Romanian Biotechnological Letters *Vol. , No. x,*

ORIGINAL PAPER

Effects of production system on the content of organic acids in Bio rhubarb (*Rheum rhabarbarum* L.)

Received for publication, December, 28, 2016
Accepted, October, 10, 2017

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Abstract

Rhubarb is a perennial vegetable crop well adapted to the temperate climate but it is not well known and cultivated as a traditional crop. The aim of the present study is to highlight the influence of the rhubarb cultivar and crop density on the content of some organic acids (malic, tartaric, oxalic, citric, ascorbic) in an organic crop. The highest content of organic acids was determined in a local population from Moldova at a density of 9090 plants·ha^{-1}; the following quantities of acids for 100 g fresh weight (FW) were determined: tartaric – 486 mg, oxalic – 343 mg, citric – 41 mg, malic – 686 mg and ascorbic –437 mg.

In this present paper, we have demonstrated that content of organic acids from edible rhubarb parts varies according to crop density and cultivars, except for malic acid.

Keywords: *Rheum rhabarbarum*, cultivar, density, tartaric, oxalic, citric, malic, ascorbic compounds

1. Introduction

The rhubarb (*Rheum rhabarbarum L.*) is a perennial species which grows best in a cold and temperate climate [1], but it is not well known and cultivated as a crop in Romania [2]. Rhubarb plants are cultivated as vegetable for their leaves and stems. Fresh rhubarb is generally available across from April to October, in open fields, harvested from March to December. Rhubarb is one of those iconic vegetables that transport us from one season to the next, from the dull days of winter to the rebirth of spring [3,4]. Interest from the European consumer in recent times for this vegetable is a good opportunity to study the influence of some technological factors on the quantity and quality of the yields [5].

The main quality characteristics are the taste and aroma, and these depends on the chemical composition. The chemical composition in organic acids, minerals, carbohydrates, proteins and vitamins highly depends on the cultivation and harvesting period [6]. A special interest has been shown by scientists regarding the quantitative and qualitative content of the organic acid.

Gherghi, 1985 identified 32 organic acids in vegetable rhubarb products. Although malic, citric and oxalic acids have been known since the time of Scheele. The tartaric acid, at least in the form of its salts, the exact position and length of these widely distributed substances in the general scheme of metabolism in plants is still a matter of speculation and debate [8, 9, 10].

Bio–synthesis of organic acids in plants is depending on biotic and abiotic factors that in organic cultivated crops are very important elements which determine the organoleptic qualities of the edible part, since synthetic fertilizers are prohibited [11]. A balance content in organic acids provide or increase immunity in the plants to the harmful organisms, which is an important fact when it comes to organic crops because of the limited application of the chemical synthesis pesticide [12]. Dynamics of acidity is an important technological indicator for harvesting of the yield, knowing that a balance between acids reduces the changes during ripening which could decrease organoleptic quality [11, 13]. Such biochemical analysis opens a new horizon to establish the optimal time to harvest the edible parts.

The organic acids have a well-established role in the plant's growth, such as the cell wall structure, storage of phosphates and building blocks of lignin. Sometimes these present functions of antibacterial, antifungal, anti-parasitic; they contribute to the appearance, color and smell of food they contain [14, 15].

Malic acid is a predominant one, and citric acid and oxalic acid are present in a smaller quantity in the rhubarb plants [16]. Because the malic acid is degraded more quickly than other acids, it is also clear that the rhubarb's edible parts lose their acidity and therefore storage lasts a short period [17].

The citric and tartaric acids in fruits and vegetables are the hardest to degrade, and a higher content in these acids favors and maintains a higher percentage of the ascorbic acid content [9, 11, 18].

It is known that oxalic acid is found in large quantities in plants of the *Polygonaceae* and *Chenopodiaceae* families, where the content can be up to 40–50 mg/100 g [19, 20]. In these plants, a high content of oxalic acid may cause in-solubilisation of the other organic acids in the human body's cells and deposition of crystallized calcium oxalate. Oxalic acid is an anti-nutritive substance, the in–solubilizing part of calcium and magnesium, thus causing toxic effects [21].

