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Research Article

Effects of Mineral and Organic Nitrogen on Chemical Characteristics of *Physalis* Fruits

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Abstract

Background and Objective: In order to improve the *Physalis* fruit quality, the organic fertilization can be a better alternative, once it reduces the production costs, recycling the wastes produced in the rural properties and the environmental pollution, by avoiding the use of mineral fertilizers and the inappropriate disposal of animal wastes. This study aimed to evaluate the fruit characteristics of two *Physalis* species, *Physalis peruviana* L. and *Physalis pubescens* L., cultivated in different sources of nitrogen-mineral and organic fertilizer (poultry litter), under field conditions in the Southwest of Paraná. **Materials and Methods:** The experimental design used was randomized blocks, with two species, three fertilizer treatments and five blocks, being characterized as a factorial experiment (2×3) with two qualitative variables. The variable evaluated were fruit productivity, diameter and weight, total soluble sugars levels, reducing sugar, total soluble proteins, ascorbic acid, total phenolic compounds and flavonoids, pH and total soluble solids in fruits. Comparison of mean was performed using Duncan and Tukey's tests ($p < 0.05$). **Results:** The experiment showed that the treatment organic fertilizer significantly increased ($p < 0.05$) the amounts of total soluble sugar (7.9 mg glucose g^{-1} fruit), reducing sugar (49.7 μg glucose g^{-1} fruit), total soluble solids in fruits (0.6° Brix), protein levels (6.8 μg protein g^{-1} fruit), total phenols (0.9 mg tyrosine g^{-1} fruit) flavonoids (64.1 μg tyrosine g^{-1} fruit), in relation to mineral fertilization in the fruits (average of both *Physalis* species). **Conclusion:** These results demonstrated that organic fertilization is the best alternative as a source of nutrients for both agronomic and biochemical parameters, ensuring good fruit quality. Both species had good development in the environment conditions where they were cultivated, being alternatives to be exploited by small farmers.

Key words: Flavonoids, organic fertilizer, phenolic compounds, *Physalis* fruits, total and reducing sugars

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The use of fertilizers and soil correctives in fruticulture can represent more than 25% of the production costs. Thinking in cost reduction, the use of organic compounds is a promising alternative to reduce or eliminate the utilization of mineral fertilizers¹. Furthermore, the organic fertilization allows environment sustainability, preserving the natural resources, avoiding water contamination and recycling materials and wastes available in the farm, which economically enables this activity².

In Brazil, the fruticulture has had more and more economic expression, with wide expansion possibilities and diversification of crops due to the favorable weather conditions and crop diversification. In this context, the growth of small-fruit crops is getting popular due to the promotion of their nutraceutical properties, increasing the interest of people who looking for healthier food¹.

The genus *Physalis*, member of the plant family Solanaceae, includes about 120 species³ and despite of this diversity, only five genus are cultivated: *P. pruinosa*, *P. longifolia*, *P. angulata*, *P. philadelphica* and *P. peruviana*⁴ being the last one, the only genus produced in commercial scale, which the top producer is Colombia, followed by others like Kenya, Australia and Peru⁵. In Brazil, the production is not significant but in some states, such as Santa Catarina, Minas Gerais and Rio Grande do Sul, it is increasing⁶. It is an herbaceous and shrubby plant, with annual cycle. However, depending on the weather conditions, especially in subtropical zones, some species can be considered perennial⁷. Its fruits are covered by a capsule and have significant amounts of vitamin A, B and C, besides minerals, tocopherol and carotenoids^{8,9}. Recent studies have demonstrated the antioxidant activity, primarily by the presence of phenolic compounds and ascorbic acid¹⁰. Some species are standing out by the production of poly oxygenated compounds, known as physalis, which has biologic activity¹¹.

Therefore, the aim of this study was to evaluate both agronomic and biochemical parameters of fruits from two *Physalis* species: *Physalis peruviana* L. and *P. pubescens* L., subjected to mineral and organic fertilization.

MATERIALS AND METHODS

The *Physalis* seeds were obtained from *P. peruviana* L. and *P. pubescens* L. plants maintained in greenhouse at UTFPR, Câmpus Pato Branco, Paraná (PR), Brazil. In order to obtain seedlings, three seeds of each species were sowed in 250 mL plastic cups, which contained fertile humus as growing media.

