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ACCUMULATION OF NITRATE AND VITAMIN C CONTENT IN CURLY LETTUCE PRODUCED IN CONVENTIONAL AND HYDROPONIC SYSTEMS, SOLD IN THE PUBLIC MARKETS OF SÃO LUÍS-MA

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ABSTRACT
Food quality is essential for food and nutritional security, covering its production, availability, and promotion of consumer health. Thus, the main objective of the research was to determine the accumulation of nitrate content in curly lettuce produced in hydroponic and conventional cultivation and sold in public markets in São Luís – Maranhão. 60 lettuce samples were collected in August 2024 in the Public Markets of six neighborhoods in São Luís: Cohab, Vicente Fialho, São Francisco, Liberdade, João Paulo, and Santo Antônio. Five samples of hydroponic lettuce and five of conventional lettuce were obtained in each neighborhood. The samples were packed in identified plastic bags and sent to the Beverage Laboratory of the Federal Institute of Maranhão for analysis of the characteristics: number of leaves, total fresh mass, soluble solids, ascorbic acid, and nitrate. The results indicate that the lettuce produced in the hydroponic and conventional systems in São Luís-MA is of good quality for consumption, without risk to health. The hydroponic system resulted in higher production of total fresh mass and number of leaves,

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while lettuces from conventional cultivation showed lower nitrate concentration. However, the vitamin C content was higher in the lettuce of the hydroponic system.

Keywords: Production. Nutritional Security. Ascorbic acid. Nitrate.

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INTRODUCTION

Lettuce cultivation can be carried out through four main systems, each with different management practices: the conventional system, organic cultivation in the open field, protected cultivation in the soil, and the hydroponic system (Filgueira, 2013).

When comparing the different lettuce production systems, it is observed that each one has characteristics that can influence both the productivity and the chemical properties of the vegetable, such as the levels of nitrate present in the leaves. This variation may be related to the different sources of nitrogen used in the fertilization of each cropping system (Silva *et al*., 2011).

In the hydroponic system, fertilization is done with nitrate, while in conventional cultivation, amide fertilization is used. In the organic system, the application of ammonia-based organic fertilizers predominates. In high concentrations, nitrate, which is converted to nitrite in the human and animal body, can increase the risk of disease and, in severe cases, lead to death (Barth *et al*., 2019).

The accumulation of nitrate in the cell vacuole is influenced by genetic and environmental factors, such as the concentration of ions in the nutrient solution, irradiance, molybdenum availability, temperature, relative humidity, cultivation system, time and time of harvest, with the concentration of ions and irradiance being the most determining factors (Alvarenga *et al*., 2000; Maynard *et al*., 1976).

According to Oliveira & Hoffmann (2015), for a food to be considered safe, it must be totally free of substances or chemicals that may threaten human health. In addition to pesticide contamination, there is also the danger of changes in the chemical composition of plants, such as an increase in the concentration of nitrate, a carcinogenic substance.

Currently, there is a growing concern with food quality, especially in relation to sanitary, organoleptic, and nutritional aspects. One of the main points of attention in nutritional characteristics is the concentration of nitrate in lettuce due to its potential risk to human health (Luz *et al*., 2008). Food quality is a fundamental aspect of food and nutritional security, involving not only the production and availability of food but also the promotion of consumer health (Mendonça *et al*., 2014).

Thus, the research aimed to determine the accumulation of nitrate and vitamin C content in curly lettuce produced in hydroponic and conventional cultivation and sold in public markets in São Luís – Maranhão.

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METHODOLOGY

The experiment took place in the municipality of São Luís, Maranhão, located at coordinates 2º36'35.94" S and 44º15'52.02"W, at an altitude of 34 meters. The average annual temperature in the region is 27ºC, with an average of 2000 mm per year. The local climate is classified as B1 WA, considered humid, with a slight water deficit during the winter between the months of June and September (GEPLAN, 2002).

The lettuces were collected in August 2024, during the morning, in Public Markets located in six neighborhoods in the city of São Luís: Cohab, Vicente Fialho, São Francisco, Liberdade, João Paulo, and Santo Antônio. In each of these neighborhoods, five samples of lettuce grown by the hydroponic system and five samples of lettuce produced by the conventional method were obtained, totaling 60 samples in all. After collecting the lettuce, they were packed in previously identified plastic bags and sent to the Beverage and Water Laboratory of the Federal Institute of Maranhão for analysis.

