Influence of refrigeration system parameters during storage of Lactuca Sativa l under non-isothermal conditions

Influência dos parâmetros de refrigeração durante armazenamento de Lactuca Sativa l sob condições não isotérmicas

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ABSTRACT

Lettuce (Lactuca sativa L.) is a leaf vegetable rich in vitamins, minerals, fiber and bioactive compounds. Its preservation after harvest usually occurs under refrigeration, slowing the chemical and microbiological reactions. The objective of this study was to verify the influence of the refrigeration parameters (set temperature and oscillation range) on the preservation of hydroponic crispy lettuce (Lactuca sativa L.), variety Solaris, in terms of weight loss and moisture, vitamin C and total chlorophyll contents. Samples were analyzed during 14 days of storage packed in expanded polystyrene (EPS) packaging wrapped with polyvinyl chloride (PVC). Different conditions of set temperature (2 °C, 6 °C and 10 °C) and oscillation range (±1 °C, ±3 °C and ±5 °C) were tested, using a 3² experimental design. The analysis revealed losses in the mass and moisture content after 14 days, with the greatest reductions being observed for the treatments with a set temperature of 10 °C and all oscillation ranges (±1 °C, ±3 °C and ±5 °C). However, the vitamin C and total chlorophyll contents showed variations during storage for all treatments studied. Based on the results of this study, it was concluded that applying a low temperature (2 °C), with all 3 oscillation ranges studied, can prolong the lettuce shelf life.

Keywords: Lettuce, Shelf life, Refrigeration, Set temperature, temperature oscillations, Chemical aspects.

RESUMO

A alface (Lactuca sativa L.) é um vegetal de folhas rico em vitaminas, minerais, fibras e compostos bioativos. Sua preservação após a colheita geralmente ocorre sob refrigeração, retardando as reações químicas e microbiológicas. O objetivo deste estudo foi verificar a influência dos parâmetros de refrigeração (temperatura definida e faixa de oscilação) na preservação da alface crocante hidropônica (Lactuca sativa L.), variedade Solaris, em termos de perda de peso e umidade, vitamina C e total conteúdo de clorofila. As amostras foram analisadas durante 14 dias de armazenamento acondicionados em embalagens de poliestireno expandido (EPS) envolvidas com cloreto de polivinil (PVC). Diferentes condições de temperatura definida (2 °C, 6 °C e 10 °C) e faixa de oscilação (±1 °C, ±3 °C e ±5 °C) foram testadas, utilizando um delineamento experimental de 3². A análise revelou perdas no conteúdo de massa e umidade após 14 dias, sendo observadas as maiores reduções para os tratamentos com temperatura definida de 10 °C e todas as faixas de oscilação (±1 °C, ±3 °C e ±5 °C). No entanto, o conteúdo de vitamina C e clorofila total apresentou variações durante o armazenamento para todos os tratamentos estudados. Com base nos resultados deste estudo, concluiu-se que a aplicação de baixa temperatura (2 °C), com todas as três faixas de oscilação estudadas, pode prolongar a vida útil da alface.

Palavras-Chave: Alface, Prazo de validade, Refrigeração, Temperatura definida, oscilações de temperatura, Aspectos químicos.
1 INTRODUCTION

Lettuce (Lactuca sativa) is a leaf vegetable that is consumed worldwide, with China being the leader in terms of production (Mou, 2008). In Brazil it is considered the most important leaf vegetable, with the consumption of the crispy type predominating (Sala, 2012). Depending on the lettuce type, its composition can be equivalent to vegetables considered nutritious (Kim et al., 2016).

Lettuce can be considered as a food present in the diet of a large part of the population, which has led to advances in the technologies development for the production and maintenance of the quality of this vegetable, to prolong its shelf life (Halbe et al., 2015; Khlaed et al., 2017). For consumers, food quality is defined according to the visual and nutritional characteristic, particularly in the case vegetables, where acceptance is based on general appearance, color, brightness, odor, signs of wilting, texture and nutritional factors (Butz et al., 2005; Clement et al., 2013).

Physical and chemical characteristics, such as mass and moisture loss, can also be used as quality indicators for leaf vegetables stored under refrigeration (Agüero et al., 2011). To maintain the desirable lettuce characteristics for longer after harvesting, refrigeration is used in order to reduce microbiological activity, enzymatic activity and respiration/evapotranspiration, because these factors influence the loss of quality (Rickman et al., 2007).