Ascorbic acid has a strong reducing effect, passing to dehydro-ascorbic acid. The role of ascorbic acids is very complex, since in plants they cause the formation of unsaturated fatty acids, degradation of amino acids or carbohydrates, affecting the iron metabolism etc. [7, 11, 21]. In the last 20 years, increasing experimental evidence has associated the metabolism of organic acids with plant tolerance to environmental stress.

This new perspective of organic acid metabolism and its potential manipulation may represent a way to understand the fundamental aspects of plant physiology and lead to develop new technologies [22, 23]. Content of vitamins, minerals, organic acids of rhubarb petioles is directly influenced by these technological benefactors, including: cultivar, planting distance and the interaction of these vital role factors [25].

The aim of the research was to evaluate the organic acid content from rhubarb plants cultivated in an organic system in relation with cultivar, crop density and the combination of cultivar x density.

2. Materials and Methods

2.1. Experiment trial

This research was carried out in the experimental field of the Agricultural University from Iasi, managed in the organic system on a rhubarb crop established in 2013. To achieve the goal and objectives of this research, a bi-factorial trial was established: Factor A–cultivar (cv.) with two graduations, "Glaskin's perpetual" (GP) and "Local population" (LP) from Moldova, and factor B–plant density, with two graduation, 9,090 plants•ha^{-1} and 12,120 plants•ha^{-1}. The trial was organized in a split plot design, with three replicates. A plot has a surface of 16.50 m^2 (2.2 m x 7.5 m).

2.2. Cultivation practical

The cultivation practices were done according to recommendations of adequate literature [5, 24, 25], using only organic and biological measures for fertilizing, disease and pest control according to our previous study [12]. Petioles harvesting was carried out once every five days.

2.3. Identification of organic acids

The analyses were carried out at the Chemistry & Biochemistry Laboratory of Banat's University, using methods recommended by [26]. 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. Based on this property, the extract is brought to the boiling point of the solvent, which will condense the refrigerant and returned to the cartridge containing the sample to be extracted. Through achievement of several cycles of extraction, the process efficiency can be controlled so that the extraction efficiency is maximized. The obtained extract was centrifuged at 3000 rpm for 10 minutes, and the supernatant is diluted in a 1:50 ratio for the determination of the citric acid in a ratio of 1:5 acids. The dilutions were filtered before determination of the concentration using colorimetric analysis & Beer's Law. The two samples are analysed in duplicate. A mixed standard solution was prepared containing 1000 mg·L^{-1} citric acid, 2000 mg·L^{-1} of malic acid, 300 mg·L^{-1} oxalic acid and ascorbic acid, 700 mg·L^{-1} of tartaric acid and 400 mg·L^{-1} lactic acid. The standard solution and appropriate dilutions were prepared with distilled water and kept in a dark place at low temperature (+4^{0}C). The organic acids in the samples were separated and determined using absorbance, then quantified with the calibration graphs, on wavelengths of λ = 254 nm for ascorbic acid, and λ = 214 nm for other organic acids [27].

The method recommended by Ionel in 2013 was used for the identification of organic acids. The sample is titrated with a sodium hydroxide solution, from a burette, with the order of 0.1 cm^3, 0.5 cm^3 portions and toward the end portion than 0.1 cm^3. A preliminary probe is determined when passing portions of 0.1 cm^3. After each additional portion of NaOH, mixed with a glass rod, a drop of titration is passed on a porcelain mesh plate, in which one drop of phenolphthalein is added. The titration is terminated, when at drop wise addition of the solution changes to pink colour [29]. The results were expressed as mg·100 g^{-1} fresh product of rhubarb.

2.4. Data processing and statistical analyses

The experimental data processing was carried out using specific mathematical and statistical methods [30]. All analyses were carried out in the three replications. 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 [31, 32], 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. To determine the degree of correlation between the combinations of the studied factors and content of organic acids the regression coefficient (r^2) was determined.

3. Results and Conclusions

3.1. Influence of the cultivar and planting distances on the total content of organic acids

The cultivar and planting density are two technological factors which directly influences the quantity and quality of the yield. Four experimental combinations of the two factors appear in the interaction study (V_1, V_2, V_3, V_4, with three replication) (Table 1).