According to Teixeira *et al.* (2020), evaluating the following characteristics:

- **Number of Leaves (NF) and Total Fresh Mass (MFT)**

To evaluate the TFM, a precision scale was used, and an entire plant was weighed to obtain the TFM. In the same plant, the leaf count was then carried out, considering the total value of leaves from the plant contained in each sample.

- **Soluble Solids (SS) °Brix**

For the evaluation of Soluble Solids, a fresh lettuce leaf from each sample was macerated in a Becker to extract the solution, from which with the aid of a dropper, 0.15mL (3 drops) was removed and the reading was performed in a refractometer on the scale of 0-30%.

- **Vitamin C**

The concentration of vitamin C in the form of ascorbic acid was determined according to the methodology used by the Adolfo Lutz Institute (2008). To carry out the procedures recommended in the methodology, 3 g of the fresh lettuce sample was weighed on an analytical scale (model: AW220), and with the aid of a glass rod, the sample was macerated until a paste-like material was obtained. Then, 50 mL of distilled water were added, stirring well until a homogenized sample was obtained.

To this sample, 10 mL of 20% H2SO4 (sulfuric acid) solution was added and then transferred to an Erlenmeyer solution. Then, 1 mL of the 10% KI (Potassium Iodide) solution and 1 mL of the 1% starch solution were added. It was titrated with the Potassium

Iodate (KIO3) solution to a bluish color. To obtain a safe and consistent result, the analyses were repeated in triplicate.

The equation used for the final result of Vitamin C was:

$$\frac{100 \times V \times Fc}{P} \text{mg\%} \quad \text{(Equation 1)}$$

Where

V= volume of potassium iodate spent on titration;

Fc=8.806 or 0.8806, respectively for KIO3 0.02M or 0.002M;

P No. of gram or mL of the sample.

- **Nitrate Content**

The extraction of nitrate from lettuce leaves was carried out using the method of Follett & Ratcliff (1963), for this the upper parts of the lettuce plants were removed, packed in identified paper bags and placed for drying in a forced circulation oven at 60 °C for 48 hours, then the samples were crushed in a blender (Philco 800W). To the ground dry matter (0.5 g), 50 mL of distilled water was added, and the solution was kept in a water bath, with magnetic stirring and heating at a temperature of approximately 60 °C for 01 hour. The agitation was not continuous but rather divided into periods of agitation of 05 minutes, followed by 15 minutes of rest. The samples were filtered on a slow-filtering quantitative filter paper.

On the other hand, the determination of nitrate present in the plants was carried out through the "Salicylic Acid Procedure" (Cataldo *et al.*, 1975), in which aliquots of 0.2 mL of extract received 0.8 mL of salicylic acid solution (C7H6O3) at 5%, after 20 minutes at room temperature, 19 mL of 2 mol NaOH was added. L-1 slowly with a pipette to raise the pH to basic (above 12).

After the addition of NaOH, heat was released in the reaction, so the samples were cooled again to room temperature and analyzed in a visible spectrophotometer (DR/2000V, Quimis®) at a wavelength of 410 nm, along with its calibration curve. This was done from known patterns of NaNO3 that had the same treatment given to the samples. Nitrate concentrations were calculated by inserting nitrate absorbance values into the calibration curve.

Calculation used for the final result of the N-NO3^{-} content: (Equation 2)

µg x 25/0, 1 x 1 / Sample Weight (Result in Fresh Weight µg N-NO3^{-}/g)

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Standard: µg $N\text{-}NO_3^-$/0.1mL

Alcohol Extract Volume: 25 mL

To construct the standard nitrate curve, the following concentrations were used: (µg $N\text{-}NO_3^-$/0.1mL) 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0. From the stock solution, the readings were performed using a spectrophotometer of the brand (Spectrophotometer - SP 2000 UV).

The standard curve was constructed by means of a linear regression obtained between the concentration values and the mean absorbances of the standard solutions. For this, the Excel 2010® software was used, which allowed the calculation of the surface coefficients and the determination of the line investigation.

- **Data analysis**

The data obtained were submitted to an analysis of variance using Tukey's test at a 5% probability level. The analyses were carried out using the AgroStat software (Barbosa; Maldonado, 2015).

