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PHYSICOCHEMICAL ANALYSIS OF BRAZILIAN SPINACH GROWN UNDER DOSES OF UREA

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ABSTRACT - The *Althernanthera sessilis* L. is a non-conventional food plant (PANC) popularly known as Brazilian spinach, rich in minerals and proteins, contributing to human nutrition. In view of the above, the present work aimed to analyze physicochemically Brazilian spinach plants cultivated under doses of nitrogen, in the form of urea. The experiment was carried out at the Experimental Garden of the Universidade Federal do Acre (UFAC), from November 25, 2017 to February 3, 2018 and the laboratory analyzes were carried out at the Unidade de Tecnologia de Alimentos (UTAL/UFAC). The delimitation used was completely randomized, containing 4 treatments (0; 1.5; 3.0 and 5.0 g), five replications and one plant per replication. The vegetative material was obtained in Porto Velho (RO) and the seedlings obtained by cuttings were cultivated in 500 mL polyethylene cups for 15 days and then transferred to pots with a capacity of 9 L. On February 3, leaves and stems of the plant were collected and analyzes were performed on a wet basis, except ash, such as pH, titratable acidity, ascorbic acid, soluble solids and proteins. Significant differences were observed for pH, titratable acidity, ascorbic acid and proteins and a linear increase of the contents with the increase of the urea dose. Nitrogen fertilization up to a dose of 5 g was efficient for the variables of titratable acidity, proteins and ascorbic acid. The highest dose of urea provided an increase of about 24% in protein content, however, even without fertilization, Brazilian spinach showed great potential in terms of protein content, being able to be consumed to supply part of the protein demand.
Keywords: *Alternanthera sessilis* L., nitrogen, PANC, protein.

ANÁLISES FÍSICO-QUÍMICA DE ESPINAFRE-DA-AMAZÔNIA CULTIVADO SOB DOSES DE UREIA

RESUMO - A *Althernanthera sessilis* L. é uma planta alimentícia não convencional popularmente conhecida como espinafre-amazônico ou orelha-de-macaco, rica em minerais e proteínas, contribuindo para nutrição humana. Diante do exposto, o presente trabalho teve como objetivo analisar físico-quimicamente plantas de espinafre-da-Amazônia cultivadas sob doses de nitrogênio, na forma de ureia. O experimento foi realizado na Horta Experimental da Universidade Federal do Acre (UFAC), de 25 de novembro de 2017 a 3 de fevereiro de 2018 e as análises laboratoriais na Unidade de Tecnologia de Alimentos (UTAL/UFAC). O delineamento utilizado foi inteiramente casualizado, contendo 4 tratamentos (0; 1,5; 3,0 e 5,0 g), cinco repetições e uma planta por repetição. O material vegetativo foi obtido em Porto Velho (RO) e as mudas obtidas por estaquia foram cultivadas em copos de polietileno de 500 mL, por 15 dias e em seguida transferidas para vasos com capacidade de 9 L. No dia 3 de fevereiro foram coletados folhas e caules da planta e efetuadas as análises em base úmida, exceto cinzas, como pH, acidez titulável, ácido ascórbico, sólidos solúveis e proteínas. Foram verificadas diferenças significativas para pH, acidez titulável, ácido ascórbico e proteínas e aumento linear dos teores com a elevação da dose de ureia. A adubação nitrogenada até a dose 5 g, se mostrou eficiente para as variáveis de acidez titulável, proteínas e ácido ascórbico. A maior dose de ureia proporcionou um incremento de cerca de 24% no teor proteico, porém, mesmo sem adubação o espinafre-amazônico mostrou-se com grande potencial com relação ao teor de proteína, podendo ser consumido para suprir parte da demanda proteica.
Palavras-chave: *Alternanthera sessilis* L., nitrogênio, PANC, proteína.

INTRODUCTION

The vegetables are food plants that have nutritional importance, as they are a source of carbohydrates, vitamins, fibers and proteins (BRASIL, 2010). However, even with all the health benefits, the population's eating habits are based on industrialized products, which leads to a diet rich in sugars and fats, and poor in essential nutrients, culminating in the elevation of diseases such as diabetes, heart diseases, as well as a state called “hidden hunger” in which essential nutrients for human metabolism are not consumed (SOUZA et al., 2012).