Table 1**.** The organic acid content of rhubarb

Variants	**Average content of organic acids (mg·100 g^{-1} FW)**					
	tartaric	**oxalic**	**citric**	**malic**	**ascorbic**	**Total acid**
V_1 (GP x d_1)	230^b	256^b	**534^a**	670	339^b	2029
V_2 (GP x d_2)	324	340	500^a	676	359^b	2199
V_3 (LP x d_1)	388	**377^a**	465^b	683	386	2299
V_4 (LP x d_2)	**520^a**	343	441^b	**687**	**438^a**	2429
$\overline{x}$	**365.5**	**329**	**485**	**679**	**380.5**	2239
p<0.05%	88.33	39.58	10.78	14.91	25.06	-

GP- Glaskin's perpetual; LP - Local population; d_1 - 12,120 plants•ha^{-1}; d_2- 9,090 plants•ha^{-1}; a–positive significant difference at p<0.05; b–negative significant difference at p<0.05

In general, depending on the combination of the two factors, except malic acid, the total content of organic acids varies widely, from 2029 mg•100 g^{-1} FW on V_1 till to 2429 mg•100 g^{-1} FW on V_4. Tartaric acid varied between 230 and 520 mg•100 g^{-1}, oxalic acid varied between 256 and 377 mg•100 g^{-1}, citric acid varied between 441 and 534 mg•100 g^{-1} and ascorbic acid between 339 and 438 mg•100 g^{-1}.

3.2. Tartaric acid content in rhubarb

The trend is upward from V_1 to V_4, with R^2 = 0.9811, which indicates that LP planted at a density of 9,090 plants•ha^{-1} accumulates a larger amount of tartaric acid (Fig.1). In the terms of the tartaric acid content, it can be seen that it ranged between 230 mg•100 g^{-1} FW in V_1 and 520 mg•100 g^{-1} FW in V_4. Concerning the tartaric content, the variation depends on the combination of two factors analyzed.

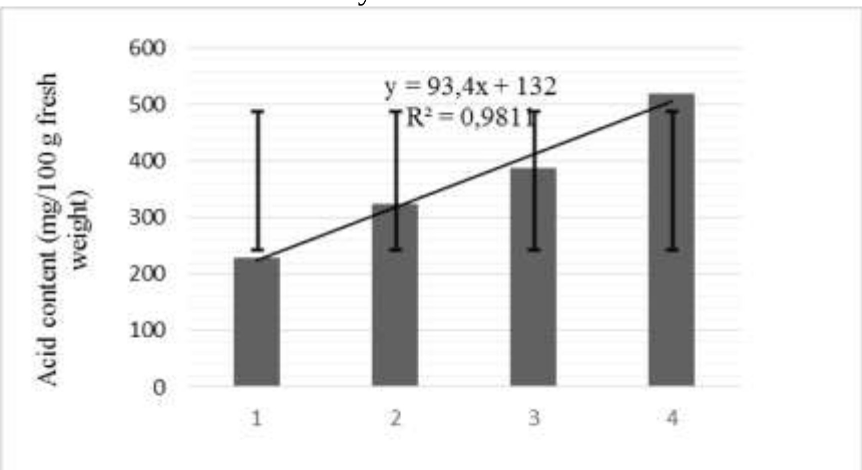

Fig. 1. The tartaric acid content according to cultivar and plant density

3.3. Oxalic acid content in rhubarb

The oxalic acid content is an indicator that depends on the quality of the edible part of rhubarb. In the experiment the oxalic acid content ranged from 257 mg•100 g^{-1}FW in V_1 up to 377 mg•100 g^{-1} FW in V_3, with an average from 329 mg•100 g^{-1} FW. Referring to the oxalic acid content compared with tartaric acid, the variation content is lower with R^2= 0.5585 (Fig. 2).