RESULTS AND DISCUSSIONS

According to the analysis of variance (Table 1), it was observed that the trait, Number of Leaves, was significantly influenced by the cropping systems, while the vitamin C content was not influenced by the cropping systems. The hydroponic cultivation system influenced the characteristics: Total Fresh Mass, Soluble Solids and Nitrate content, while the conventional cultivation system did not influence the characteristics: MFT, Soluble Solids and Nitrate content. However, the Coefficient of Variation showed good experimental precision; values lower than the CV indicate a greater uniformity between the data.

Table 1. Average values observed for the number of leaves, total fresh mass, soluble solids, vitamin C, and nitrate content in lettuce grown in conventional and hydroponic cultivation systems, marketed in São Luís – MA, 2024.

			Features		
Cultivation systems	**NF** (leaf plant-1)	**MFT** (g plant-1)	**Soluble Solids** (%)	**Vitamin C** (mg.100g-1)	**Nitrate** (µg.g-1)
Conventional F1	21.00 abs	400.80a	2.00 to	27.27 to	33.77 to
Conventional F2	21.00 abs	395.80 to	2.10 to	27.29 to	31.35 to
Conventional F3	17.00 b	344.40 to	1.80 to	26.63 to	31.94th
Conventional F4	22.00 abs	383.60 to	2.10 to	26.61 to	32.73th
Conventional F5	23.00 abs	435.20 to	1.40 to	26.99 to	33.85 to

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Conventional F6	26.00 to	416.80 to	2.30 to	26.86 to	32.46 to
CV%	13,64	17,53	31,75	3,74	6,35
F	4,33**	01 ^{NS}	1.29 ^{NS}	0.44 ^{NS}	1.14 ^{NS}
Hydroponic F1	26.00 to	385.00 abs	2,320 b	29.21 to	37.13 to
Hydroponic F2	18.00 b	280.40 b	3.00 AB	28.88 to	37.43 to
Hydroponic F3	24.00 abs	369.60 abs	2.96 abs	29.09 to	37.57 to
Hydroponic F4	23.00 abs	434.60 to	3.08 to	28.88 to	33.34 b
Hydroponic F5	18.00 b	360.20 abs	2.56 abs	29.02 to	33.72 b
Hydroponic F6	25.00 to	443.80 to	2.84 abs	29.17 to	34.93 b
CV%	14,46	14,53	13,30	0,83	2,92
F	6,01**	5,77**	3,14*	1.75 ^{NS}	17,18**

Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at the 5% probability level. Where ^{NS} = not significant; * significant at 5% probability by the F-Test.

The number of leaves varied between 17.00 leaves $^{plant-1}$ to 26.00 leaves $^{plant-1}$, observing in the plants from fair 6 of the conventional system and in fair 1 of the hydroponic system, with the highest number of leaves (26.00 leaves $^{plant-1}$, respectively) and the smallest number of leaves (17.00 leaves $^{plant-1}$), was observed in plants acquired at fair 3, from the conventional system. The cultivation method and the environment affect the yield of the cultivars, with specific advantages for the hydroponic system and the greenhouses (Blat *et al*., 2011).

A similar result was verified by Ferreira (2024) evaluating the performance of curly lettuce cultivars grown in a hydroponic system, the Caipira cultivar with 22.4 leaves per plant.

Smaller results were observed by Ribeiro & Ferreira (2023) in a hydroponic system; they observed 17.63 leaves per plant and in the conventional system 13.80 leaves per plant. These results suggest that the cultivar and the cultivation method affect the number of lettuce leaves. Queiroz *et al*. (2014), evaluating five lettuce cultivars grown in the summer, obtained an average value of 17.15 leaves per plant.

Different results, both about the number of plant leaves and the cropping system, were observed by Teixeira *et al.* (2020). The number of lettuce leaves (46.70) produced in a conventional system was higher than the number of leaves (34.10) of plants produced in the hydroponic system.

According to Diamante *et al*. (2013), the number of leaves is important because it reflects the adaptation of genetic material to the environment and is linked to commercialization. Santos *et al.* (2010) state that the number of leaves can be influenced by the environment, which, in combination with the genetic component, causes physiological and morphological changes in plants.

The highest production of fresh matter of the plant (443.80 g $^{plant-1}$) was obtained by lettuce plants produced in the hydroponic system, acquired at fair 6, followed by plants produced in the conventional system (435.20 g $^{plant-1}$), acquired at fair 5. These results indicate that the cropping system influences the performance of the cultivars.

