

Influence of packaging on the conservation of quality attributes of lamb's-ear leaves during storage

Influência da embalagem na conservação dos atributos de qualidade das folhas de peixinho durante o armazenamento

Influencia del embalaje en la conservación de los atributos de calidad de las hojas de oreja de conejo durante el almacenamiento

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Abstract

The objective of this study was to evaluate the influence of the packaging system on the useful life and post-harvest quality of lamb's-ear leaves (*Stachys byzantina* L.). The sanitized sheets were packaged in four different packaging systems: (A) rigid polyethylene (PET), (B) PET together with ethylene-absorbing sachet, (C) PET wrapped with high barrier plastic film, and (D) PET together with an ethylene-absorbing sachet and wrapped with high barrier plastic film and stored at 7 °C. Samples for physical-chemical analysis, bioactive compounds and antioxidant activity were removed before storing and at 5, 10 and 15 days of storage. The leaves contained in packages C showed less loss of mass and in system D they showed greater preservation of color attributes (L*, C* and °H). The packaging did not influence the contents of bioactive compounds, which showed higher values at 5 days of storage. The leaves stored in A, B, C and D showed high antioxidant capacity until the 15th day of storage. It was concluded that the quality attributes of the lamb's-ear leaves were kept in all packaging systems throughout 15 days of cold storage. The contents of bioactive compounds and antioxidant activity showed the highest values up to 5 days of storage at 7 °C. Thus, considering the economic aspects and practicality in handling, packaging A is the most suitable for refrigerated storage (7 °C) and commercialization of fresh lamb's-ear leaves up to 15 days of storage.

Keywords: *Stachys byzantina* L.; Bioactive compounds; Natural antioxidants; DPPH; ABTS.

Resumo

Objetivou-se avaliar a influencia do sistema de embalagem sobre o tempo de vida útil e na qualidade pós-colheita de das folhas de peixinho (*Stachys byzantina* L.). As folhas sanitizadas foram acondicionadas em quatro diferentes sistemas de embalagem: (A) polietileno rígido (PET), (B) PET em conjunto com sachê absorvedor de etileno, (C) PET envolta com filme plástico de alta barreira, e (D) PET em conjunto com sachê absorvedor de etileno e envolta com filme plástico de alta barreira e foram armazenadas à 7°C. As amostras para as análises físico-químicas, de compostos bioativos e atividade antioxidante foram retiradas antes do armazenamento e aos 5, 10 e 15 dias de armazenamento. As folhas contidas nas embalagens C apresentaram menor perda de massa e no sistema D mostraram maior preservação dos atributos de cor (L*, C* e °H). As embalagens não influenciaram nos teores de compostos bioativos, que apresentaram maiores valores aos 5 dias de armazenamento. As folhas armazenadas em A, B, C e D apresentaram alta capacidade antioxidante até o 15º dia. Conclui-se que os atributos de qualidade das folhas de peixinho foram mantidos em todos os sistemas de embalagem durante os 15 dias de armazenamento refrigerado. Os teores de compostos bioativos e a atividade antioxidante apresentaram os maiores valores até 5 dias de armazenamento a 7 °C. Assim, considerando os aspectos econômicos e praticidade no manuseio, a embalagem A é a mais indicada para armazenamento refrigerado (7 °C) e comercialização de folhas de peixinho *in natura* até 15 dias de armazenamento.

Palavras-chave: *Stachys byzantina* L.; Compostos bioativos; Antioxidantes naturais; DPPH; ABTS.

