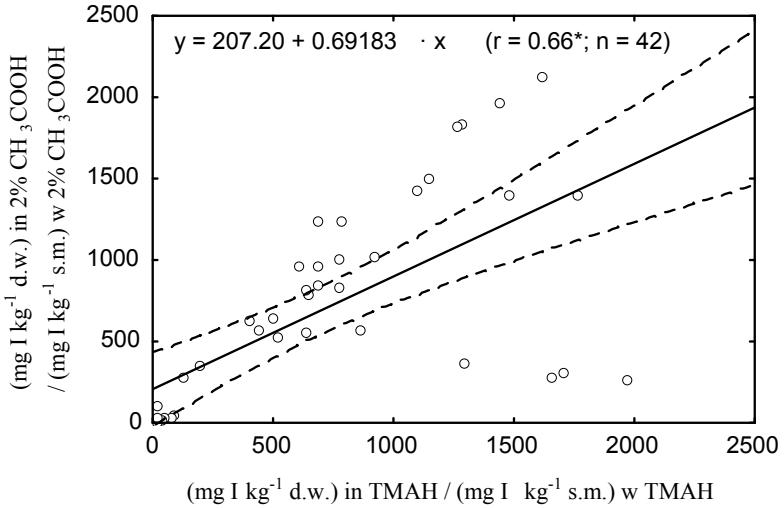

Fig. 1. The scatter graph for iodine content in spinach (determined in TMAH versus 2% CH_3COOH) in pot experiment – results from 2006–2007 for $P < 0.05$.

Ryc. 1. Wykres rozrzutu zawartości jodu w szpinaku (oznaczonego w TMAH versus zawartość jodu oznaczanego w 2% CH_3COOH) uzyskany w doświadczeniu wazonowym – wyniki z lat 2006–2007 dla $P < 0,05$.

In the pot experiment, iodine concentration in spinach assayed in 2% CH_3COOH was slightly higher than its values detected using TMAH (Table 1). Similar relation was found for plants with foliar application of KIO_3 grown in field. Adversely, in the case of foliar nutrition with KI, iodine content determined after incubation with TMAH was higher than in 2% acetic acid. Particularly interesting was the relation between iodine content assayed in TMAH (*x* variable) and 2% acetic acid (*y* variable). In the pot trial the value of correlation coefficient (r) for tested variables was equal to r = 0.66 (Fig. 1). Similarly, in the field experiment correlation coefficients for group of plants treated with KI or KIO_3 were statistically significant and equal to r = 0.99 and r = 1.00, respec-

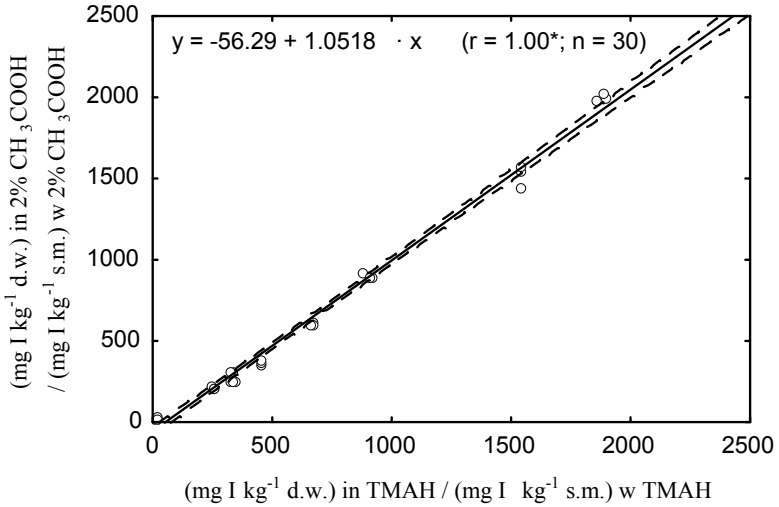

Fig. 2. The scatter graph for iodine content in spinach (determined in TMAH versus 2% CH_3COOH) in field experiment for $P < 0.05$.

Ryc. 2 Wykres rozrzutu zawartości jodu w szpinaku (oznaczonego w TMAH versus zawartość jodu oznaczanego w 2% CH_3COOH) uzyskany w doświadczeniu polowym dla $P < 0,05$.

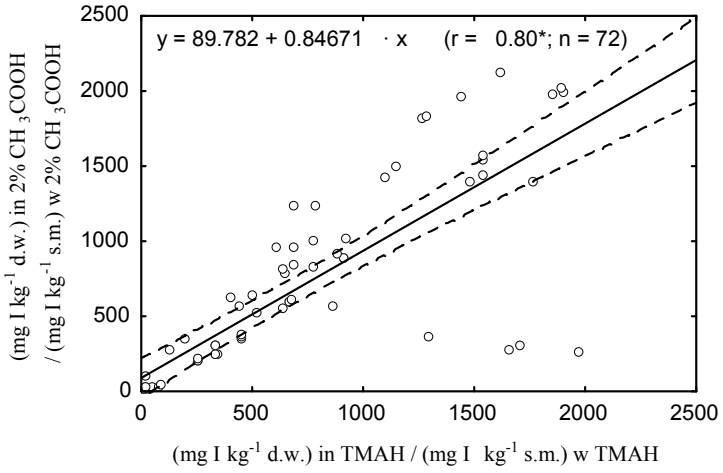

Fig. 3. The scatter graph for iodine content in spinach (determined in TMAH versus 2% CH_3COOH) – summarized results from pot and field experiments for $P < 0.05$.