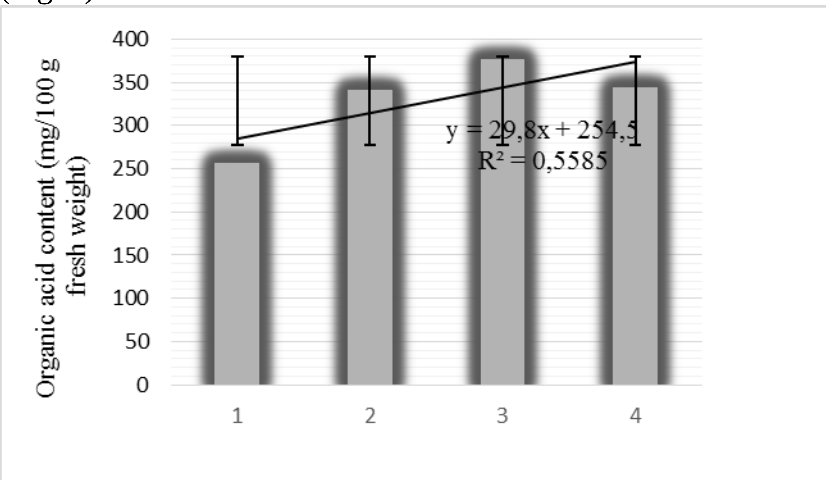

Fig. 2. The oxalic acid content according to cultivar and plant density

3.4. Citric acid content in rhubarb

The content of citric acid in rhubarb ranged between 441 mg•100 g^{-1} FW in V_4 and 534 mg•100 g^{-1} FW in V_1, with an average content of 485 mg•100 g^{-1} fresh weight. The higher content in oxalic acid was obtained on GP cv., but we can say that the variation in the content of citric acid in the edible parts differ, depending on the distances from planting, so the highest content was observed in variants with higher density, respectively 12.120 pl•ha^{-1}. Compared with the other four analyzed acids from rhubarb, citric acid is one in which the regression line descends, from GP x 12,120 plants•ha^{-1} by the LP x 9,090 plants•ha^{-1}, with a probability of 99.35% (Fig. 3).

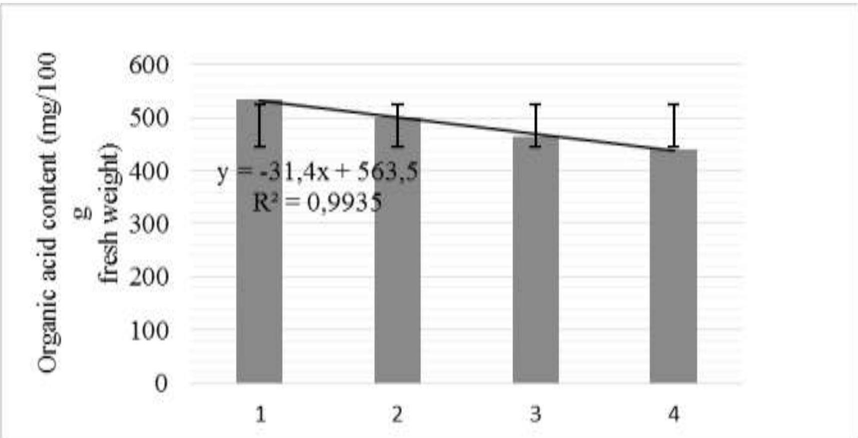

Fig. 3. The citric acid content according to cultivar and plant density

3.5. Malic acid content in rhubarb

Among the organic acids, the highest content was found in the case of malic acid, with average values from 679±2.88 mg•100 g^{-1} fresh weight, with very small differences between the experimental variants; this shows that the four variants of this content are not influenced by cultivar and planting density, that fact being confirmed also by other studies [33].

However, the highest content in malic acid was found in the LP planted at a density of 9,090 plants•ha^{-1}.

3.6. Ascorbic acid content in rhubarb

In the case of ascorbic acid, we can say that the values of this acid in rhubarb are close to the values of oxalic acid. The ascorbic acid content ranged from 339 mg•100 g^{-1} FW in GP cv. with a density of 12,120 plants•ha^{-1} to 438 mg•100 g^{-1} FW in the case of LP cv.at density of 9,090 plants•ha^{-1}.

Perspective to accumulation the ascorbic acid is upward from a high density to a lower crop density, especially in the LP, which supports the assertion with a probability of 95.07% (Fig. 4). So, the higher content of ascorbic acid of 438 mg•100 g^{-1} obtained by LP cv.at density of 9,090 plants•ha^{-1}.