Different results with lower TFM production were observed by other authors. Teixeira *et al*. (2020), evaluating lettuce plants produced in three cropping systems under the climatic conditions of the region of São Luís, MA, obtained MFT of 239.90 g in the conventional system and 201.60 g in the hydroponic system. Ramos *et al*. (2003), evaluating seven cultivars of curly lettuce, observed a total fresh weight of 213.5 g per plant, and Sousa *et al*. (2018) evaluated the performance of loose curly lettuce cultivars grown in the summer and found that the total fresh mass ranged from 291.8 to 397.3 g per plant.

Also in table 1, the highest number of leaves was (26.00 leaves $^{plant-1}$) and (25.00 leaves $^{plant-1}$), possibly influenced lettuce plants to a higher production of fresh mass (435.20 g $^{plant-1}$ and 443.80 g $^{plant-1}$), plants produced in the conventional system and hydroponic system, respectively. According to Araújo *et al.* (2011), for the lettuce vegetable, a higher number of leaves per plant provides a greater increase in fresh mass and, thus, greater productivity. For Souza *et al*.. (2014), the relationship between the number of leaves and the production of fresh mass may be associated with the fact that the greater the number of leaves, the greater the photosynthetic capacity of the plant. This results in a higher production of carbohydrates, which are essential for the growth and accumulation of biomass.

According to Table 1, the values observed for oBrix were higher (3.08%) in plants produced in the hydroponic system. Martins *et al*. (2016) state that the content of total soluble solids is strongly affected by environmental factors, such as temperature, fertilization, luminosity, and planting density, among others.

The soluble solids content (oBrix) is considered by Varoquaux *et al.* (1996), as an important attribute for predicting the post-harvest shelf life of lettuce, the higher the soluble solids content of the freshly harvested lettuce, the longer the period in which its quality can be

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preserved, although this is not a quality characteristic, as the consumer does not expect to taste a sweet lettuce, unlike typical dessert fruits.

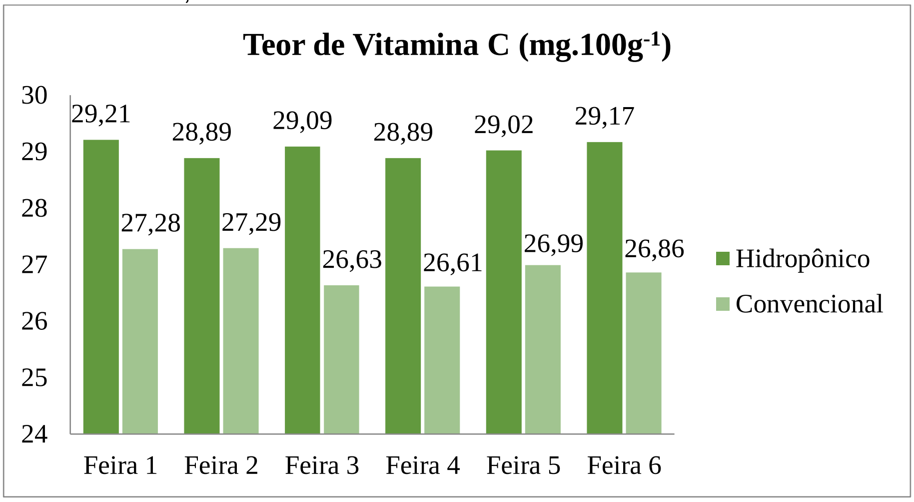
Different results were observed by other authors, Silva *et al*. (2011) evaluating the quality of lettuce Vera, in organic, conventional and hydroponic cultivation systems, found that the best percentage of soluble solids was in the conventional cultivation system 4.0%, And for Teixeira *et al*. (2020), where the plants of the conventional system had the highest content, 3.33%, while the hydroponic system had the lowest content, with 3.04%.

Santos *et al.* (2010), evaluating the quality of lettuce produced in different production systems and marketed in the municipality of Botucatu-SP, observed that the levels of soluble solids did not differ statistically, indicating that it is a product with low levels of sugars and acidity as indicated for the establishment of diets.

According to Moraes *et al*. (2011), lettuce oBrix can increase with cold storage. They noted that average soluble solids rose from 2.88% on the day of harvest to 3.37% after four days of cold storage.