Resumen

El objetivo de este estudio fue evaluar la influencia del sistema de empaque en la vida útil y la calidad poscosecha de las hojas de oreja de conejo (*Stachys byzantina* L.). Las hojas desinfectadas se empaquetaron en cuatro sistemas de empaque diferentes: (A) polietileno rígido (PET), (B) PET junto con una bolsita absorbente de etileno, (C) PET envuelto con una película de plástico de alta barrera y (D) PET junto con un sobre absorbente de etileno y envuelto con película plástica de alta barrera y almacenado a 7 °C. Las muestras para análisis físico-químico, compuestos bioactivos y actividad antioxidante se retiraron antes del almacenamiento y a los 5, 10 y 15 días de almacenamiento. Las hojas contenidas en los paquetes C mostraron menor pérdida de masa y en el sistema D mostraron mayor preservación de los atributos de color (L *, C * y °H). El envasado no influyó en el contenido de compuestos bioactivos, que mostraron valores superiores a los 5 días de almacenamiento. Las hojas almacenadas en A, B, C y D mostraron alta capacidad antioxidante hasta el día 15. Se concluye que los atributos de calidad de las hojas de oreja de conejo se mantuvieron em todos los sistemas de empaque durante los 15 días de almacenamiento refrigerado. Los contenidos de compuestos bioactivos y actividad antioxidante mostraron los valores máas altos hasta los 5 días de almacenamiento a 7 °C. Así, considerando los aspectos económicos y la practicidad en el manejo, el envase A es el más adecuado para almacenamiento refrigerado (7 °C) y comercialización de hojas de oreja de conejo fresco hasta 15 días de almacenamiento.

Palabras clave: *Stachys byzantina* L.; Compuestos bioactivos; Antioxidantes naturales; DPPH; ABTS.

1. Introduction

With the growing search for healthier eating habits by the population, it is necessary to introduce new foods in the diet and some that have already been part of it, but at some point have lost space in the market for other foods.

Unconventional vegetables are vegetables that grow spontaneously, alone or among other crops. They are regional vegetables that are part of the culture of traditional populations, with species that are not organized in a productive chain as the conventional vegetables. Most of the time, these plants are treated as weeds for agronomically standing out over conventional vegetables, due to their high rusticity, requiring few cultural practices along their cultivation, and higher cost-benefit ratio, meeting even the poorest classes of society thanks to its low cost of acquisition (Tomou et al., 2020; Kinupp & Lorenzi, 2014; Brasil, 2010). Consequently, they are eliminated from the production field and remain unknown by the population, who neglects their food and nutritional importance.

These unconventional vegetables include lamb's-ear (*Stachys byzantina* L), which belongs to the genus *Stachys*, family Lamiaceae, and is an herbaceous, perennial plant that grows in clumps and is widespread throughout the world, mainly in regions of mild climate of Europe and Asia. Under Brazilian conditions, it hardly blooms, so it is usually propagated vegetatively (Souza et al., 2021; Silva et al., 2021; Tomou et al., 2020; Pirbalouti & Mohammadi, 2013; MAPA, 2010). However, despite being unknown by most of the Brazilian population, lamb's-ear has a high nutritional value and can be a source of mineral salts, vitamins, carbohydrates, lipids, proteins and phenolic compounds (Bahadori, 2020; Brasil, 2019; Viana

et al., 2015). It is considered a medicinal plant and is used in the treatment of infections, asthma, rheumatism and other inflammatory disorders. In addition, it is considered a functional food, because it has essential vitamins, fibers, antioxidants and minerals in its composition (Tomou et al., 2020; Salimi et al., 2011).

Vegetables are functional foods that constitute a strategy to promote health and well-being for humans. Their nutritional benefits are immeasurable, since they are sources of bioactive compounds and antioxidants capable of combating free radicals, which can cause various types of damage to organisms (Fernandes et al., 2020; Souza et al., 2018; Dos Santos et al., 2016). These bioactive compounds are non-nutritive components present in vegetables and other foods that, when ingested, help in the prevention and/or combat against degenerative diseases and originate from the secondary metabolism of plants (Hangan et al., 2020; Wang et al., 2018).

With the need for keeping food safe, increasing its shelf life and with a better cost-benefit ratio, several packages and storage techniques have been used in recent decades, including the use of modified-atmosphere packaging (MAP), which consists of replacing the natural atmosphere with another mixture of gases of known composition, optimized for each type of product, as it slows down the degradation and preserves the quality characteristics for a longer period of time. Before using a certain type of packaging, one must observe the characteristics of the food, as well as the form of processing, the shelf life and the cost of this process (Santos & Oliveira, 2012).

Many flexible plastic films are employed in modified-atmosphere packaging systems. This system is a supplement of refrigeration, reducing the respiratory rate by the increase of CO₂ and decrease of O₂, a characteristic that can increase the shelf life of vegetables (Russo et al., 2012).