Water activity and moisture content are directly related to water loss, which results in wilting of the leaves, so it is very important to store vegetables under optimal temperature conditions, between 0 and 4 °C (Agüero et al., 2011; Kader, 2002; Jacxsens et al., 2000, 2002). Parameters such as the relative humidity (RH), air circulation velocity and atmospheric composition in packages are also important factors when dealing with refrigerated storage because, along with the temperature, they affect the chemical, physical, sensorial, microbiological and organoleptic characteristics (Álvares et. al., 2007).

Temperatures between 0 and 2 °C are ideal for storing vegetables, but the temperature must be above the food freezing point. However, care should be taken when regulating the temperature since, in practice, there may be fluctuations (due to the control achieved by the regulator) which can lead to the temperature being higher or lower than required, resulting in unfavorable storage conditions, such as food freezing (Baptista, 2006).

Relative humidity (RH) should also be monitored during the storage period, because low relative humidity inside refrigerators promotes humidity loss in vegetables, causing...
wilting, color changes and alterations in other quality characteristics, such as a reduction in vitamins and the sensory characteristics. In contrast, high relative humidity may favor the multiplication of microorganisms on the food surface, causing changes in texture, color and odor (Agüero et al., 2011; Cantillano, 2013).

In this context, the aim of this study was to evaluate the changes in hydroponic crispy lettuce samples stored in a household refrigerator for 14 days. Different set refrigeration temperatures and oscillation ranges were applied to verify the best conditions in terms of prolonging the lettuce shelf life. The quality parameters evaluated were variations in the mass and the moisture, vitamin C and total chlorophyll contents.

2 MATERIAL AND METHODS

2.1 SAMPLE PREPARATION

Heads of hydroponic crispy lettuce (Lactuca sativa L.), variety Solaris, were harvested in the morning from a production field in Blumenau, Santa Catarina, Brazil. The harvesting was performed at the commercial-point stage, which means that the lettuce heads were fully formed, approximately 30 days after transplanting. After harvesting, the samples were placed in polystyrene boxes and transported to the laboratory.

To perform the experiment, six leaves with similar size and color were selected, with no flaws, and placed in styrofoam packages wrapped with polyvinyl chloride (PVC) film and stored for 14 days in a household refrigerator at specific set temperatures and oscillation ranges.

2.2 EXPERIMENTAL APPARATUS

A Consul refrigerator, model CRD37, with an on/off compressor was used. In order to monitor the internal cabinet temperature and relative humidity, 15 thermocouples (3 on each of the 4 shelves and in the drawer) and 4 humidity sensors (one in the drawer and one on each of 3 different shelves) were installed.

For each experimental treatment, the control of the temperature (minimum to maximum amplitude), acquisition of the temperature data and the monitoring of the internal relative humidity were carried out using the software Labview 15.0. Combinations of set temperatures of 2 °C, 6 °C and 10 °C and oscillation ranges of ±1 °C, ±3 °C and ±5 °C were studied.
2.3 CHEMICAL ANALYSIS

2.3.1 Mass loss

The mass loss was quantified in relation to the initial leaf mass (Agüero et al. 2011). Each lettuce sample was weighed immediately after harvest and on days 3, 5, 7, 10 and 14 using a Sartorius semi-analytical balance with an accuracy of ± 0.01. The results were expressed as percentage of mass loss.

2.3.2 Moisture content

The moisture content was determined by keeping the lettuce samples in an oven at 105 °C ± 2 °C until constant weight (AOAC, 2000).

2.3.3 Vitamin C

The vitamin C content was determined by redox titration of the samples with a solution of 2,6-dichloroindophenol, using the methodology of AOAC (2000). Assays were performed in triplicate and results were expressed in mg of ascorbic acid per 100 grams.

2.3.4 Total chlorophyll

The total chlorophyll content was evaluated during the storage, in triplicate, according to the method of Lichtenthaler (1983), through the pigment extraction with 80% acetone. In this procedure, 0.5 g of crushed lettuce was homogenized with 10 mL of 80% (v/v) acetone. This solution was kept at 3 °C for 24 h, protected from light. After this period the solution was filtered and absorbance readings were taken on a HACH UV-vis spectrophotometer (model DR/2010) at 652 nm, using only acetone as the blank sample. The total chlorophyll content was calculated according to Eq. (1) (Witham, 1971).

\[
\text{Total chlorophyll (mg.g}^{-1}) = \frac{[A_{652} \times 1000] \times (V(1000 \times W))}{34.5} \quad \text{Eq. (1)}
\]

where \(A_{652}\) represents the absorbance reading at a wavelength of 652 nm, \(V\) the volume of the extract after filtration (mL) and \(W\) the weight of the lettuce sample used (g).