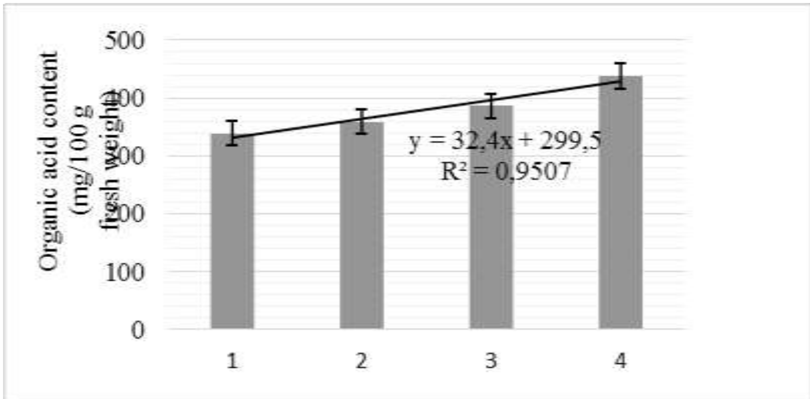

Fig. 4. The ascorbic acid content according to cultivar and plant density

3.7. Effect of cultivar and planting distance on rhubarb yield

The cultivar and planting distance, as technological measures influenced the yield of rhubarb, cultivated under organic growing conditions, in the second year after established. The production dates are presented in Table 2.

Table 2. Effect of cultivar and planting distance on rhubarb yield (n=3)

Experimental version	Yield (t/ha)	Differences *vs.* mean (t/ha)	Significances of the differences *vs.* mean
V_1 (GP x d_1)	39.08±5.21	7,37	**
V_2 (GP x d_2)	33.85±1.51	2,14	ns
V_3 (LP x d_1)	27.52±2.96	-4,19	ns
V_4 (LP x d_2)	26.39±3.76	-5,32	o
Average $\bar{x}$	31.71±0.00	-	

LSD 5%=4.45 t/ha; LSD 1%=6.75 t/ha; LSD 0.1%=10.84 t/ha; GP- Glaskin's perpetual; LP - Local population; d_1 - 12,120 plants•ha^{-1}; d_2- 9,090 plants•ha^{-1}; ns-no significant differences; ** distinct significant positive differences at p<0.1; o-significant negative differences at p<0.05.

The distinct significant positive differences was obtained in the experimental version were GP cv. planted at a density of 12,120 plants•ha^{-1}. Local population planted at distance of 12,120 plants•ha^{-1} (26.39 t/ha) and 9,090 plants•ha^{-1} (27.52 t/ha) obtained productions less than average experiment (31.71 t/ha).

Discussions

The technological measures were applied on a rhubarb crop, to ensure the optimum plant growth and development as well as the good quality of the vegetable product under natural circumstances, according to the requirements of plants [12, 34]. The role of the optimization of technological factors is to achieve sufficient production in quantitative terms but mostly qualitative.

Analysis of the variance (ANOVA) of the experimental data revealed the importance of the studied factors: cultivar, crop density and cultivar x crop density on the organic acid content. The Fisher test for $p<0.05$ level of significance highlighted that the cultivar is a significant factor for the following organic acids: tartaric, oxalic, citric, malic and ascorbic.

The crop density influence, according to the same test, is positive significant influenced for the following content of organic acids: tartaric, citric, and ascorbic.

Except for the analyzed citric acid, all four acids have a smaller quantity in GP, compared to LP, underscoring the influence is determined by cultivar. This result might be explained by a genetic adaptation of the LP to certain abiotic or biotic stress factors, during the long period of cultivation, which favors a higher acidity [34].

Thus, we can say that tartaric, oxalic and ascorbic contents are lower in GP than the mean value of experiment, regardless of plant density per hectare. Higher values are an indication of this acid, which shows that the LP from Moldova are ready faster than GP this is a very important clue in storage balance among the acids in petioles from rhubarb.

Regarding the variation of organic acids in varieties, based on calculated statistical values, we can say that the value of this coefficient is higher in GP cv. ($r^2 = 0.236$) compared to LP where $r^2 = 0.0937$, which means that the differences between acid contents are lower in LP cv. This is of practical interest because, LP is more balanced in terms of acidity.

The distance between plants is a technological factor that directly influences the crop density, nutrition space, light regime and, as a consequence of organic acid content.