The objective of this study was to evaluate the shelf life and postharvest quality of the unconventional vegetable *Stachys byzantina* L. (lamb's-ear) packaged in different packaging systems.

2. Material and Methods

The experiment was conducted in the Food Conservation Laboratory, Department of Food Engineering, Federal University of São João del-Rei, Sete Lagoas *campus*, in Sete Lagoas – MG, Brazil. The experiment used leaves of lamb's-ear (*Stachys byzantina* L.) plants cultivated in the Vegetable Bank of the Agricultural Research Company of Minas Gerais (EPAMIG) – Santa Rita Farm, located in Prudente de Morais (19° 45' 41.35" S; 44° 15' 73.7" W). PET packages and high-barrier plastic films were purchased in the local market, and the sachets with ethylene absorber (potassium permanganate, net weight of 9 grams) were purchased from the Bioconservacion Food Safety Technology company (São Paulo, SP).

Young leaves of lamb's-ear in homogenous maturity state were randomly harvested in the morning period and transported under refrigeration to the laboratory, where they were selected, washed in running water, sanitized with aqueous solution of sodium hypochlorite (150 to 200 ppm of active chlorine) and drained to remove excess water.

100 g portions of leaves were packed in four different systems: (A) rigid polyethylene terephthalate (PET) package, used for protection against mechanical damage, (B) PET package containing an ethylene absorber sachet, (C) PET package wrapped with hermetically sealed high-barrier plastic film, and (D) PET package containing an ethylene absorber sachet and wrapped with high-barrier plastic film hermetically sealed with manual sealing machine.

The leaves packaged in the different packaging systems were stored in B.O.D. (Biochemical Oxygen Demand) incubator (LT 320 T), at a temperature of 7 °C ± 2.

The samples for evaluation of physicochemical characteristics, contents of bioactive compounds and antioxidant activity were removed before storage (T0) and at 5 (T1), 10 (T2) and 15 (T3) days of storage.

A split-plot arrangement was used (4 x 4), with four types of packaging (A, B, C and D) and four times of storage (0, 5, 10 and 15 days), in a completely randomized design with 3 replicates. All analyses were carried out in triplicate (Pereira et al., 2018).

Before applying the statistical tests under the proposed model, normality and homogeneity of variance assumptions were checked using Lilliefors' test and Levene's test, respectively.

The data were subjected to analysis of variance (ANOVA) at 0.05 probability level and, when there was significance, the means of qualitative factors were compared by Tukey test ($p < 0.05$) whereas those of quantitative factors were analyzed by regression ($p < 0.05$), through the statistical program R Core Team (2017).

2.1 Evaluation of physicochemical characteristics

2.1.1 Color

The colorimetric parameters were evaluated using a Konica Minolta CR410 colorimeter, in the color space L^* , C and $^{\circ}H$, where L^* indicates lightness, ranging from 0 (black) to 100 (white), C indicates chromaticity, which represents the saturation and purity of the color (near 0 - neutral colors and close to 60 - vivid colors) and $^{\circ}H$ indicates the hue angle, which represents the actual color at a 270° angle. Readings were taken at 3 distinct points of the upper part of the leaves, and the results obtained from the mean, according to the protocol of AOAC (2016).

2.1.2 Total Soluble Solids

The soluble solids content was determined using an R2mini digital refractometer, and the results were expressed in $^{\circ}$ Brix. The samples were ground in a mortar and filtered. Subsequently, drops of the filtrate were placed directly on the prism in the refractometer for direct Reading, according to the protocol of AOAC (2016).

2.1.3 Total solids

Total solids (dry matter) were determined using about 2 grams of the ground and homogenized lamb's-ear leaves, which were placed in aluminum crucibles and subjected to a temperature of 105°C in a sterilization and drying oven, until obtaining a constant mass. The percentage of total solids was calculated based on the difference between the initial and final masses, according to the protocol of AOAC (2016).

2.1.4 Hydrogen Potential (pH)

The pH was determined by potentiometry (AOAC, 2016) with a digital pH meter, by direct immersion of the electrode in a solution composed of ground and homogenized lamb's-ear leaves and 50 mL of distilled water.