According to Figure 1, the highest levels of vitamin C were observed in the leaves of the plants grown in the hydroponic system (28.89 to 29.21 mg.100g-1), and the lowest levels were observed in the plants of the conventional system (26.61 to 27.29 mg.100g-1). Brecht *et al*. (2010), the acid content in food varies with the variety grown and the growing conditions.

Figure 1. Vitamin C content in curly lettuce plants produced in conventional and hydroponic cropping systems, sold in markets of São Luís-MA, 2024.



Source: the author, 2024.

As Chitarra & Chitarra (2005) report, after harvest, the concentration of organic acids in lettuce usually decreases due to their conversion into sugars. In addition, the

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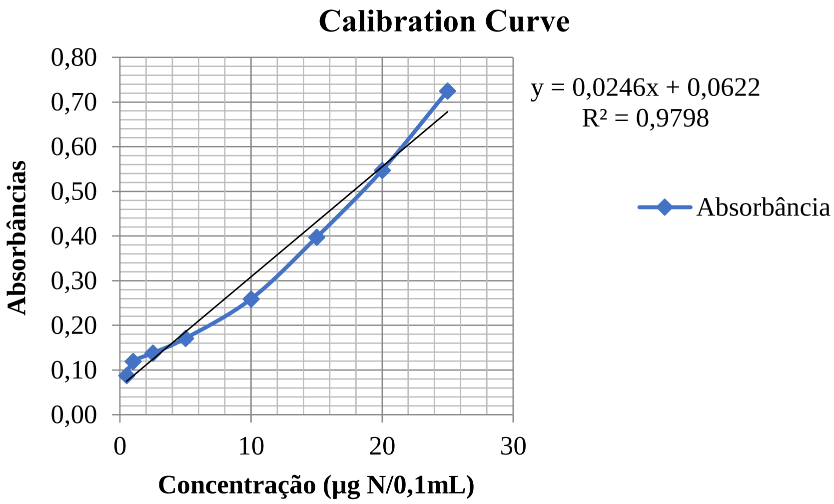
chemical composition of plants varies between species and within the same species, depending on environmental conditions (Taiz; Zeiger, 2004).

Teixeira *et al*. (2020) observed vitamin C levels lower than those observed in this research. They found in their research that the highest content of vitamin C was found in the leaves of the plants of the organic system (14.97 mg/100g), while the lowest content was observed in the plants of the hydroponic system (7.75 mg/100g). The low levels of vitamin C in the conventional and hydroponic systems can be attributed to the immediate availability of nitrogen to the plants.

Sousa (2012) investigated the performance of lettuce cultivars of the smooth, curly, and American types during summer cultivation and found variations in ascorbic acid levels. The curly cultivar showed a vitamin C content of 30.59 mg.100g-1, with no significant differences compared to the other cultivars.

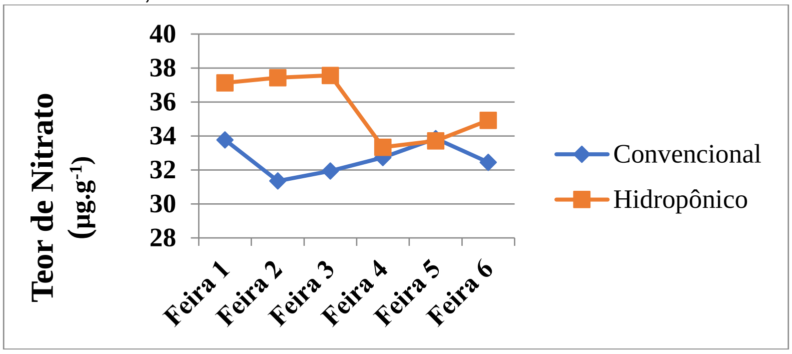
Figure 2 shows the standard calibration curve obtained using the $NaNO_3$ standard; a linear growth between absorbance and concentration is observed. The equation of the standard curve line was determined as y=0.0246x+0.0622, with a correlation coefficient (R2) of 0.9798, a value very close to 1. This coefficient indicates an excellent fit of the curve to the experimental data, which is considered satisfactory for calibration and, therefore, suitable for sample analysis.

Figure 2. Calibration curve obtained with NaNO3 standard in a UV-visible spectrophotometer at a length of 410 nm.



The nitrate contents in lettuce leaves ranged from 31.35 µg.g-1 to 37.47 µg.g-1 in the conventional and hydroponic systems, respectively (Figure 3). These nitrate levels are below the maximum limit allowed for the lettuce vegetable, so the lettuces produced in the two cropping systems studied are considered lettuce of good quality for consumption.