Ryc. 3 Wykres rozrzutu zawartości jodu w szpinaku (oznaczonego w TMAH versus zawartość jodu oznaczanego w 2% CH_3COOH) – sumaryczne wyniki z doświadczenia wazonowego i polowego dla $P < 0,05$.

tively (Table 1). Correlation coefficient calculated for all spinach samples from the whole field experiment (foliar application of both forms of iodine - KI and KIO_3) was 1.00 (Fig. 2). Comparative analysis of data derived from both trials revealed that correlation coefficient between variables *x* and *y* was statistically significant: r = 0.80, P < 0.05 (Fig. 3).

Table 1. Results of iodine determination in spinach
Tabela 1. Wyniki oznaczenia jodu w szpinaku

Mean iodine content in spinach Średnia zawartość jodu w szpinaku	Concentration of iodine (mg I kg^{-1} d.w.) determined in: Zawartość jodu (mg I kg^{-1} s.m.) oznaczona w:		Correlation coefficient (r) *x* versus y Współczynnik korelacji (r) *x* wersus y
	TMAH *x* variable – zmienna *x*	2% CH_3COOH *y* variable – zmienna *y*	
In pot experiment W doświadczeniu wazonowym	640.8	668.9	0.66*
In field experiment: W doświadczeniu polowym:			
– plants with foliar application of KI dla roślin dokarmianych dolistnie KI	345.7	288.5	0.99*
– plants with foliar application of KIO_3 dla roślin dokarmianych dolistnie KIO_3	935.3	946.3	1.00*

* correlation coefficient significant for P < 0.05 – * współczynnik korelacji istotny dla P < 0,05.

Differentiated values of correlation coefficient in field and pot experiments indicate that the strength and statistical significance of the relation between tested variables depended on iodine form, its dose and method of application in vegetation experiments with spinach cultivation. Comparative analysis of results obtained in both trials suggests that the applicability of 2% acetic acid for iodine determination in spinach was better in the case of plants treated with higher doses of iodine, especially when applied foliarly.

It should be mentioned that mean iodine recovery from fortified samples after its incubation with 2% CH_3COOH was 91.1% ±17.7% SE (n = 15) while for samples incubated with TMAH – 89.3% ±30.7 SE (n = 15). Obtained results demonstrated a relatively high usefulness of sample incubation with 2% acetic acid for iodine determination in spinach. A relatively high value of standard error obtained for iodine recovery could have been caused by the fact that these analyses were performed on samples derived from both the control plants as well as spinach enriched with I. In the latter one, iodine content was from several dozen to several hundred times higher than in the control.

CONCLUSIONS

Presented studies revealed a relatively good applicability of plant sample incubation with 2% acetic acid for iodine determination in spinach. The applicability increased along with increasing iodine dose applied to plants – particularly through foliar nutrition.

Sample incubation with 2% CH_3COOH can be defined as an alternative method of sample preparation of spinach plants or other leafy vegetables for iodine determination.

Sample incubation with 2% CH_3COOH can be particularly useful for iodine analysis in biofortified plants with recommended iodine concentration within the range of 500 to 1300 mg I kg^{-1} d.w.

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PORÓWNANIE OZNACZENIA JODU W SZPINAKU ZA POMOCĄ 2% CH_3COOH ORAZ TMAH

Streszczenie. Najczęściej do oznaczenia jodu w próbach środowiskowych wykorzystuje się tetrametyloaminę (TMAH) – związek szkodliwy dla zdrowia i życia człowieka. Dlatego też otwartą kwestią jest poszukiwanie innych, równie szybkich i łatwych do wykonania (ale mniej szkodliwych dla zdrowia człowieka niż TMAH) metod umożliwiających porównywalne oznaczenie jodu w próbach środowiskowych. Celem badań było określenie przydatności oznaczenia jodu w szpinaku po inkubacji prób z 2% kwasem octowym w porównaniu do standardowej procedury analitycznej z TMAH (tetramethylammonium

hydroxide). Badaniami objęto próby szpinaku z dwóch doświadczeń wegetacyjnych (wazonowego i polowego) obejmujących zróżnicowane pod względem dawki nawożenie doglebowe i dokarmianie dolistne roślin jodem w formie KI i KIO_3. Badania wykazały stosunkowo dobrą przydatność inkubacji prób z 2% kwasem octowym do oznaczania zawartości jodu w szpinaku. Jednakże na statystyczną istotność zależności (współczynników korelacji), pomiędzy zawartością jodu oznaczanego w TMAH (zmienna *x*) i w 2% kwasem octowym (zmienna *y*), decydujący wpływ miała forma, dawka i sposób aplikacji jodu podawanego roślinom w czasie uprawy. W doświadczeniu wazonowym wartość współczynnika korelacji pomiędzy zmiennymi była statystycznie istotna i wynosiła r = 0,66. W doświadczeniu polowym wartości współczynników korelacji (pomiędzy zmienną *x* i *y*) dla grupy roślin dokarmianych dolistnie KI oraz KIO_3 były statystycznie istotne i wynosiły odpowiednio r = 0,99 i r = 1,00. Łączna analiza porównawcza dla danych z obydwu doświadczeń wykazała, że współczynnik korelacji pomiędzy zmienną *x* i *y* był istotny statystycznie i wynosił r = 0,80. Średni odzysk jodu z prób fortyfikowanych po inkubacji z 2% kwasem octowym wyniósł 91,1% ±17,7% (n = 15), a po inkubacji z TMAH kształtował się na poziomie 89,3% ±30,7 (n = 15).

Słowa kluczowe: oznaczanie jodu, TMAH, 2% kwas octowy, biofortyfikacja, szpinak

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