Biochemical and Production of Rhubarb Under Growing Technological Factors

ALEXANDRU COJOCARU[1], NECULAI MUNTEANU[1], BRINDUSA ALINA PETRE[2], TEODOR STAN[1], GABRIEL CIPRIAN TELIBAN[1], CATALIN VINTU[1], VASILE STOLERU[1*]

[1]University of Agriculture Sciences and Veterinary Medicine, 3 M. Sadoveanu, 700440, Iasi, Romania

[2] University Al.I.Cuza, 11 Carol I Blvd., 700506, Iasi, Romania

The aim of the present study was to assess the influence of two varieties of rhubarb (Glanskin's Perpetual and De Moldova) and two densities, 10.000 and 13.300 pl/ha on the content of macro (K, Ca, P, Mg, Fe and S), micro-elements (Al, Si and Mn), organic acids (tartaric, oxalic, citric, malic and ascorbic) and total production. The content of oxalic acid in the four versions ranged from 2.3 mg/mL juice in the case of Glanskin's perpetual cultivation, with the density of 13.300 pl/ha at 3.8 mg/mL in the case of the Moldova variety at the same density. The content of tartaric acid varied in wide limits from 1.75 mg/mL juice to 6.5 mg/mL juice. The content of macro-elements varied within wide limits, the report being: Fe > K > Mg >S > P > Ca. The largest production was obtained at the variety De Moldova with density of 13.300 pl/ha.

Keywords: rhubarb, macro and microelements, organic acids, technological factors

Rhubard (*Rheum rhabarbarum L.*) is a perennial vegetable species [1], cultivated only for its petiole [2], its leaves being toxic due to their content of oxalic acid [3]. In the world, this vegetable species is less known, being spread out mainly in the Asian countries, for medical purposes [4]. In Europe, it is cultivated more in Germany, France and England [5, 6].

Rhubarb contains main active components (chrysophanol $[C_{15}H_{10}O_4]$, aloe-emodin $[C_{15}H_{10}O_5]$, rhein $[C_{15}H_8O_6]$, emodin $[C_{15}H_{10}O_5]$ and physcion $[C_{16}H_{12}O_5]$), with pharmacodynamic effects [7].

The production and composition of petioles is clearly influenced by the technology applied to the crop, especially by the variety, the planting distance and the nutritional regimen [8, 9].

Organic acids play a biochemical role in maintaining the nutritional value and the quality of a vegetable species, and therefore they are among the frequently quantified compounds [10].

The major acid present in the rhubarb petioles is the malic acid, followed by oxalic acid and citric acid [11, 12].

Rhubarb juice can be used as an agent to blanch fruits [13] as well as an antioxidant to preserve fruits [14]. Products made of rhubarb have a favorable taste due to a high content of organic acids and rhubarb stalks taste best in early spring when they are ripe [15, 16].

Previous studies have shown that the root fresh weight and root dry weight increased significantly at the wilting stage following treatment with 90 kg/ha P_2O_5 (100% and 59%, respectively) and 75 kg/ha K_2O (43% and 41%, respectively) compared to the control [17].

The high content of potassium in rhubarb juice ranges from 2814 to 4000 mg/L. Also, magnesium concentrations were comparably high and ranged from 53 to 136 mg/L. In contrast to other vegetables, rhubarb juice does not contain calcium in high levels, because the exceeding oxalic acid precipitated rapidly with calcium and form insoluble calcium oxalate. Particularly, the natural sodium values are very high in comparison to other fruit and vegetable juices (5–202 mg/L). The antioxidant capacity varied from 2.6 to 25 mmol/L Trolox [18, 19].

The data present in the literature regarding the establishment of the rhubarb crop highlight the fact that in the first year after the crop is established, there may be obtained productions of 0.7 kg/pl.at a density of 8.000 pl/ha [20]. At densities of 13.300 pl/ha, the Victoria cultivar has obtained overall productions of 46.64 t/ha, comparatively with the density of 8.000 pl/ha, where obtained production was of 21.72 t/ha [3]. By the tissue culture, in the first year after the crop was established, early yield achieved 11.2 t/ha and plants accounted for 25% of the total petiole yield [21].