2.3.5 Statistical analysis

The results are presented as the mean and standard deviation (SE). The Tukey test to determine an honestly significant difference (HSD) was used to compare the means, at a 5%
level of significance, of the values obtained in the chemical analysis performed at different periods during the refrigerated storage and for each treatment on the days analyzed, to determine the best conditions.

The chemical analysis results were evaluated through analysis of variance (ANOVA) to verify the suitability of the models and analysis of the contour curves. The results were statistically analyzed using the program Statistica 7.0 for Windows, Statsoft.

3 RESULTS AND DISCUSSION
3.1 LETTUCE CHARACTERIZATION (DAY OF HARVEST)

Table 1 shows the chemical characteristics of the lettuce samples “in natura” determined after harvest.

Table 1 - Chemical characterization of Lactuca sativa L. “in natura” on day of harvest (day 0).

| Treatment | Temperature (°C) | Amplitude (°C) | RH (%)   | Mass (g) | Vitamin C (mg.100g⁻¹) | Total Chlorophyll (mg.g⁻¹) | Moisture (%) |
|-----------|------------------|----------------|----------|----------|----------------------|---------------------------|--------------|
| 1         | -1               | -1             | 50 ± 30  | 87.77    | 22.90                | 0.08                      | 94.51        |
| 2         | -1               | 0              | 70 ± 35  | 139.60   | 36.62                | 0.08                      | 95.22        |
| 3         | -1               | +1             | 50 ± 35  | 86.81    | 31.60                | 0.12                      | 94.56        |
| 4         | 0                | -1             | 60 ± 25  | 53.43    | 47.06                | 0.12                      | 94.02        |
| 5         | 0                | 0              | 57 ± 25  | 92.45    | 30.39                | 0.09                      | 96.12        |
| 6         | 0                | +1             | 60 ± 40  | 83.59    | 26.76                | 0.11                      | 95.32        |
| 7         | +1               | -1             | 74 ± 20  | 54.97    | 29.94                | 0.08                      | 95.10        |
| 8         | +1               | 0              | 70 ± 30  | 59.52    | 22.75                | 0.08                      | 96.08        |
| 9         | +1               | +1             | 60 ± 40  | 117.2    | 15.29                | 0.10                      | 96.02        |

The values for the moisture content obtained in this study are similar to those reported by Agüero et al. (2011) (93.94%) and given in the Tabela Brasileira de Composição de Alimentos (2011), that is, 96.10% for the same lettuce variety.

In relation to the vitamin C content, the Tabela Brasileira de Composição dos Alimentos (2011) informs that 100 g of fresh curly lettuce has 15.5 mg of vitamin C. Kim et al. (2016), reported 2.9 mg.g⁻¹ of vitamin C in crisphead lettuce (Lactuca sativa L.), while for baby Romaine they obtained values between 21 and 40 mg.g⁻¹. Morais et al. (2011) observed a vitamin C content of 27.24 mg.100g⁻¹ in lettuce on the day of harvest (similar to the values obtained for treatments 1, 3, 5, 6, 7 and 8).
3.2 MASS LOSS

Table 2 shows that during the 14 days of refrigerated storage, for all treatments, a mass reduction ($p < 0.05$) occurred in the lettuce samples, and from day 5 the decrease in this parameter is higher for all treatments. Up to day 3, the treatments did not show a large variation in mass loss, except treatment 1, for which the mass decreased from 100% to 83.43% ± 0.01 and at the end of the storage period this treatment presented the largest mass reduction (26.11%). At the end of the experiment (day 14), treatment 2 showed an 8.75% reduction, indicating that this is the best set temperature and oscillation range conditions for maintaining the mass of the lettuce samples during the period studied. In comparison, the other treatments had losses above 12% at the end of the study.

| Treatment | Mass loss (%) |
|-----------|---------------|
| 1         | 86.43 ± 0.01  |
| 2         | 97.24 ± 0.04  |
| 3         | 96.2 ± 0.01   |
| 4         | 92.36 ± 0.01  |
| 5         | 99.99 ± 0.01  |
| 6         | 98.27 ± 0.02  |
| 7         | 95.22 ± 0.02  |
| 8         | 96.23 ± 0.03  |
| 9         | 97.26 ± 0.01  |

Table 2 - Mean values for mass loss of hydroponic curly lettuce (Lactuca sativa L.) stored at set temperatures of 2, 6 and 10 °C with oscillation ranges of ±1, ±3 and ±5 °C over 14 days.