2.1.5 Total Titratable Acidity

Total titratable acidity was determined by titrimetry, by weighing 1 gram of ground and homogenized leaves and mixing it with 50 mL of distilled water and 3 drops of 1% phenolphthalein. The solution containing the sample was titrated with a standardized solution of 0.1 N sodium hydroxide, until reaching the turning point. The values were expressed in percentage of citric acid, according to the methodology recommended by AOAC (2016). The results were expressed in grams of citric acid 100 grams^{-1} of sample on a fresh basis.

2.1.6 Mass Loss

Mass loss was obtained by weighing the packaged material on an analytical scale (Shimadzu AUX220). The weighing procedure was carried out at the beginning and on all dates in which the samples were removed. The results were expressed in percentage of mass loss.

2.2 Evaluation of bioactive compounds:

2.2.1 Total Phenolic Compounds

The content of total phenolic compounds was quantified by the Folin-Ciocalteu method (Tugli et al., 2019, Singleton et al., 1999), with modifications. A 0.5-mL aliquot of ethanolic extracts was mixed with 2.5 mL of the Folin-Ciocalteu reagent diluted in water (1:10). After 8 minutes at room temperature, 2.0 mL of sodium carbonate solution (4%) were added and the mixture was kept at room temperature in the absence of light for 2 hours. Absorbance at 740 nm was determined in a spectrophotometer (FENTO 700S) and the total phenolic content was calculated using a standard gallic acid curve. The results were expressed in mg of gallic acid equivalent per 100 grams of sample on a dry basis (mg GAE*100g⁻¹ DB).

2.2.2 Flavonoids and Anthocyanins

The contents of flavonoids (FLA) and anthocyanins (ANT) were determined according to the method of Francis (1982), in which 0.5 g of the homogenized sample received 10 mL of acidified ethanol (ethanol-HCL 85:15 v/v). The solution was kept at rest for 24 hours under refrigeration at 12 °C. After filtration, the samples were read in spectrophotometer (Shimadzu UV-1800) at 374 nm for flavonoids (mg 100 grams⁻¹ of sample on dry basis) and 535 nm for anthocyanins (mg of cyanidin 3-glucoside per 100 grams of sample on dry basis).

2.2.3 Carotenoids

The content of total carotenoids was determined according to the methodology proposed by Rodriguez-Amaya (2001), which consists of the extraction with acetone (A.R.) and quantification by spectrophotometry at 450 nm. The results were expressed in mg of carotenoids per 100 grams of fresh mass.

2.2.4 Antioxidant Activity

Antioxidant capacity was determined by the ABTS and DPPH methods, according to Gorinstein et al. (2004), with modifications. A 0.1-mL aliquot of the extract was transferred to a test tube, where 2.9 mL of the radical (ABTS and DPPH solutions) were added. After the reaction of the solutions (30 minutes), readings were taken in the spectrophotometer (FENTO, 700S) at 734 nm for ABTS and at 515 nm for DPPH. Antioxidant capacity was calculated using the Trolox standard curve (100 to 2000 µM) and the results were expressed in µmol of Trolox equivalent per gram of sample on dry basis (µmol TE.g⁻¹ DB).

3. Results and Discussion

3.1 Physicochemical characteristics

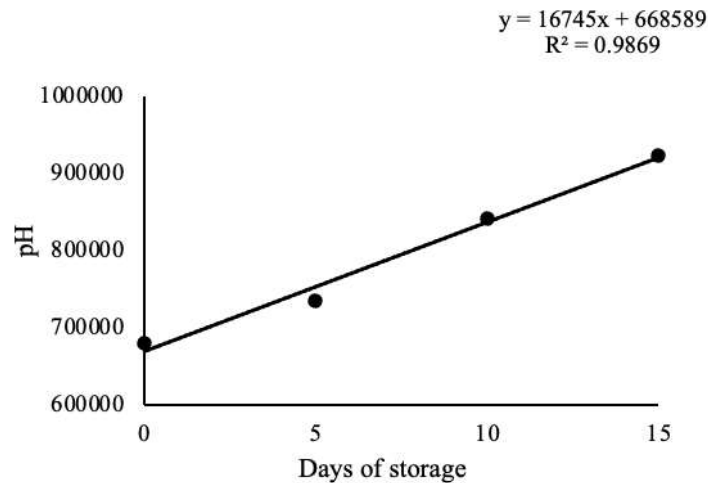
The parameters hydrogen potential (pH), total soluble solids (TSS) and total titratable acidity (TTA) were significantly affected only by the storage times. The variable lightness (L) showed significant differences for types of packaging systems and storage times, separately. There was a significant interaction ($p < 0.05$) between packaging systems and

