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INFLUENCE OF MICROWAVE FIELD ON THE ASCORBIC ACID CONTENT IN LEAVES OF SOME COMMON AROMATIC PLANTS IN ROMANIA

MANUELA STAN[a], MARIA LOREDANA SORAN[a]*, CODRUŢA VARODI[a], ILDIKO LUNG[a]

ABSTRACT. The present work reports the variation of the ascorbic acid content in leaves of parsley, dill and celery plants grown in microwave fields of two microwave frequency domains: GSM (mobile communication) and WLAN (wireless internet connection). The percentage increase in ascorbic acid content of irradiated plants reported to control plants was calculated. The ascorbic acid was identified and quantified by high-performance liquid chromatography (HPLC) and electrochemical methods. The experiments were performed on Grace Alltima C18 column (100 x 3 mm, 3 µm) thermostated at 30°C with gradient elution. The mobile phase used for chromatographic separation consists of 15 mM potassium phosphate buffer at pH 2.7 and methanol. Electrochemical experiments employed chronoamperometry using a typical three-electrode electrochemical cell. Amperometric measurements were carried out under magnetic stirring using 0.1 M phosphate buffer solution (pH 6.8) as supporting electrolyte. Our study showed different percentage increases in ascorbic acid content of microwave irradiated plants determined by HPLC and electrochemical method, respectively, with lowest values (6.8%, 11%) in parsley irradiated with WLAN frequency microwaves, and highest values (more than 200%) in celery irradiated with GSM frequency microwaves.

Keywords: *ascorbic acid, HPLC, chronoamperometry, microwave effects on plant growth, aromatic plants*

INTRODUCTION

Within the last years, there was an exponential increase in the use of mobile phones and wireless devices, causing an increased level of electromagnetic radiation that can potentially affect all living organisms [1; 2]. Consequently, many scientific reports are focused on the germination process

[a] *National Institute for Research and Development of Isotopic and Molecular Technologies, 65-103 Donath, 400293 Cluj-Napoca. Romania, * loredana.soran@itim-cj.ro*

as well as the development stages of the plants after their exposure to various magnetic fields [3-6]. As a result of their studies, authors concluded that seeds germinate quickly under magnetic field influence, and the plant roots are thicker, longer and exhibit a larger area depending on the exposure time and magnetic field strength [4]. Parsley (Petroselinum crispum), dill (Anethum graveolens) and celery (Apium graveolens), leafy vegetables belonging to the Umbelliferae (Apiaceae) family and among the most frequently used culinary plants growing in Romania, are important sources of minerals, vitamins (e.g. vitamins B, C, E), and other components such as carotenoids, chlorophylls and polyphenols [7, 8].

Vitamin C, also known as ascorbic acid (AA), L-ascorbic acid or L-ascorbate, is a valuable vitamin for human organism. Ascorbic acid is easily oxidized to form dehydroascorbic acid (DHAA), oxidation being reversible [9]. Besides its role to prevent scurvy, many other health benefits have been attributed to ascorbic acid such as antioxidant, anti-carcinogenic, immunomodulator and cold prevention [10]. Experimental studies have shown that ascorbic acid has an essential role in plant growth [11, 12].

Ascorbic acid (vitamin C) is an essential vitamin for human nutrition, thus knowing its content in diverse food sources and how it is affected by external factors has become an important research topic. Different studies reported the change in the ascorbic acid content in plants under the influence of various factors such as genotypic differences, preharvest and postharvest factors [13], drought stress [14, 15], temperature increase [16], ozone stress [17, 18], electromagnetic radiations [19, 20]. It was shown that stress factors affect different physiological and biochemical parameters of plants and activate plant defense mechanisms through their antioxidant defense systems [17].

The aim of the present study was to investigate the effect of microwaves in the GSM (mobile communication) and WLAN (wireless internet connection) frequency domains, on the ascorbic acid content in leaves of parsley, dill and celery. Our study consists of a comparative evaluation of the experimental results obtained for irradiated and control (non-irradiated) plants by HPLC and electrochemical methods, respectively.