* Storage period (days)

* Treatment 1 had a large variation in mass loss, except treatment 1, for which the mass decreased from 100% to 83.43% ± 0.01 and at the end of the storage period this treatment presented the largest mass reduction (26.11%). At the end of the experiment (day 14), treatment 2 showed an 8.75% reduction, indicating that this is the best set temperature and oscillation range conditions for maintaining the mass of the lettuce samples during the period studied. In comparison, the other treatments had losses above 12% at the end of the study.

Agüero et al. (2011), on studying the mass reduction in lettuce (cv. ‘Lores’) stored at 0-2 °C and relative humidity between 70-72%, obtained a mass decrease of around 18% on day 5. França et al. (2015) reported a lettuce mass loss of 31.58% (cv. ‘Vitória de Santo Antão’) on day 6 of storage at 5 °C and Kotsiras et al. (2016) determined a 35.8% mass loss for the same vegetable (cv. ‘Carmesi’) on day 14 at temperatures between 4 and 5 °C.

Leaf vegetables like lettuce are susceptible to water loss from the plant tissue after harvesting, because increases in the respiration and transpiration processes occur, leading to...
mass loss and consequently reduced quality of the product (Wills et al., 2004; Agüero et al., 2011; Kotsiras et al., 2016).

According to reports in the literature, respiration and transpiration rates are increased when storage occurs at inappropriate temperature and relative humidity (6 °C, 10 °C, 15 °C and RH lower than 90%) causing wilting, nutritional loss, color degradation and firmness alteration (Wills et al., 2004; Bico et al., 2009; Kotsiras et al., 2016). These parameters are very important because they influence the consumers’ decision to buy (Fillion, 2000; Chen et al., 2017).

3.3 MOISTURE CONTENT

Moisture loss occurs through the transpiration process, as discussed above, and the results obtained for this parameter can be seen in Table 3.

| Table 3 - Mean values for moisture loss of hydroponic curly lettuce (Lactuca sativa L.) stored at set temperatures of 2, 5 and 10 °C with oscillation ranges of ±1, ±3 and ±5 °C over 14 days. |
|---|---|---|---|---|---|---|
| Storage period (days) | 0 | 3 | 5 | 7 | 10 | 14 |
| Treatment | Moisture (%) | | | | | |
| 1 | 100 ± 0.23 | 99.56 ± 0.23 | 100.24 ± 0.06 | 98.53 ± 0.50 | 100.08 ± 0.14 | 99.73 ± 0.47 |
| 2 | 99.25 ± 0.50 | 99.66 ± 0.34 | 99.20 ± 0.40 | 99.55 ± 0.21 | 99.60 ± 0.13 | 99.22 ± 0.03 |
| 3 | 99.33 ± 0.19 | 99.32 ± 1.00 | 99.08 ± 0.50 | 99.39 ± 0.42 | 99.22 ± 0.03 |
| 4 | 99.83 ± 0.07 | 100.50 ± 0.12 | 100.08 ± 0.10 | 100.22 ± 0.17 | 100.35 ± 0.04 |
There is a clear variation in the moisture content on day 5 of the refrigerated storage under the different conditions of the experimental design. The Tukey test results show that for treatments 2 (decrease of 0.4%) and 3 (decrease of 0.78%) there was no significant difference (p < 0.05) between the means during the 14 days of refrigerated storage. In contrast, the means of the other treatments differed statistically (p < 0.05) throughout the period studied. In treatment 7 there was a 0.98% reduction in the moisture content, indicating that these storage conditions are not suitable for lettuce samples.

On evaluating the treatments on day 14, it was found that the mean results for moisture content for treatments 4, 8 and 1 do not differ statistically from each other. Thus, these are the best storage conditions for lettuce samples in terms of this parameter, retaining 100.35%, 99.92% and 99.73% of the moisture content, respectively.