The non-destructive methodology based on X-ray fluorescence (EDXRF) has been applied for the determination of some major and minor elements (K, Ca, Fe, Mn, Cu and Zn) in different rhubarb species: *Atriplexhortensis*, *Rumexpatienta*, *Lactuca sativa*, *Spinacea oleracea*. *Atriplexhortensis* and *Rumexpatientia* species presented higher value of potassium (K) content, ranged from 2.9 to 4.75%. The highes tiron (Fe) concentrations were 1002.5 and 952.05 ppm from *Lactuca sativa* and *Rumexpatientia species* and calcium (Ca) concentration ranges from 1.2 to 2.15% with the highest value attributed to *Spinacea oleracea* species [22].

The highest oxalate content in leaves and stems was found in plants from these families. Total oxalate content ranged from 143 to 232 mg/100 g in roots, and 874 to 1959 mg/100 g in leaves and stems. As reported in the literature, patients with calcium oxalate stone disease should be advised to avoid these oxalate rich foods [23].

In this context, the aim of the study was to assess the influence of two rhubarb varieties and two densities on the content of macro and microelements, organic acids and total yield of production.

Experimental part

The experiment was carried out in the research station at Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine of Iaºi, on a rhubarb crop established by seedlings in 2013. The cambic chernozem soil is characterized by a medium fertility, with 3% organic matter, 31% of clay and pH = 6.6.

During the experimental year, in the vegetation period, the average temperature was 18°C, the precipitation was 517.8 mm and the relative air humidity was 67% [24].

Experimental design

To achieve the main goal of this research, a bi–factorial trial was represented by cultivar (cv.) with two graduations, "Glaskin's perpetual" (GP) and "Moldova Local population" (LP) and plant density, with two graduations, 13.300 and 10.000 pl/ha. The trial was organized in a split plot design, with three replicates, and each plot had a surface of 16.50 m².

The determinations were made according to national and international standards in accordance with International Organization for Standardization (ISO) and Official Methods of Analysis of AOAC INTERNATIONAL, 20th Edition (2016). AOAC Standards Guidelines & References -AOAC International. AOAC offers the official analysis methods of AOAC INTERNATIONAL (OMA) *Official Methods of Analysis of AOAC INTERNATIONAL.*

Determination of macroelements and microelements

In order to determine the concentration of essential elements from the studied samples, the atomic absorption method was used (Contra 300), Analytik Jena [25, 26]. The component parts of the variants used in the study were dried in a drying oven at 105°C, and then there was measured between 0.5 and 1 g per each sample, and finally they were subjected to a process of disaggregation (digestive or mineralization) for bringing them into solution [27, 28].

Determination of organic acids

The analyses were carried out in collaboration with, Chemistry & Biochemistry Laboratory of Banat's University, using methods previous reported [29, 30]. Juice extract samples were obtained from each petiole variant. The samples were extracted in a Soxhlet-type apparatus in which the solvent is provided by boiling the extract. This method is based on the difference between the boiling points of the solvent and the extracted analyses [31, 32].

The aqueous extract obtained was centrifuged at 3000 rpm for 10 minutes, and the supernatant was diluted in 1:50 ratio in order to determine the citric acid and in a 1:5 ratio in order to determine the oxalic, citric, malic, tartaric and ascorbic acids. The dilutions were filtered prior to photocolorimetric determination of the acid concentration, analyzing two samples in duplicate. A standard mixed solution was prepared, containing 1000 mg/L citric acid, 2000 mg/L malic acid, 300 mg/L oxalic and ascorbic acid and 700 mg/L tartaric acid [33]. The standard solution and the corresponding dilutions were prepared with distilled water and were kept at a low temperature (4°C) in a dark place. The organic acids from the studied samples were separated and determined with the aid of the absorbance, and then quantified with calibration graphs. For the ascorbic acid, the wavelength $\lambda = 254$ nm was used, respectively $\lambda = 214$ nm for the other organic acids [34, 35].

Determination of total production

The harvesting was done manually, on a weekly basis, the petioles having a diameter ranging between 1.5–2.5 cm and the length of 30 - 35 cm. No more than 1/3 of petioles were harvested, in order to avoid weakening the plants as reported in previous studies [36].