Brazil has the world's greatest biodiversity of vegetable crops, with more than 46,000 species cataloged (FLORA DO BRASIL, 2022), most of the wealth is found in the Amazon, however, only a small portion of this biodiversity is known in the scientific world. Among all this

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range, there are non-conventional food plant or PANC, defined by Kinupp and Barros (2008), as those considered weeds that emerge in the field of production spontaneously, in backyards, non-anthropic places or are parts of other species such as stems, leaves, flowers and roots. These species, also known by traditional communities as native, spontaneous, wild or exotic, have already demonstrated high nutritional potential, however, they are not part of the commercial production chain. Some plants, however, gained space on the consumer's table, such as sorrel (*Rumex acetosa* L.) (SILVA et al, 2018), yam (*Dioscorea alata* L.) (SIQUEIRA et al., 2014) e ora-pro-nóbis (*Pereskia aculeata* MILL) (PAULA et al., 2016).

Among the non-conventional vegetables, a species with great nutritional potential, however, little studied is *Althernanthera sessilis* L., belonging to the Amaranthaceae family, popularly known as Brazilian spinach or sissoo spinach. Cultivated on a small scale, it is marketed mainly in large fairs in states in the North region, such as Amazonas and Pará (KINUPP; LORENZI, 2014).

The plant is considered a perennial herb, with an erect or decumbent stem, reaching 30 cm in height. Propagation is done by cuttings that can be rooted or not, coming from the stem. In beds, plants should be grown in the shade in order to produce larger and more tender leaves (KINUPP; LORENZI, 2014). The species comes from tropical and humid regions and has dark green and naturally curled leaves, measuring 1-6 cm in length, elliptical, small, white and sessile flowers (GOMES, 2020), and can be used both in food and medicinally (GNARAJ et al. 2011).

The demand of vegetables for nitrogen is due to being a constituent of several metabolic processes, in addition to being in greater concentration in the dry matter (DIJKSTRA et al., 2017). Deficiency due to low amounts causes chlorotic older leaves that soon spread throughout the plant, reducing roots, dry matter and growth (CAETANO et al., 2015).

Given the presented, this work aimed to analyze physicochemically and bromatologically Brazilian spinach plants, grown in pots under doses of nitrogen, in the form of urea.

MATERIAL AND METHODS

The experiment was carried out in the Experimental Garden, belonging to the Universidade Federal do Acre (UFAC), Rio Branco (Acre), under the geographical coordinates: latitude 9° 57' 35” S, longitude 67° 52' 08” W and altitude of 150 m. According to the Köppen classification, the climate of the region is *Aw* type (hot and humid), with average temperatures of 21°C to 31ºC, average annual precipitation of 1,648.94 mm and average relative humidity of 83% (PEEL et al., 2007).

The seedlings of Brazilian spinach (*Alternanthera sessilis L.*) were obtained in the municipality of Porto Velho (RO), through cuttings and planted in 500 ml plastic cups containing commercial substrate on November 25, 2017 for rooting. After 15 days, they were transplanted to 9 L polyethylene pots, containing commercial substrate, and placed on benches under shading with a 50% shade screen. Because it is a non-commercial and rustic species, the necessary cultural treatments were few, requiring only the application of natural insecticide Neem oil, applied every 15 days of cultivation, in the afternoon, at 3:00 pm, to control the attack of defoliating caterpillars and the manual removal of weeds, according to their appearance, verified every three days. Irrigation was performed daily in two shifts, during the morning (8:00 am) and afternoon (4:00 pm).

The experiment was carried out in a completely randomized design, containing four treatments and five replications, each repetition composed of one plant. The treatments consisted of the application of 4 concentrations of urea in the nitric form, granulated + control (without fertilization), being: T0 (0.0 g/pot), T1 (1.5 g/pot), T2 (3.0 g/pot) and T3 (5.0 g/pot), performing only one topdressing fertilization, with the respective treatment doses, on January 17, 2018, during the morning (8:00 am).

On February 3, 2018, samples of leaves and stems were collected in the morning and taken to the Unidade de Tecnologia de Alimentos (UTAL), of the Universidade Federal do Acre, to carry out physicochemical analyses, according to the norms of the Instituto Adolfo Lutz (IAL, 2008). Subsequently, the samples were crushed and arranged in the treatments. All analyzes were performed in triplicate, on a wet basis, except for ash. The physicochemical analyzes were: pH, soluble solids, ash, ascorbic acid and titratable acidity of proteins.