Agüero et al. (2011) studied lettuce samples stored at 0-2 °C with 70-72% of relative humidity and obtained a decrease of 0.69% of the moisture content on day 5. For samples stored at the same temperature, but with a relative humidity of 95-98%, there was an increase of 0.68% in the moisture content on day 16 of storage, which is consistent with the results in Table 3.

According to Holcroft (2015), leaf vegetables that lose between 3 to 5% moisture begin to wither and this moisture loss can affect vegetables due to water accumulation on their surfaces or in the packaging, causing defects in appearance, including color change and wilting and the development of microorganisms (Linke, 2013).
3.4 VITAMIN C

On day 5 of storage, the vitamin C levels varied significantly (p ≤ 0.05) from 100% to 44.12% in treatment 3. In treatment 4, ascorbic acid was also reduced from 100% (initially) to 57.41% on day 3 and this difference is also statistically significant (Table 4).

Table 4 - Mean values for vitamin C content of hydroponic curly lettuce (Lactuca sativa L.) stored at set temperatures of 2, 6 and 10 °C with oscillations of ±1, ±3 and ±5 °C over 14 days.

| Storage period (days) | 0   | 3   | 5   | 7   | 10 | 14 |
|-----------------------|-----|-----|-----|-----|----|----|
| Treatments            |     |     |     |     |    |    |
| 1                     | 100 ± 0  | 117.99 ± 13.21  | 125.85 ± 5.04 | 111.10 ± 13.43 | 121.69 ± 5.38 | 132.26 ± 57.05 |
| 2                     | 100 ± 0  | 85.47 ± 3.34  | 85.91 ± 3.71  | 83.02 ± 3.38  | 73.28 ± 5.74  | 66.01 ± 3.28  |
| 3                     | 100 ± 0  | 89.54 ± 3.76  | 44.12 ± 0.69  | 42.89 ± 3.07  | 44.11 ± 3.79  | 38.22 ± 1.89  |
| 4                     | 100 ± 0  | 68.61 ± 14.10 | 57.41 ± 11.57 | 68.92 ± 16.77 | 69.03 ± 2.38  | 64.79 ± 11.90 |
| 5                     | 100 ± 0  | 106.05 ± 10.61 | 91.22 ± 7.95  | 96.35 ± 6.83  | 70.93 ± 3.83  | 83.63 ± 13.59 |
| 6                     | 100 ± 0  | 87.75 ± 8.91  | 103.42 ± 7.92 | 91.54 ± 7.69  | 96.93 ± 7.54  | 83.14 ± 7.64  |
| 7                     | 100 ± 0  | 101.71 ± 15.49 | 99.68 ± 3.85  | 93.33 ± 22.72 | 101.54 ± 11.21 | 67.74 ± 3.50  |
| 8                     | 100 ± 0  | 84.31 ± 4.90  | 108.98 ± 16.05 | 104.24 ± 18.37 | 119.31 ± 30.58 | 103.30 ± 9.97  |
| 9                     | 100 ± 0  | 88.16 ± 0.10  | 91.00 ± 3.96  | 91.39 ± 4.16  | 79.41 ± 4.10  | 80.60 ± 6.71  |

Different lowercase letters on the same line indicate a significant difference at the 5% level according to the Tukey test
Different uppercase letters on the same line indicate a significant difference at the 5% level according to the Tukey test
Column 0 = control; Mean and standard deviation in triplicate

When evaluating the treatments in terms of the degradation of this vitamin, it can be noted that there is no significant difference at the 5% level between the mean values for treatments 1, 8, 6 and 9 on day 14. This indicates that under the conditions of these four...
treatments the vitamin C content of the curly lettuce samples is maintained. In these treatments the final contents are higher than the initial values, due to the loss of moisture increasing the vitamin C concentration in the lettuce (Scalon et al., 2012).

Kim et al. (2016) obtained 2.9 mg.g\(^{-1}\) of vitamin C in curly lettuce (cv. 'Batavia'), while for baby lettuce (cv. 'Mini Romaine') the values were between 21 and 40 mg.g\(^{-1}\). Morais et al. (2011) observed vitamin C contents of 27.24 mg.100g\(^{-1}\) on the day of harvest (a value similar to those obtained in treatments 1, 3, 5, 6, 7 and 8) and 25.11 mg.100g\(^{-1}\) on day 4 of the storage of hydroponic lettuce (cv. ‘Olinda’) at 7.6 ± 1 °C and 27 ± 5% RH. These values are close to those found on the same day for treatments 3 and 6. As noted by Ohse et al. (2001), lettuces cultivated in hydroponic systems present a higher vitamin C content.