The plants were maintained in a green house, with an average temperature of $24 \pm 2^\circ\text{C}$, where the watering was done manually every day, in early morning and in late afternoon, trying to avoid overwatering and water deficiency. After 20 days, the thinning was done, leaving only one seedling per cup. After 50 days after sowing, when the seedlings reached 15 cm of height, they were transplanted to the experiment location, with a spacing of 3.0×0.5 m. Each experimental unit consisted of 12 plants and borders rows were not used for the evaluations.

The cultural practices consisted of manual watering, keeping the soil at its field capacity, weed control, mainly at the early stages of development, with manual hoeing and pest and diseases monitoring, without the need of any control method. When the plants were 30 cm tall, they were supported using bamboo stakes.

The experiment was conducted from 2013-2014 in Itapejara d'Oeste-PR, located at $26^\circ 3' 30.54''$ S and $52^\circ 48' 7.06''$ O, altitude of 530 m, where the climate is classified as Cfa (humid subtropical climate), according to Köppen. The average temperature during the experiment was 23.5°C (the data were collected daily at the experiment location). The soil was analyzed, before planting and, with soil corrections, presented pH 5.8 and base saturation (V%) above 70%.

The experimental design used was randomized blocks, with two species, three treatments and five blocks, being characterized as a factorial experiment (2×3) with two qualitative variables.

Each *Physalis* received three treatments: control (without fertilizer), mineral fertilizer (N) and organic fertilizer (poultry litter), considering previous assays with 300 kg ha^{-1} of N, where the fertilizers were applied directly in the planting rows. For the mineral fertilization, the nitrogen source used was urea (45% N). The organic material was obtained from the farm where the experiment was conducted. This material was composted during 90 days before its utilization and it was chemically analyzed at the end of this period, presenting 2.62% of nitrogen, 1.50% of phosphorus, 2.29% of potassium, 2.25% of calcium and 0.51% of magnesium.

In the mineral treatment, the values of phosphorus and potassium were based on the ones determined in the poultry litter and the complementation was done with single superphosphate (18% P_2O_5) e potassium chlorate (60% K_2O). The plant fructification has started approximately 70 days after planting. The fruits were daily collected when the capsules were of yellow coloration. After obtaining the average weight of each fruit, they were stored in ultra-freezer (-38°C) and posteriorly, processed in order to obtain the juice using a mixer.

The juice was filtered to determination of Total Soluble Sugars (TSS) levels, by the phenol-sulfuric method¹² and Reducing Sugar (RS) as described by Miller¹³, using glucose (anhydrous D-glucose PA ACS-Vetec) as standard, Total Soluble Proteins (TSP) by the Bradford method¹⁴, with albumin from bovine serum (Sigma), Total Phenolic Compounds (TPC)¹⁵ and flavonoids¹⁶ with curve pattern of tyrosine (L-tyrosine 98.5%-Vetec), all of these all these utilizing spectrophotometer (Shimadzu UV-1800); ascorbic acid (Vitamin C), by titration¹⁷, pH and total soluble solids in fruits (°Brix), by direct measurements using pH meter (Tecnopon) and digital refractometer (Instrutherm), respectively. The values were expressed in mass per gram of fruit (μg or mg g^{-1} of fruit).

Statistical analysis: The statistical analysis of the data was performed using Genes software version 2013.5.1.

Comparison of mean was performed using Duncan and Tukey's tests ($p < 0.05$)¹⁸.