The pH determination was performed using 5 mL of the sample, added to 50 mL of distilled water, and the reading was determined using a digital potentiometer previously calibrated with standard solutions of pH 7.0 and 4.0. The analysis of soluble solids (SS) was determined by using two drops of the liquid sample, without dilution, to determine the direct reading in a digital refractometer, previously calibrated with distilled water, with a range from 0 to 32 and expressed in ºBrix.

The ash (mineral) content was determined by carbonizing 2.5 g of the crushed sample, placed in a porcelain crucible, then incinerated in a regulated muffle furnace at a temperature of 600ºC, until reaching constant mass, obtained on a precision balance. The ascorbic acid content was determined by weighing 5 mL of the liquid sample in an Erlenmeyer flask, to which 20 mL of sulfuric acid (20%) was added, followed by the addition of 1 mL of potassium iodide (0.1 M) and 3 mL of starch (1%) and titrated with potassium iodate (0.1 M) until reaching a pink color.

The titratable acidity was obtained by weighing 5 mL of the liquid sample in a Becker, later being transferred to a 125 mL Erlenmeyer flask, adding 50 mL of distilled water. Using five drops of 1% phenolphthalein, titration with sodium hydroxide solution (0.1 M) was performed until reaching a dark brown color. The protein content was obtained by determining the percentage of total nitrogen in the sample, according to the Kjeldahl method, then transformed by the conversion factor 5.75 to vegetable protein, according to Anvisa Resolution n.360, of December 23, 2003 (BRAZIL, 2003).

The data obtained were tabulated and submitted to the normality and homogeneity test, using the Shapiro-Whilk and Bartlet tests, respectively. Afterwards, they were submitted to analysis of variance and to the quantitative data applied to regression, at 5% error probability, using the statistical program Sisvar (FERREIRA, 2011).

RESULTS AND DISCUSSION

Table 1 shows a significant difference at 5% error probability for pH, titratable acidity, ascorbic acid and proteins, while for soluble solids and ash there was no significant difference. In relation to pH, a linear reduction in the values was observed, with the increase in the used concentrations of urea (Figure 1). This result is directly linked to the titratable acidity, as the two variables are inversely correlated, that is, when the titratable acidity is increased, there is a reduction in pH (CHITARRA; CHITARRA, 2005). The pH is measured by the hydrogenic concentration of the solution, allowing the evaluation of spoilage, growth of microorganisms, taste and odor of the food. However, even with the reduction of pH, the values remained in the alkaline range.

TABLE 1 - Physicochemical and chemical characteristics in the composition of leaves and stems of Brazilian spinach plants, cultivated under urea concentrations.

Variables	Treatments				CV(%)
	T0	T1	T2	T3	
pH	8.03 a*	7.76 ab	7.84 ab	7.63 b	2.50
Titratable acidity	1.61 c	1.94 bc	2.33 b	2.91 a	10.51
Soluble solids (°Brix)	2.66 a	2.14 a	2.82 a	2.50 a	24.05
Proteins (g 100 g^{-1})	19.50 b	21.53 ab	24.7 a	25.03 a	11.58
Ascorbic acid (mg 100 g^{-1})	23.07 b	24.45 b	39.04 a	42.30 a	14.52
Ashes (g 100 g^{-1})	12.27 a	12.48 a	10.41 a	12.05 a	9.87

*Means followed by the same letter on the line do not differ statistically from each other by the Tukey Test, at 5% error probability. T0 = (0.0 g/urea/pot), T1 = (1.5 g/urea/pot), T2 = (3.0 g/urea/pot) and T3 = (5.0 g/urea/pot). CV = coefficient of variation.

Viana et al., (2015) working with four non-conventional vegetables, such as purslane (*Portulaca oleracea*), Indian spinach (*Basella alba*), slender amaranth (*Amaranthus viridis*) and goldfish plant (*Nematanthus gregarius*), found that all of them presented in the analysis physics, pH<7.0. In the evaluation of fresh leaves of ora-pro-nóbis, Martinevski et al., (2013) found pH=5.10, a value considered low acid. The pH in the alkaline range is a desirable characteristic from a commercial point of view, influencing on the flavor, as the less acidic, the less bitter the vegetable leaf will be (PEREIRA et al., 2016).