According to Scalon et al. (2012), the increase in this vitamin observed in some treatments may be related to the mass reduction (dehydration) increasing the ascorbic acid concentration in the vegetables. Vitamin C oscillations observed in this study could be related to instability due to sensitivity to light, oxygen, heat and physicochemical agents and to vegetable processing (Klein, 1987). A reduction in the vitamin C content of foods can also occur due to the presence of enzymes, such as ascorbic acid oxidase, since these are vulnerable to the oxidizing and enzymatic agents present in vegetables (Phillips et al., 2016).

The presence of oxygen, metal ions, alkaline pH, light, high temperatures and mechanical stress such as cuts (plant tissue rupture) favors the vitamin C degradation process by non-enzymatic oxidation and/or an enzymatic process (Phillips et al., 2016). Some of these factors, such as higher storage temperatures and cutting of the plant tissue, may be responsible for the reduction in the vitamin C content of the crispy lettuce samples observed in this study. Vitamin C stability increases at lower temperatures, as observed in treatments 1 and 4; however, in some cases a reduction can occurs during frozen storage or at very low temperatures (Pinedo, 2007), as in the case of treatments 2 and 3, where negative temperatures were reached during the refrigerated storage.

### 3.5 TOTAL CHLOROPHYLL

In relation to the variation in the chlorophyll content of the lettuce samples, it can be observed that the treatments, except for 3 and 8, showed a significant difference at the 5% level over the storage time (Table 5).
Table 5 - Mean values for total chlorophyll of hydroponic curly lettuce (*Lactuca sativa* L.) stored at temperatures of 2, 6 and 10 °C and amplitudes of ±1, ±3 and ±5 °C over 14 days.

| Storage period (days) | Treatment | Total chlorophyll (%) |
|-----------------------|-----------|-----------------------|
|                       | 0         | 3                     | 5         | 7                     | 10        | 14        |
| 0°                    | 100 ± 125.96 ± 3.94<sub>bc</sub> | 117.59 ± 3.46<sub>abc</sub> | 103.05 ± 12.41<sub>ABC</sub> | 136.73 ± 10.53<sub>ab</sub> | 155.46 ± 21.87<sub>a</sub> |
| 0°                    | 100 ± 116.59 ± 31.02<sub>ab</sub> | 125.24 ± 29.52<sub>AB</sub> | 163.03 ± 12.30<sub>ab</sub> | 125.60 ± 33.69<sub>AB</sub> | 121.01 ± 5.65<sub>b</sub> |
| 0°                    | 100 ± 97.07 ± 16.55<sub>ab</sub> | 123.65 ± 13.74<sub>ABC</sub> | 117.52 ± 8.62<sub>ABCD</sub> | 119.95 ± 9.05<sub>AB</sub> | 96.05 ± 12.91<sub>bCD</sub> |
| 0°                    | 100 ± 70.78 ± 21.78<sub>ab</sub> | 67.27 ± 6.47<sub>ab</sub> | 71.61 ± 19.27<sub>bc</sub> | 54.11 ± 7.77<sub>aD</sub> | 37.23 ± 3.25<sub>D</sub> |
| 0°                    | 100 ± 78.98 ± 14.47<sub>ab</sub> | 76.28 ± 20.44<sub>ab</sub> | 107.50 ± 11.51<sub>bc</sub> | 106.71 ± 15.33<sub>ab</sub> | 90.02 ± 8.95<sub>bCD</sub> |
| 0°                    | 100 ± 78.99 ± 2.85<sub>bc</sub> | 107.90 ± 6.35<sub>ab</sub> | 88.68 ± 4.97<sub>bCD</sub> | 136.35 ± 11.69<sub>a</sub> | 69.41 ± 11.86<sub>bCD</sub> |
| 0°                    | 100 ± 94.64 ± 11.57<sub>bc</sub> | 88.86 ± 8.64<sub>bCD</sub> | 142.47 ± 9.95<sub>ab</sub> | 123.08 ± 11.88<sub>ab</sub> | 95.82 ± 8.34<sub>bCD</sub> |
| 0°                    | 100 ± 100.95 ± 19.84<sub>ab</sub> | 84.17 ± 10.24<sub>ab</sub> | 108.87 ± 12.45<sub>bCD</sub> | 84.51 ± 25.32<sub>bc</sub> | 76.17 ± 23.32<sub>bCD</sub> |
| 0°                    | 100 ± 119.57 ± 31.39<sub>ab</sub> | 147.96 ± 14.17<sub>ab</sub> | 107.01 ± 38.29<sub>bCD</sub> | 19.33 ± 5.73<sub>bCD</sub> | 85.95 ± 5.73<sub>bCD</sub> |