RESULTS AND DISCUSSION

Experimental results showed that fruits from plants cultivated with organic fertilizer (poultry litter) presented significantly higher amounts of Total Soluble Sugar (TSS) ($p < 0.005$) than fruits from plants under mineral fertilizer and control group. Furthermore, plants of *P. peruviana* presented a higher increment in the synthesis of TSS in comparison with *P. pubescens* plants (Fig. 1a). Regarding to the mineral fertilizer, it was possible to observe a significantly increase of TSS concentration in fruits ($p < 0.005$) when compared to the control group but when comparing the effects of the mineral fertilizer treatment on both species, there were not

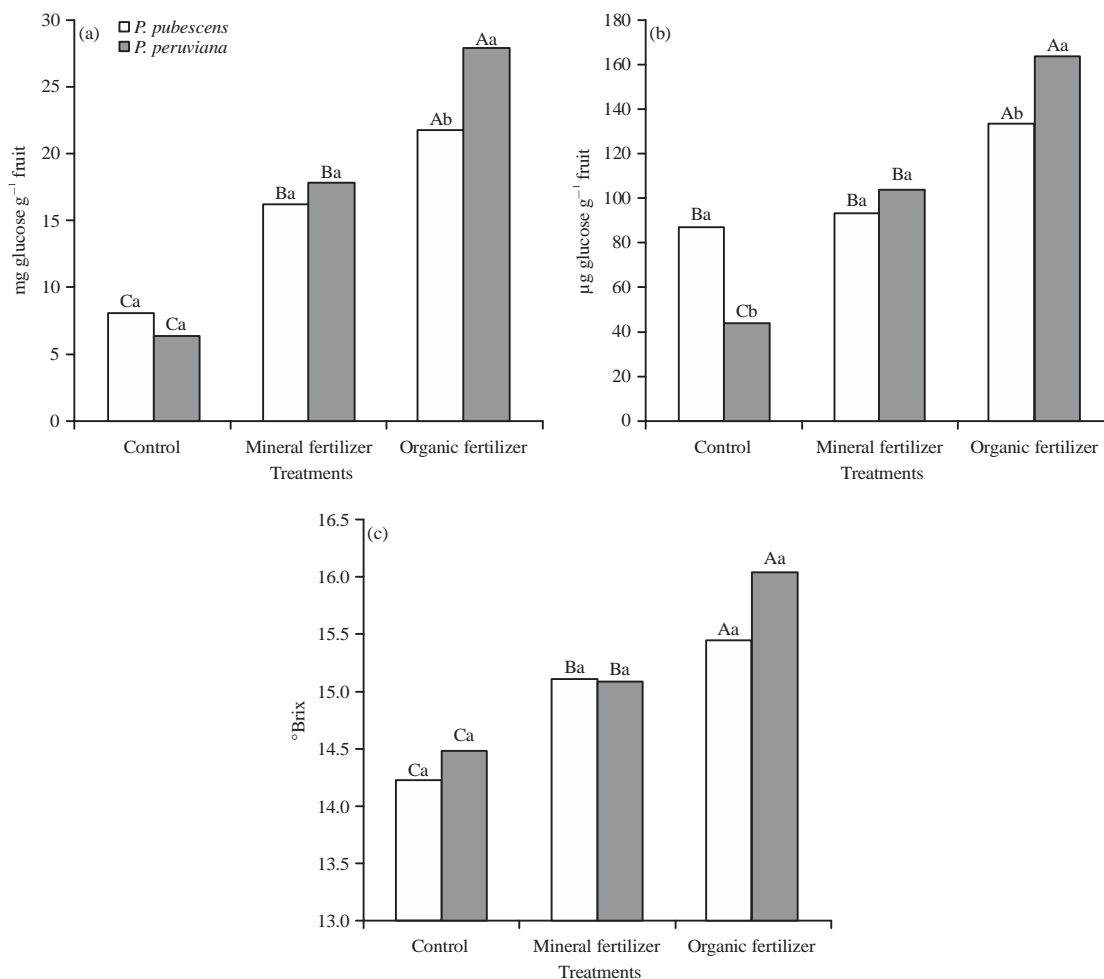


Fig. 1(a-c): (a) Total soluble sugars, (b) Reducing sugar and (c) Total soluble solids, in two species of *Physalis*, under different nutritional sources

Means followed by capital letter are comparing the different nutritional sources and means followed by lowercase are comparing the two *Physalis* species (Duncan's test, $p < 0.05$)

significantly differences. Both species presented the similar results, increasing the concentration of TSS, when compared with the respective control groups but with no significant differences. In the treatment with no fertilizer (control group), despite the low content of TSS, the plants of *P. pubescens* stood out but if no significant difference with *P. peruviana* (Fig. 1a). In a greenhouse environment, the plants under mineral fertilization presented higher content of TSS in *P. pubescens* when compared with *P. peruviana*¹⁹.