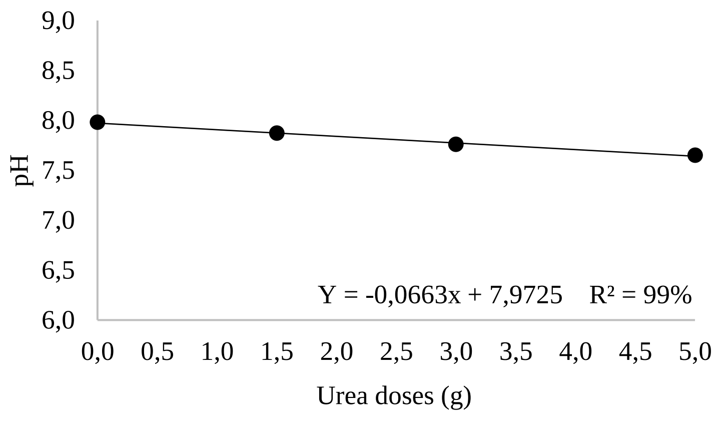

FIGURE 1 - pH of samples of leaves + stalks of Brazilian spinach plants, cultivated under urea concentrations.

For titratable acidity, the results showed that Brazilian spinach presented levels ranging from 1.61 to 2.91 g 100 g^{-1}, with a linear increase in relation to the increase in urea concentrations (Figure 2). With the addition of nitrogen to the substrate, the levels of organic acids present in the plant also increase. The values were similar to those found by Silva et al. (2013), who found levels of 1.26 and 2.23 g 100 g^{-1} for types I and II of sorrel, respectively. In the present work, the presence of bacteria on the leaves was not evaluated, but it is known that most vegetables have low acidity, being more susceptible to deterioration by microorganisms (CHITARRA; CHITARRA, 2005).

For the contents of soluble solids, there was no significant difference between treatments. Meinerz et al. (2021) found mean values of 4.45°Brix and 3.94°Brix for organic arugula. The value of soluble solids is calculated by the constitution of sugars (mainly sucrose, fructose and glucose) and other substances in smaller proportions (pectins, phenolics, vitamins, salts, acids, amino acids and organic acids) (LUCENA et al., 2007).

Regarding the protein content, a significant difference can be observed between the treatments, with a linear increase according to the increase in urea concentrations (Figure 3). Comparing the averages of the treatments (Table 1) it was verified that the T3 treatment was superior to the others, therefore, the highest dose of urea provided a greater increase of protein to the plant.

The results were promising, as Brazilian spinach showed high levels, up to 25 g 100 g^{-1}. It is worth mentioning that, even in T0 (control), presenting 19.5 g 100 g^{-1} of protein was higher when compared to other vegetables, conventional or non-conventional (Table 2). Still in Table 2, it is observed that New Zealand spinach has levels very close to those found in Brazilian spinach plants, thus evidencing the great nutritional potential of these vegetables. When compared to lettuce, the most consumed vegetable in the world, it is observed that Brazilian spinach, grown with the addition of 5 g of urea, has protein levels 10 times higher. According to Kinupp and Barros (2008) proteins from animal origin overlap in relation to those of plant origin, but they are more expensive to acquire. Thus, it is possible to use Brazilian spinach to complement the food diet.

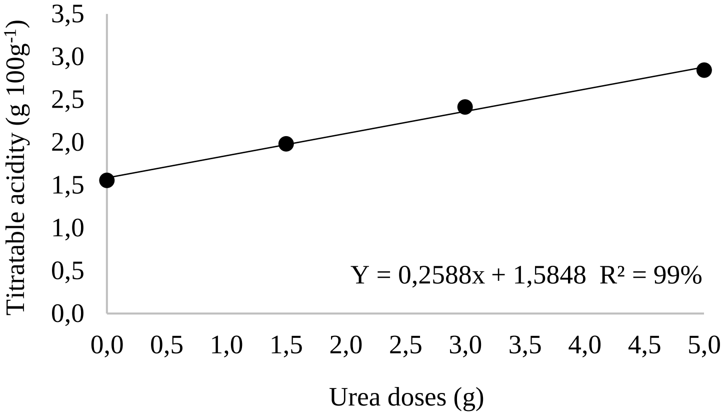

FIGURE 2 - Titratable acidity (g 100 g^{-1}) of leaves + stems of Brazilian spinach plants, cultivated under urea concentrations.