storage time for the variables chroma (C), hue (H°) and mass (M). There was no significative interaction between the factors. The hydrogen potential was transformed according to Boxcox ($\lambda = 7.5$) in order to correct normality and homogeneity.

3.1.1 Hydrogen Potential (pH)

In response to storage time, the mean pH values were described by a linear model, in which the highest pH value was obtained at 15 days of storage, corresponding to 6.24 (value transformed for statistics = 900000) (Figure 1).

Figure 1. Variation of the transformed mean value of hydrogen potential (pH) in lamb's-ear leaves in response to the different days of storage in the packaging system C, under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



Source: Authors.

The increase in pH has also been observed in other vegetables. Nasser et al. (2018), evaluating the effect of different O_2 and CO_2 concentrations in minimally processed eggplant stored for 10 days, observed that the pH values changed over the days, but there was a small increase at the end of the storage period, as occurred in the present study. These same authors cite that the pH value may increase in response to an increase in the microbiota during storage.

Another reason that can lead to an increase of pH along the storage is the elevation of metabolic activity in the leaves, which results in a higher consumption of organic acids and acid radicals to maintain the respiration. These acids are metabolized and transformed into non-acidic molecules (Pech et al., 2002).

In the present study, there was a considerable increase in pH from the first to the last day of storage. Kluge et al. (2014), analyzing the effect of different antioxidant compounds on the conservation of the qualitative, microbiological and nutritional characteristics of minimally processed yellow bell peppers kept under cooling, observed a decrease in pH, with subsequent increase from the 10th day of storage. These authors cite that acidification at the end of storage may occur due to the increase in the concentration of intracellular carbon dioxide, resulting from the increase in respiratory rates.

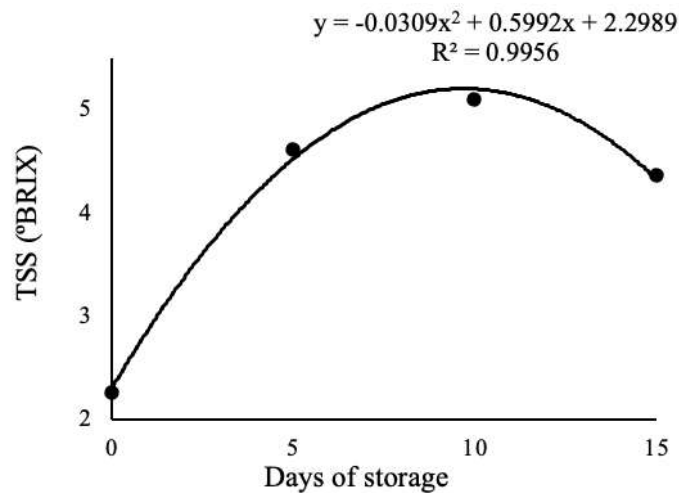
In a study conducted analyzing the pH variation of several arugula cultivars during storage, Jardina et al. (2017) observed that pH values fluctuated and tended to increase from 6 days of storage for all cultivars, which may be due to the elevation in the respiratory rate and the intense consumption of organic acids due to the minimal processing, a cut that leads to greater deterioration.

The total concentration of organic acids tends to decrease along the storage, and the post-harvest alterations differ according to the species and acids in question, type of tissue, management, storage conditions, cultivar and period of production (Kays, 1997).

3.1.2 Total Soluble Solids (TSS)

For the TSS content in lamb's-ear leaves, there was an initial increase in the values and, after approximately 10 days, these values began to decrease again, with no difference between the packaging systems used (Figure 2).