As well as to TSS, the organic fertilization allowed a higher Reducing Sugar (RS) content, which significantly differed of mineral and the control group ($p < 0.005$). Again, *P. peruviana* had better results under organic fertilization than *P. pubescens* (Fig. 1b). However, when they were cultivated with no fertilization (control group), the species *P. pubescens* stood out, possibly because it is more rustic and able to respond a relatively low nutrient level. These results are in accordance with those obtained with TSS because, in the same growing conditions, there were increases, which were not significant ($p < 0.005$) but showed similar results in both cases. Furthermore, *P. pubescens*, without fertilization, showed significantly higher ($p < 0.005$) sugar levels, probably due to its natural capacity of growing, once it is considered a weed in many regions of Brazil²⁰.

The sugar levels present in the fruits can vary among species and within the same one, depending on the weather conditions, soil fertility, season, fruit maturity stage and other factors. In the organic fertilization treatment, was possible to observe a higher photosynthetic capacity of the plants and, consequently, a higher accumulation of sugars in the fruit possibly, because in this treatment, the nutrient amount was greater and was able to supply the nutritional plant's needs more efficiently.

The reducing sugars are represented, mainly, by the monosaccharide glucose and fructose, which are the first stable organic compounds synthesized by the plant during the photosynthetic process²¹, being their levels in the fruit influenced by the growing conditions where the plant is cultivated.

As a complementation, the total soluble solids test ($^{\circ}$ Brix) of the fruits was carried out with means of 14.3° Brix (control group), 15.1° Brix (mineral fertilization) and 15.8° Brix (organic fertilization), with significant differences ($p < 0.005$) among the treatments but not among species (Fig. 1c). In a study performed by Muniz *et al.*²², was also observed higher levels of total soluble solids in fruits of *P. peruviana* cultivated with organic fertilization (14.87° Brix), with a cattle and swine manure mixture, whereas the mineral fertilization presented the lowest value, 13.73° Brix, with no significant difference of the control group (13.90° Brix).

In consonance with the rule of Codex Stan²³, *Physalis* fruits must present a minimum total soluble solids level of 14° Brix in order to be commercialized. Thus, the results analyzed in this study demonstrate that the fruits would be in accordance with the commercialization rules of one of the main *Physalis* producer and exporter countries in the world. The determination of Total Soluble Proteins (TSP) in the fruits did not present any significant difference ($p < 0.005$) between the two species, differing only by the nutritional sources used. Again, the organic fertilization was more efficient in the inducing of photosynthesis synthesis, when compared to the mineral fertilization and control group (Fig. 2a). Regarding the proteins, is possible to observe a different behavior, where *P. pubescens* had higher values, even though there were not significant differences ($p < 0.005$) among the species. These results seem to be related to the capacity of this species and its rusticity.

It is possible to affirm that the protein concentrations are relatively low, in accordance with the studies made by Licodiedoff *et al.*²⁴, who analyzed fruits of *P. peruviana*, concluding that the fruit is not rich in proteins as well as lipids and minerals. However, De Oliveira *et al.*²⁵ found protein levels of 0.85% in fruits of *P. angulata* and they compared with fruits of guava and pineapple, which presented values of 0.6 and 0.4%, respectively, demonstrating that the protein content in other different *Physalis* species can be higher than very known and consumed fruits by the population.

In other studies, Kinupp and de Barros²⁶ analyzed many plants and fruits in relation to the protein content and they found in *P. angulata* and *P. pubescens*, levels of 6.9 and 10.35% (dry basis), respectively. Regarding to the analyzed fruits in the same author's study, them presented relatively low levels but they overcome the levels of many other fruits. Other authors found 1.66% of protein in *P. peruviana*, cultivated on field²⁷.

Regarding to the determination of pH of fruits, none significant difference ($p < 0.005$) was observed due to the type of fertilization, being the values among species and treatments between 3.68 and 3.76. Muniz *et al.*²² also did not observed any significant differences when analyzing the pH of juice from plants under chemical and mineral fertilization. Thus, the nutrition cannot influence in this fruit's trait.