Different lowercase letters on the same line indicate a significant difference at the 5% level according to the Tukey test. Different uppercase letters on the same line indicate a significant difference at the 5% level according to the Tukey test. Column 0 = control; Mean and standard deviation in triplicate.

Treatment 1 offered the best conditions in terms of maintaining the total chlorophyll content, with a value of 155.46%. This treatment did not differ significantly from treatment 3, with a total chlorophyll content of 121% on day 14. Treatment 5 also showed an increase in this pigment at the end of the storage period, with a value of 104.52%, and the mean had no significant difference (p < 0.05) with treatment 3, indicating that these set temperatures and oscillation ranges are also good conditions for the conservation of total chlorophyll in the vegetable under study (Table 5).
Agüero et al. (2011) evaluated lettuce (cv. ‘Lores’) and found initial values of approximately 0.59 mg.g\(^{-1}\), which differs from the results reported herein and during 20 days of storage at 0-2 °C and 95-98% relative humidity the total chlorophyll content showed little change. The values for chlorophyll content differ according to the species and the genotypes of the same species (Lee, 1988). Shen et al. (2016) observed an increase in the total chlorophyll content of wheat leaves of approximately 0.25 mg.g\(^{-1}\) during storage.

According to Streit et al. (2005), negative temperatures tend to increase the precipitation of food proteins due to a decrease in the pH and thus the rates of acid catalysis reactions (pheophytinization) are increased. An increase in the chlorophyll content could also be due to the formation and synthesis of the compound pheophytin, which has a characteristic olive green color.

A tendency toward a loss in chlorophyll content can occur due to the activation of the chlorophyllase enzyme and in treatments with a considerable loss of chlorophyll it has been verified that the metabolic activity is accelerated. When a vegetable is exposed to some type of mechanical stress, in this case, the cutting of the vegetable tissue, organic acids are released, which decrease the pH and the activity of enzymes that increase the degradation rate of the protein-chlorophyll complex is stimulated (Darezzo, 2004). This process may have occurred in our study, since the treatments with higher levels of chlorophyll correspond to those with higher pH values (treatments 3, 5, 9).

The depigmentation of some lettuce leaves in an unsaturated atmosphere may be related to a greater exposure to light, oxygen and low RH. In addition, leaves with higher moisture loss favor increased chlorophyll loss (Agüero et al., 2011).

The contour surface (Figure 1) shows that the best sample preservation conditions may occur on the minimum set temperature and oscillation range limits studied, with temperatures between 2 °C and 3.5 °C with oscillation ranges of ±1 °C at ±1.5 °C resulting in a lower mass variation (20%) after 14 days of refrigerated storage. However, according to Streit et al. (2005) and Pinedo (2007), in practice, some of these conditions may lead to a decrease in vitamin C and total chlorophyll, causing consumer rejection due to chemical losses, due to low storage conditions.
4 CONCLUSIONS

The hydroponic crispy lettuce samples conditioned in EPS packaging with PVC film evaluated showed higher mass variations during storage applying treatments 2 (set temperature of 2 °C with oscillation range of ±3 °C) and 3 (set temperature of 2 °C with oscillation range of ±5 °C), with mass preservation values of 91.24% and 85.86%, respectively. The remaining moisture content was 99.82% in treatment 8 and 99.73% in treatment 1, indicating that both treatments favored the maintenance of this parameter in the samples studied during 14 days of refrigeration.

The vitamin C content showed a variation over the period of 14 days in all treatments, but the conditions which led to the lowest vitamin fluctuation was treatment 1. The total chlorophyll was conserved at the lowest temperature investigated, that is, in treatments 1, 2 and 3 (2 °C ± 1 °C, 2 °C ± 3 °C, 2 °C ± 5 °C, respectively).

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