RESULTS AND DISCUSSION

HPLC method

The HPLC method coupled with diode array detection (HPLC-DAD) was developed for identification and quantification of ascorbic acid in parsley, dill and celery leaf extracts. The ascorbic acid was quantified by external standard method following the validation protocol described in a previous work

[21]. The HPLC detection was carried out at 243 nm. In a previous work [21], the retention time of ascorbic acid in plant extracts was found to be 1.85 min. The regression equation was expressed as y = 6E+07x - 9E+06 (where x–concentration, y–area), and the coefficient of correlation (R^2) was 0.9986. The limit of detection (0.2 μg/mL) and the limit of quantification (0.22 μg/mL) were determined by a method previously described [22]. Working ascorbic acid standard solutions in the concentration range 0.3 – 1 μg/mL were prepared by successive dilutions with ultrapure water from a stock solution of ascorbic acid (1 μg/mL).

Overlay of HPLC chromatograms of the extracts (the peaks corresponding to ascorbic acid) registered for irradiated and non-irradiated plants have shown an increase in the ascorbic acid content of plants grown in microwave fields (Fig. 1).

Quantitative determinations were performed in order to establish the percentage increase (%).The amount of ascorbic acid per 100 g of fresh plant was determined for both irradiated and non-irradiated plants (Table 1).

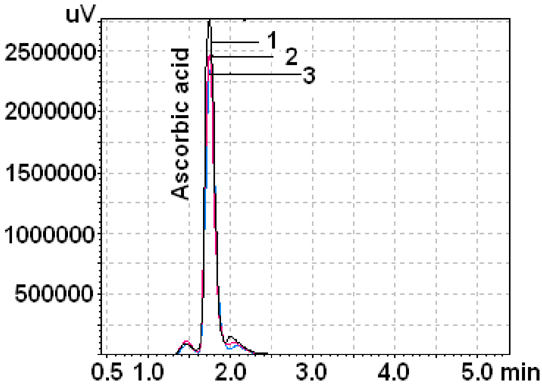

Figure 1. HPLC chromatograms of celery extracts. GSM-irradiated (1), WLAN-irradiated (2), and non-irradiated (3).

Table 1. The amount of ascorbic acid (AA) and the percentage increase (+%) reported to control plants determined by HPLC method.

Plant lot	Ascorbic acid content (mg AA/100 g plant) ± RSD (%)		
	Parsley	Dill	Celery
M1[a]	285 ± 0.78 (+8%)	201 ± 0.63 (+66%)	320 ± 0.56 (+211%)
M2[b]	282 ± 0.50 (+6.8%)	180 ± 0.95 (+49%)	290 ± 1.03 (+181%)
R[c]	264 ± 1.32	121 ± 1.41	103 ± 1.51

[a] plants irradiated with GSM frequency microwaves; [b] plants irradiated with WLAN frequency microwaves; [c] control plants.

For non-irradiated (control) plants, the comparative evaluation of experimental data showed the highest amount of ascorbic acid in parsley.

In irradiated plants, a greater increase in levels of ascorbic acid was determined in plants irradiated with GSM microwaves compared to WLAN irradiated plants. The highest quantity of ascorbic acid was found in celery plants irradiated with GSM microwaves. Ascorbic acid concentration in celery leaves increased 3.1 times after three weeks of GSM microwave treatment.

Electrochemical method

Analysis of the extracts of control and irradiated plants were also performed by electrochemical method. For this purpose, a new calibration curve was drawn (y = 1.88E-08x + 1.04E-4) with a good correlation factor (R^2 = 0.9987) using ascorbic acid standards (between 1 µg/mL - 1800 µg/mL) prepared in ultrapure water by successive dilutions. The limit of detection (1.29 µg/mL) and the limit of quantification (2.57 µg/mL) were calculated [22]. For determination of precision were selected two standard solutions (8.80 and 17.60 mg/L) with concentration in the calibration range and were added to the extracts. The precision was expressed as RSD (%) and calculated as (standard deviation / mean) × 100. The precision for intra- and inter-day values was less than 5%. The precision and accuracy are in good agreement because low values of RSD (%) and high values for recovery (Table 2) were obtained.