Figure 3. Nitrate levels in curly lettuce obtained by conventional and hydroponic cultivation systems, sold in the public market of São Luís-MA, 2024.



Source: the author, 2024.

According to McCall & Willumsen (1998), the European Community has established maximum permitted nitrate limits for lettuce produced in greenhouses: 3,500 mg/kg for the summer period, 4,500 mg/kg for the winter period, and 2,500 mg/kg for lettuce grown in the open field.

From a nutritional point of view, for a 70 kg individual who consumes, on average, up to 50 g of fresh lettuce per day, and considering that the acceptable daily limit is 3.6 mg $N\text{-}NO3^{-}$/kg of body weight, the nitrate concentration in lettuce would need to be higher than 5000 µg $N\text{-}NO3^{-}$/g of fresh mass to exceed this limit (Cometti *et al.*, 2005).

Santos *et al.* (2010) clarify that the variations in the absolute values of nitrate can be attributed to the methodology employed and to factors such as light intensity, temperature, relative humidity, cultivation time, and harvest time, which affect the accumulation of nitrate in lettuce leaves.

Several authors have also analyzed the nitrate content in lettuce. Felipe & Pereira (2020) observed that the nitrate content ranged from 904.01 $^{mg.kg-1}$ to 29,775.79 $^{mg.kg-1}$. The nitrate content in lettuce grown in hydroponics is much higher than in other crops. Teixeira *et al*. (2020) found that nitrate content in lettuce leaves ranged from 40 µg/g in the organic system to 90 µg/g in the conventional and hydroponic systems, respectively. Sousa (2012) observed that the curly cultivar presented a content of 141.88 $^{mg.kg-1}$, with significant variations about the other cultivars. Silva *et al.* (2011) found that the highest nitrate content was observed in the hydroponic system (331.8 mg/kg) compared to the conventional

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system (113.6 mg/kg). Santos *et al.* (2010) evaluated curly lettuce sold in Botucatu-SP and found low levels of nitrate.

Roorda Vaneysinga (1984) observed significant differences in nitrate contents between cropping systems, highlighting the ability of lettuce to accumulate nitrate in its leaves, especially when grown in hydroponic systems, where the concentration in the nutrient solution is higher.

Relating the nitrate content with the vitamin C content, the research showed that lettuce plants grown in the hydroponic system had higher nitrate levels and consequently had lower vitamin C contents, according to Lee & Kader (2000), who state that fertilizers with high nitrogen rates tend to reduce the vitamin C content in fruits and vegetables. In addition, factors such as prolonged storage, exposure to heat, light, and oxygen, as well as processes such as cooking and freezing, also contribute to this decrease.

The high availability of nitrogen favors the synthesis of proteins and carbohydrates, reducing the use of photoassimilates to produce compounds of secondary metabolism, such as ascorbic acid. In addition, the increase in leaf area due to nitrogen fertilization can decrease the intensity of light in the plant canopy, reducing the production of ascorbic acid in shaded areas, especially in hydroponic systems, where nitrogen is more accessible (Ramos, 2006).

The main factors influencing nitrate accumulation in vegetables are NO3- reduction capacity and excessive nitrate consumption. Stress factors, such as temperature, luminosity, and drought, reduce the plant's ability to reduce $NO3^-$ (Silva *et al.,* 2011).

From a nutritional point of view, for a 70 kg individual who consumes, on average, up to 50 g of fresh lettuce per day, and considering that the acceptable daily limit is 3.6 mg $N\text{-}NO3^-$/kg of body weight, the nitrate concentration in lettuce would need to be higher than 5000 µg $N\text{-}NO3^-$/g of fresh mass to exceed this limit (Cometti *et al.*, 2005).

CONCLUSIONS

- Due to the results obtained in this research, the lettuce produced in the hydroponic and conventional cultivation system sold in public markets in São Luís-MA can be considered of good quality for consumption, not presenting a risk to the health of the consumer.

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- The hydroponic environment provided plants with a higher production of Total Fresh Mass and Number of Leaves. However, with higher levels of nitrate accumulated.
- Lettuce plants grown in the conventional cropping system showed higher quality with lower nitrate concentration. However, the highest vitamin C content was in the hydroponic system.

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