For the ascorbic acid levels (Vitamin C), no significant differences ($p < 0.005$) among the two species evaluated were observed but increases occurred according to the nutritional sources. The species of *P. pubescens* under organic fertilization responded with higher production of ascorbic acid than *P. peruviana*, even with no significant differences between the sources (Fig. 2b). Similar results were found by

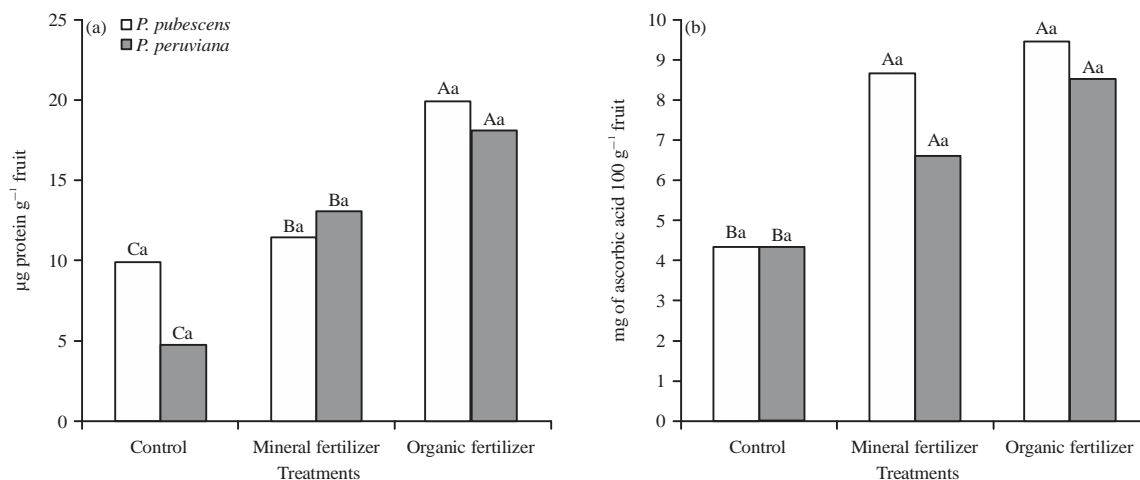


Fig. 2(a-b): (a) Protein levels and (b) Ascorbic acid in two *Physalis* species, under different nutritional sources
Means followed by capital letter are comparing the different nutritional sources and means followed by lowercase are comparing the two *Physalis* species (Tukey 5%)

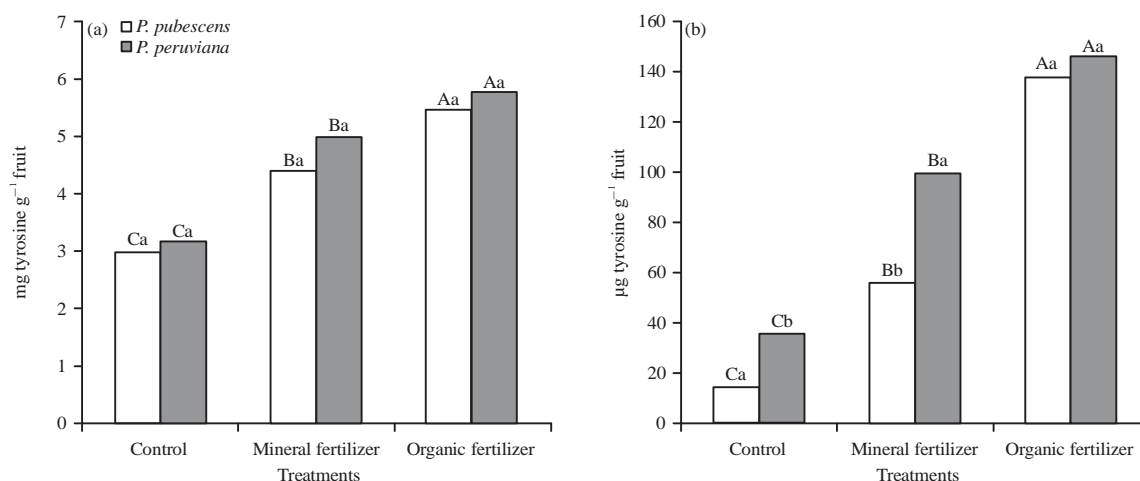


Fig. 3(a-b): Levels of (a) Total phenols and (b) Flavonoids, in two *Physalis* species, under different nutritional sources
Means followed by capital letter are comparing the different nutritional sources and means followed by lowercase are comparing the two *Physalis* species (Tukey 5%)