Table 2. Intra- and inter-day precisions and accuracies for determination of ascorbic acid by electrochemical method.

Concentration of ascorbic acid (mg/L)	Intra-day (n = 3)		Inter-day (n = 3)	
	Precision (RSD, %)	Recovery (%)	Precision (RSD, %)	Recovery (%)
8.80	1.34	105.20	4.10	107.10
17.60	3.47	97.60	2.76	93.31

In order to evaluate the matrix effect on analysis, a recovery test was performed. Standard AA solution was added to all three plant extracts at two different levels of concentration (88.0 and 176.0 mg AA/L) and analyzed in triplicate. The mean recovery ranged from 93.9% to 107.6%, with higher variation in the case of parsley (Table 3).

Table 3. Ascorbic acid (AA) recovery in aromatic plants studied determined by electrochemical method.

Plant	Initial amount of AA (mg/L)	Added amount of AA (mg/L)	Mean of found amount of AA (mg/L)	Recovery (%)
Parsley	70.4	88.0	170.0	107.6
		176.0	231.0	93.9
Dill	35.2	88.0	130.0	105.7
		176.0	210.0	99.5
Celery	21.1	88.0	95.6	106.1
		176.0	196.0	99.4

The results obtained for control and irradiated plants using electrochemical method were in good agreement with the results obtained by HPLC technique (Table 4). The quantitative differences between the two analysis methods may be due to matrix effects occurring in electrochemical determinations due to acetic acid solution used to prepare the plant extracts.

Table 4. The amount of ascorbic acid (AA) and the percentage increase (+%) reported to control plants determined by electrochemical method.

Plant lot	Ascorbic acid content (mg AA/100 g plant) ± RSD (%)		
	Parsley	Dill	Celery
M1[a]	215 ± 1.47 (+36%)	149 ± 1.50 (+70%)	235 ± 1.35 (+280%)
M2[b]	176 ± 1.27 (+11%)	106 ± 1.49 (+20%)	109 ± 1.45 (+76%)
R[c]	158 ± 1.42	88 ± 1.80	62 ± 1.54

[a] plants irradiated with GSM frequency microwaves; [b] plants irradiated with WLAN frequency microwaves; [c] control plants.

Chen and co-workers suggest that efficient antioxidant defense plays an important role in microwave treated Nannochloropsis oculata algae, the algal cells could improve their antioxidant ability through the enhancement of enzymatic and non-enzymatic preventive substances and cause resistance to environmental stresses like microwave irradiation [23].

As an antioxidant, ascorbic acid has an important role in protecting against oxidative stress [24]. As previously shown by literature reports, plants react differently under the influence of similar stress factors. It was shown that water deficit induced oxidative stress and significantly increased the ascorbic acid content in leaves of Jonagold wilmuta apple tree compared to control Jonagold wilmuta apple tree, while the content of ascorbic acid did not indicate oxidative stress in Elstar apple tree [14]. Similar trends were obtained by Lee, whose experimental results indicated that superior ozone tolerance in the Hood soybean cultivar (compared with Hark) was associated with a greater increase in endogenous levels of ascorbic acid [17]. Likewise, other studies related to the effect of drought stress on plants showed that ascorbic acid content was higher in tolerant genotypes [15].

As result of our studies, celery was less affected by microwaves than parsley and dill. Elevated ascorbic acid under microwave stress could be related to higher protection against microwaves in irradiated plants.

CONCLUSIONS

On the basis of the results obtained, according to our studies plants respond differently to microwave stress. Our experimental results show an increase in the content of ascorbic acid in the stressed leaves of celery and dill subjected to microwave fields, while the ascorbic acid content was not significantly different in control and stressed leaves of parsley. Overall, GSM frequency microwaves showed more significant influence on the ascorbic acid levels of studied plants than WLAN frequency microwaves. The lowest percent increase in the amount of ascorbic acid was detected, by HPLC and electrochemical method, respectively, in parsley grown in WLAN microwave field (6.8%, 11%), while the highest percent increase was determined in celery grown in GSM microwave field (211%, 280%).