The content total un-azotic organic acids depend on the distance of planting. Thus, in terms of larger nutritional space content of organic acids, it is 2,239 mg•100 g^{-1} FW compared to a smaller space where the nutritional content of organic acids is lower at 2164 mg•100 g^{-1} FW. From the data, it follows that to obtain products with higher acidity, less crop densities or greater distances between plants should be used.

At a density of 12,120 plants•ha^{-1}, the organic acid content was higher compared to the situation when rhubarb is planted at lower densities. On the basis of the statistical factor r^2 regarding how plant density plays an important technological factor when it comes to creating optimal conditions for balanced accumulation of acids, a last resort is influenced by light and photosynthesis.

This could be explain by the fact that the rhubarb petioles overall did not reach maturity from consumption and thus there is an imbalance between acid [20, 35].

The total content of non-azotic acids varies according to the interaction between cultivar and planting distances. The highest content of organic acids was obtained in the experimental variant V_4 (LP x d2).

Concerning to tartaric acid content, variation depends on the combination of two factors analyzed. The trend with $r^2 = 0.9811$ indicate that LP cv. planted at a density of 9,090 plants•ha^{-1} accumulates a larger amount of tartaric acid.

The oxalic acid content is an indicator that depends on the quality of the edible part of rhubarb. The higher content of oxalic acid is observed in the LP from Moldova, but we can say that the variation in the oxalic acid content did not differ significantly according with planting distances. Even though the literature notes that in some cases oxalic acid can be 50% of the total acidity in rhubarb [20]. The results of oxalic acid are in accordance with Son, (2000) which showed in rhubarb a content of 336 mg•100 g^{-1} FW. Through technology applied and varieties, this content is reduced which means a lower presence oxalate in the body and therefore a lower risk to human health. Oxalic acid content in rhubarb higher than our results was obtained by [37]. Other studies and surveys reported oxalic acid contents higher in other plants, as *Amaranthus sp.*, *Tea Chinese*, *Atriplex hortense*, *Tetragonia tetragonoides*, *Spinacia oleracea* [1] or less in tomato, parsley, apple, cabbage or lettuce [37, 38].

The higher content in oxalic acid was obtained on GP cv., but we can say that the variation in the content of citric acid in the edible parts differ, depending on the distances from planting, so the highest content was observed in variants with higher density, respectively 12,120 pl•ha^{-1}.

However, the highest content in malic acid was found in the LPs from Moldova, planted at a density of 9,090 plants•ha^{-1}. In the case of ascorbic acid, we can say that the values of this acid in rhubarb are close to the values of oxalic acid. Generally, in major type of vegetable the contents of malic acid are less in rhubarb [39, 40].The ascorbic acid content ranged from 339±14.00 mg•100 g^{-1} FW in GP cv. with a density of 12,120 plants•ha^{-1} to 438±35.23 mg•100 g^{-1} FW in the case of LP cv.at density of 9,090 plants•ha^{-1}. Lower contents of ascorbic acid can be found in other leaf (spinach, cabbage) and root vegetables [40].

In general, the pleasant sweet and sour taste from the fruit is given by high total free acidity, like in rhubarb [20]. Concerning the total yield of rhubarb there is not a positive correlation between the cultivars production and total content of organic acids. LP get lower production than GP cv., but organic acid content is higher at lower plant density. Similar results of production, on Victoria variety to the mulching conditions were obtained [41]. Rumpunen (1999) based on a research study to 71 commercial cultivars mentions productions from 3 to 7 t/ha at density of 8870 plants•ha^{-1}. The higher yield in GP cv. is clearly influenced by density, but the total production does not positively correlate with a higher content in organic acids.

Conclusions

This study demonstrated the clear impact of the cultivars and planting density as well on the organic acid content and yield.

The better suited variety to environmental conditions planted at less distances favored getting a higher total acidity rate of rhubarb stalks and thus in a balance between acids.

In terms of quality exception to the rule, citric acid content is higher in improved cultivars and small planting distances. Malic acid, regardless of the cultivar and planting distance was found in the same quantity. The average ratio of tartaric, oxalic and ascorbic acids is approximately 1.

The tartaric and oxalic acids are lower in improved cultivar planted a higher density, it should be considered the establishment for future rhubarb crops, as a possible design solutions.

4. Acknowledgements

We thank Dean Hufstetler for revised English.

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