Data processing and statistical analyses

The experimental data processing was carried out using statistical methods. Standard deviation (± SD) was calculated for each data series as an indicator of dataset scatter (n = 3). The significance of acid content and yield, due to cultivar x density combinations was established by ANOVA [37, 38], based on the Fisher test. The differences among the average values for each experimental variant were compared by using the Student test and the least significant difference (LSD) test at $p < 0.05$ probability level, computed by the SPSS version 20.

Results and discussions

The results obtained in the case of macroelements by spectrophotometry with atomic absorption are presented in table 1. The main content of macroelements is represented by Fe, followed by K and Mg. According to the specialized literature, the Ca content is relatively reduced, because it precipitates in the presence of oxalates [39].

The content of Fe, K, Mg, P and Ca varies within small limits depending on the variety, with the exception of S, where in the case of the GP cultivar, the content was of 0.53 g/100 g fw comparatively with LP, where the average was of 0.70 g/100 g fw. The content of K, Ca, P and Fe is much higher in the case of higher densities, comparatively with Mg and S, which are accumulated in higher quantities, at more reduced densities.

The results obtained in the case of microelements (Si, Al and Ti), studied by spectrophotometry with atomic absorption are presented in table 2. According to specialized literature, a high content of Si is also present in the case of the species from the Polygonaceae family, such as the sorrel, where this element varies between 1.8 and 17 g/100g fw [40]. The content of Si and Ti varies within very small limits, depending on the variety and density.

Manganese is an element that varies in the petiole within higher limits depending on the variety, so in the GP cultivar, the average content is of 0.34 g/100 g fw comparatively with the LP, where the average content was of 0.24 g/100 g fw.

The aluminum content varies in the plant's petiole, both depending on the variety and on the density, so the highest

Variant	Macronutrients contents					
	K wt%	Ca wt%	P wt%	Mg wt%	Fe wt%	S wt%
V₁	1.93±0.01	0.52±0.00	0.53±0.01	1.03±0.01	5.19±0.02	0.54±0.01
V₂	1.84±0.01	0.54±0.01	0.54±0.01	1.11±0.01	4.61±0.01	0.53±0.00
V₃	1.85±0.01	0.49±0.00	0.52±0.00	1.13±0.01	4.77±0.04	0.69±0.01
V₄	1.94±0.01	0.51±0.01	0.60±0.01	1.00±0.06	5.20±0.05	0.71±0.01
x̄	1.89±0.01	0.51±0.01	0.55±0.01	1.07±0.02	4.94±0.03	0.62±0.01
V₁ – Glaskin's perpetual x 13.300 pl./ha; V₂ – Glaskin's perpetual x 10.000 pl./ha; V₃ – Local population De Moldov" x 13.300 pl./ha; V₄ – Local population"De Moldova x 10.000 pl./ha.						

Table 1
CONTENT OF MACROELEMENTS IN THE RHUBARB PETIOLE (n = ±3)

Variant	Micronutrients contents			
	Al (ppm)	Si (ppm)	Ti (ppm)	Mn (ppm)
V_1	7.40±0.01	28.48±0.46	0.48±0.01	0.35±0.01
V_2	7.52±0.01	28.80±0.81	0.42±0.01	0.33±0.01
V_3	7.46±0.03	28.74±0.15	0.43±0.02	0.26±0.02
V_4	6.79±0.09	28.94±0.81	0.47±0.02	0.22±0.02
$\bar{x}$	7.29±0.03	28.74±0.56	0.45±0.01	0.29±0.02

V_1 – Glanskin's perpetual x 13.300 pl./ha; V_2 – Glanskin's perpetual x 10.000 pl./ha; V_3– Local population De Moldova x 13.300 pl./ha; V_4 – Local population De Moldov x 10.000 pl./ha.

content was obtained in the GP cultivar, at the lowest density, namely 7.52 g/100g fw.

The high content of microelements in rhubarb, comparatively with other vegetable species is also determined by the fact that the rhizome of this vegetable species explores a large volume of soil, therefore the content is higher in the petioles of this plant, compared with other vegetables [41].

The main acids analyzed from the aqueous extract of rhubarb were: the malic, citric, tartaric, ascorbic and oxalic acids. The malic acid is the most important acid from the aqueous extract of rhubarb, with an average content of 6.79 mg/mL juice. According to the data presented in Table3, this acid does not vary in the petiole depending on the cultivar and density.