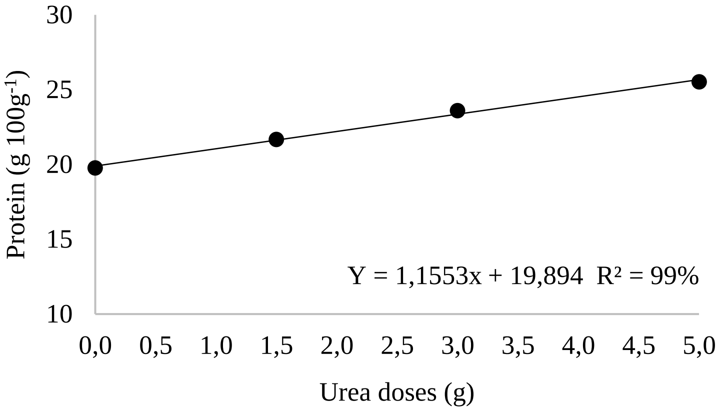

FIGURE 3 - Protein content of leaves + stalks of Brazilian spinach plants, cultivated under urea concentrations.

TABLE 2 - Comparison between protein contents of some vegetables, with the appropriate references.

Vegetables	Protein contents (%)	Sources
Type I sorrel	15.82	SILVA et al. (2013)
Type II sorrel	15.88	SILVA et al. (2013)
Purslane	12.82	VIANA et al. (2015)
Indian spinach	17.44	VIANA et al. (2015)
New Zealand spinach	24.70	NEPA (2011)
Ora-pro-nóbis	28.59	TAKEITI et al. (2009)
Lettuce	2.29	PEREIRA et al. (2016)

As for the ascorbic acid content, there was a significant difference between the treatments, at a 5% error probability ($p<0.05$), due to the increase in urea concentrations (Figure 4). The results presented in this work differ from those performed by Moraes (2006), who observed a reduction in vitamin C when the nitrogen concentration was increased, this was possibly due to the constant growth of the plant which consequently, dilutes the vitamin in plant tissues. The daily need for vitamin C is estimated at 45 mg (FAO, 2000), so Brazilian spinach, despite having intermediate values of vitamin C, is able to meet this need for an adult.

As for the ash content, there was no significant difference between the treatments, therefore, the urea concentration did not influence the amount contained in the leaves + stalks of Brazilian spinach. Thus, the amount of ash found represents a significant value, exceeding the percentage found in cauliflower and broccoli leaves, being 1.38 ± 0.00a and 0.80 ± 0.00a, respectively. The ash of an aliment is the inorganic residues that remain, after the burning by incineration of organic matter (HOFFMANN et al., 2021). Even with favorable results for the variables analyzed, it is necessary to carry out further work with the species, as it is an non-conventional food plant, there are no published works regarding its cultivation and propagation.

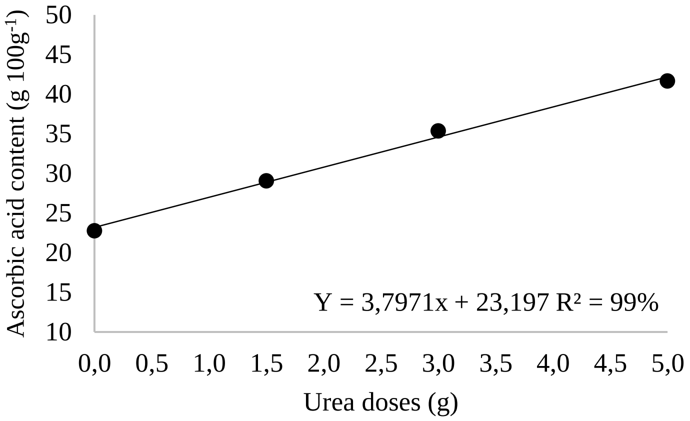

FIGURE 4 - Ascorbic acid content of leaves + stems of Brazilian spinach plants, cultivated under urea concentrations.

CONCLUSIONS

Nitrogen fertilization up to a dose of 5 g was efficient for the variables of titratable acidity, proteins and ascorbic acid.

The highest dose of urea provided an increase of about 24% in protein content, however, even without fertilization, Brazilian spinach showed great potential in terms of protein content, being able to be consumed to supply part of the protein demand.

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