EXPERIMENTAL SECTION

Plant material

The plant seedlings (parsley, dill and celery) were grown in laboratory from Agrosel seeds (Câmpia Turzii, Romania). Quantities of 10 seeds were planted in each of the 150 mL identical plastic pots filled with equal quantities of commercial garden soil. Three weeks after seeding the plant vessels were placed in three identical anechoic chambers [25], a reference chamber (R) and two microwave irradiation chambers for plant irradiation on two microwave domains, namely GSM (mobile communication), using a generator with 860 – 910 MHz frequency range (M1) and WLAN (wireless internet connection), using a 802.11 g router generator with operating frequency ranging between 2.412 – 2.48 GHz and main operating channel at 2.42 GHz (M2). A number of 50 plants per chamber were considered for the study. Plants inside the chambers were illuminated at an intensity of 700 lux and the internal temperature of chambers was maintained at room temperature (25°C). Both control and irradiated plants were watered daily with 10 mL distilled water. After exposure to microwaves during three weeks, the plants were removed from the chambers and the fresh leaf material was analyzed.

Reagents and standards

L(+)-ascorbic acid was purchased from J.T. Baker (Holland), potassium phosphates, phosphoric acid, acetic acid and methanol of HPLC grade were purchased from Merck (Germany). All chemicals were analytical reagent grade. Standard L(+)-ascorbic acid stock solutions in the concentration range 0.3 – 1 μg/mL were prepared in ultrapure water.

Extraction procedure

The sonication method using an ultrasonic bath (Elmasonic S 15H, 37 kHz) was employed for extraction of ascorbic acid, and a modified version of the procedure presented by Yıldız and co-workers was followed [8]. The fresh leaf material of control and irradiated plants was finely chopped, weighed in portions of 1 g and after 10 minutes soaking with 8% (v/v) aqueous acetic acid was extracted two times by sonication with 5 mL extraction agent for 30 min at room temperature, thus the whole ultrasound extraction process taking 1 h. The supernatants were decanted and the combined extracts were rapidly filtered through a Buchner funnel using filter paper, then through nylon syringe filters (0.45 μm) and the total volume was adjusted to 10 mL. All extractions were carried out in triplicate. The plant extracts were stored at 2-4°C in tightly stoppered dark glass vials. It was previously shown that the role of the acid used in extraction of vitamin C is to protect against rapid oxidative reactions which could occur due to active enzymes released from cells during the extraction process [26]. The ascorbic acid from parsley, celery and dill was identified and quantified by HPLC and electrochemical methods, using the same plant extracts.

HPLC analysis

The analyses were carried out on a Shimadzu HPLC model LC-2010 with PDA detector. The chromatographic separation was performed using a Grace Alltima C18 column (100 x 3 mm, 3 μm) thermostated at 30°C. As eluent A, 15 mM phosphate buffer at pH 2.7 and eluent B, methanol, were used. The gradient elution program was as follows: 10% B (min 0), 20% B (min 5), 10% B (min 10). The injection volume was 20 μL and the flow rate of mobile phase was set to 0.4 mL/min. The injection of the plant extracts into HPLC system was performed three times. The HPLC detection was carried out at 243 nm, the maximum absorption wavelength of ascorbic acid.

Electrochemical analysis

The chronoamperometric measurements were performed with an Autolab Potentiostat 302N (EcoChemie, Netherlands) connected with a traditional three-electrode system. A platinum electrode was used as the auxiliary electrode, carbon paste electrode (CPE) and Ag|AgCl/KClsat were used as the working and reference electrodes, respectively. Batch amperometric measurements were carried out under magnetic stirring, using 0.1 M phosphate buffer solution (pH 6.8) as supporting electrolyte and +600 mV as working potential. The pH of the buffer solution was measured with the digital Hanna Instruments HI 255 Combined Meter.

The traditional CPE was prepared by hand-mixing of graphite powder with silicon oil in 70:30 ratio using an agate mortar. The homogeneous carbon paste electrode was packed into a cavity of a homemade carbon paste electrode (3.0 mm in diameter).

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