The tartaric acid varies both depending on the variety and on the density. Therefore, then content of tartaric acid varies from 2.77 mg/mL juice in the GP cultivar, up to 5.04 mg/mL juice in the LP cultivar. Tartaric acids is accumulated in higher quantities in the case of more reduced densities, so, for 10.000 pl./ha, the content was of 4.22 mg/mL juice, comparatively with the higher density, where the content was of 3.59 mg/mL.

The same trend regarding the higher level of oxalic, citric and ascorbic acids may be highlighted in the case of the GP and LP varieties, where the population De Moldova presents higher content levels of organic acids.

Similar data is presented in other references as well, where the local populations, well adapted to the climatic conditions, accumulate higher quantities of acids [42]. The content of tartaric and ascorbic acids is higher in the case of more reduced densities, 5.20 mg/mL juice for LP, and 4.38 mg/mL juice for LP respectively.

The production obtained in the case of the rhubarb cultivars is influenced both by the variety and the density. The total production has varied between 27.20 t/ha in the case of the GP cultivar, at a reduced density, up to 39.02 t/ha in the case of the LP cultivar, at a high density, the differences obtained in this case compared to the average, being processed statistically (p < 0.01) (table 4).

In the case of the GP variety, the production was of 30.00 t/ha, 35.15 t/ha respectively, in the case of the LP cultivar. Higher productions are also obtained in the case of high densities, respectively 35.91 t/ha, comparatively with the density of 10000 pl/ha where the total production was of 29.24 t/ha.

Table 3
CONTENT OF ORGANIC ACIDS IN THE RHUBARB PETIOLE (n = ±3)

Variant	Tartaric acid (mg/mLsuc)	Oxalic acid (mg/mLsuc)	Citric acid (mg/mLsuc)	Malic acid (mg/mLsuc)	Ascorbic acid (mg/mLsuc)
V_1	2.30±0.04	2.56±0.10	5.34±0.02	6.70±0.06	3.39±0.03
V_2	3.24±0.03	3.40±0.06	5.01±0.04	6.77±0.08	3.59±0.04
V_3	4.88±0.05	3.77±0.07	4.65±0.04	6.83±0.06	3.87±0.03
V_4	5.20±0.05	3.43±0.04	4.41±0.04	6.87±0.03	4.38±0.03
$\bar{x}$	3.91±0.04	3.29±0.06	4.85±0.04	6.79±0.05	3.81±0.03

V_1 – Glanskin's perpetual x 13.300 pl./ha; V_2 – Glanskin's perpetual x 10.000 pl./ha; V_3– Local population De Moldova x 13.300 pl./ha; V_4 – Local population "De Moldova" x 10.000 pl./ha;.

Table 4
INFLUENCE OF CULTIVAR X DENSITY FACTORS ON RHUBARB YIELD (n = ±3)

Variant	Total yield (t/ha)	% to the average	Difference to average (t/ha)	Significance of differences
V_1	32.79±0.19	100.68	0.22	ns
V_2	27.20±0.36	83.51	−5.37	oo
V_3	39.02±0.30	119.80	6.45	**
V_4	31.28±0.67	96.04	−1.29	ns
$\bar{x}$	32.57±0.38	100.00	0.00	−

LSD 5% = 3.18 t/ha; LSD 1% = 4.82 t/ha; LSD 0,01% = 7.74 t/ha; V_1 – Glanskin's perpetual x 13.300 pl./ha; V_2 – Glanskin's perpetual x 10.000 pl./ha; V_3 – Local population; Ns – nonsignificant; oo – negative significantly different; ** – positive significantly distinct

Conclusions

The macroelements analyzed in the rhubarb petiole were found to be in the following series Fe > K > Mg > S> P > Ca. Among the microelements, the highest determined content was represented by Si and Al, higher at a lower density, in the case of Si, with an average content of 28.87 g/100g fw, comparatively with Al, where the highest content was obtained at a higher density of an average value of 7.42 g/100g fw.

The organic acids present in the aqueous extract of rhubarb vary within wide limits, depending on the nature of each acid and the content is influenced mainly by the variety and less by the density. The largest production of rhubarb petioles was obtained at the variety De Moldova with a density of 13,300 pl/ha. Our studies proved the micro and macro elemental composition of different varieties of rhubarb, a well known traditional Chinese herb, with anti-inflammatory and antimicrobial properties.

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