Muniz *et al.*²², evaluating the effects of mineral and organic fertilization on acid ascorbic levels in *P. peruviana*. The vitamin C amount found in this study, which was in average of $8.31 \text{ mg of ascorbic acid in } 100 \text{ g}^{-1}$ of fruits was much inferior than those described for other authors: Licodiedoff *et al.*²⁴ obtained $102.68 \text{ mg in } 100 \text{ g}^{-1}$ of fruits of *P. peruviana* and Moura *et al.*²⁸, $43.30 \text{ mg } 100 \text{ g}^{-1}$ of fruits. The low amounts of vitamin C found in the *Physalis* fruits is probably due to the loss occurred during the storage period, at low temperatures. According to Campelo *et al.*²⁹, ascorbic acid is unstable in the presence of some factors such as oxygen, light, temperature and enzymes. In their study, the authors noticed the vitamin C degradation at cold storage in fruits of acerola (*Malpighia glabra* L.), once there was and increasing decrease

from the eight days of freezing up to twelve months. Therefore, it is likely that the maintenance of fruits for relatively long periods was responsible for the loss of that compounds. Furthermore, the vitamin C levels tend to decrease according to the fruit ripeness, being influenced by the weather and cultivation conditions, soil humidity and species³⁰.

Due to the importance of the phenolic compounds, these analysis in *Physalis* fruits according to the fertilization used, showed interesting results. The organic fertilization allowed higher production of those compounds in comparison to the mineral fertilization and control group but there was not significant difference among the studied species (Fig. 3a). Hakkinen and Torronen³¹ noticed that the content of total

phenolics and ellagic acid were higher in strawberries organically cultivated. Besides, higher significant levels of some phenolic acids and total polyphenols were also found in peaches and pears cultivated in organic systems, in comparison with those conventionally cultivated³².

Among the phenolic compounds, the flavonoids have great importance once their biological activities-nutritional, medicinal, antioxidant, etc. The organic fertilization was responsible for higher phenolic compounds levels but with no significant difference among species, where *P. peruviana* presented slightly higher levels of flavonoids than *P. pubescens* (Fig. 3b). The flavonoids are substances present in most plants and in all parts. About 2% of the fixated carbon by the plants are converted into these substances, which is one of the biggest groups of natural phenols. Many kinds of this substance are present in *Physalis*, such as glycosides and flavones and flavonols aglycones²⁴.

According to the results presented, the treatments demonstrated the importance of the nutrients source for fruit production, because with the increasing demand for food organically produced and with high nutritional values, it is essential to search for cost reduction and products within certain standards. Another important topic, is about the functional aspect of some organic molecules, among them, the phenolic compounds, due to its activities, specially antioxidant.

CONCLUSION

The organic fertilization allowed good results to the biochemical parameters, ensuring good quality of the fruits of both species of *Physalis*. These results, make even more possible the production in a commercial scale, once it reduces the costs, resulting in a sustainable production, using resources, which in most cases, are available in the farm, avoiding wastes. Besides the economic gains, it is important to highlight the benefits to the environment, once the inappropriate disposal of the organic matter from the poultry litter, as well as the substitution of the mineral fertilization by the organic will determine less environmental contamination, mainly of the water sources.

SIGNIFICANCE STATEMENT

This study discovered the possible positive effects of organic fertilization on biochemical characteristics of two *Physalis* species. This study will make even more possible the production in a commercial scale, once it reduces the costs,

resulting in a sustainable production, using resources which are available in the farm, avoiding wastes and make this fruit more accessible to everyone.

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