Figure 2. Mean variation of total soluble solids (TSS) in °Brix in lamb's-ear leaves stored in response to the different days of storage, under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



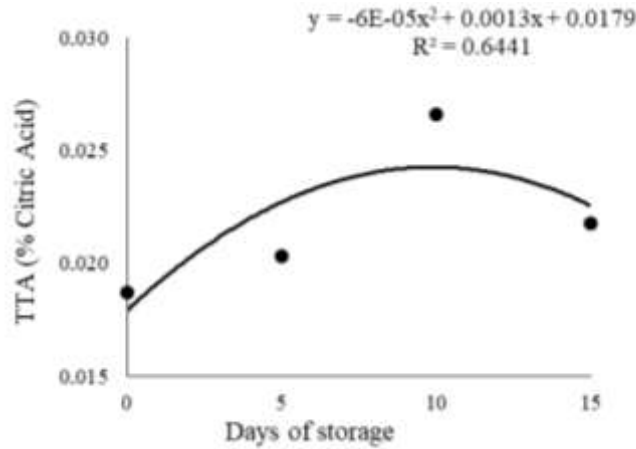
Source: Authors.

This increase at the beginning of the evaluation period may be related to the concentration of solids due to the loss of water during storage (Neres et al., 2004). Jardina et al. (2017) observed a variation in TSS contents of arugula until the 10th day of storage, and the investigated cultivar had high contents throughout the storage period, showing 5.20 °Brix on the 8th day of analysis (in comparison to 3.33 °Brix on the 1st day), indicating slow metabolism of organic compounds (sugars), which lead to deterioration, as observed in the present study. TSS content is a parameter of extreme importance for the acceptance of vegetables by consumers, since the presence of carbohydrates, and consequently of sweetness, is an important characteristic in their choice of the product (Menezes et al., 2009).

3.1.3 Total Titratable Acidity (TTA)

The mean values of TTA did not differ significantly between the packaging systems, but a significant difference was observed for the days of storage. According to the results shown in Figure 3, the highest TTA value (0.025 g citric acid.100g⁻¹ fresh mass) was obtained on the 10th day of storage.

Figure 3. Mean variation of Total Titratable Acidity (TTA), in % of Citric Acid, in lamb's-ear leaves in response to the different days of storage, under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



Source: Authors.

The acidity in vegetables is mainly attributed to organic acids located in the cell vacuoles, in a dissolved form. Higher values of acidity may indicate greater preservation of the product, considering that the acids are being less consumed in respiration due to the decrease of the time-temperature values for thermal penetration (Gocan et al., 2016).

During the senescence period, the concentration of organic acids tends to decrease, due to the possible leakage of cell fluid and volatilization of these acids (Berli et al., 2004). This effect, however, was not observed in lamb's-ear leaves in the present study.

Viana et al. (2015) found a TTA concentration of $0.11\text{ g citric acid.}100\text{ g}^{-1}$ fresh mass in Malabar spinach (*Basella rubra*) leaves. The present study found an average of $0.022\text{ g citric acid.}100\text{ g}^{-1}$ dry mass in lamb's-ear leaves. With a few exceptions, vegetables have low acidity, a characteristic that makes them highly susceptible to bacterial contamination. Based on the results, it was possible to observe the reduction in the values of this parameter, which may be related to the increase of pH.

3.1.4 Mass

For mass of lamb's-ear leaves, there was a significant interaction between packaging systems and days of storage. According to the data presented, leaves stored in C and D packaging systems showed lower losses of fresh mass until the last day of storage.

According to Mantilla et al. (2010), the atmosphere inside the package plays a major role in the conservation of vegetables. The transformation of this atmosphere aims to create a gaseous composition within the package, which can be obtained passively or actively.

For Kluge et al. (2014), several factors cause reduction in post-harvest respiratory activity, for instance, inactivation of the enzymes present in the cells when the vegetables are subjected to cuts and biochemical changes in the glycolysis pathway.

Based on the results obtained, the highest mean values of mass loss were found in leaves stored in the B packaging systems, when compared to the leaves stored in the systems A, C and D. This is due to the greater moisture lost by the leaves, which increases the relative humidity inside these packages, leading to a reduction in the water vapor pressure deficit of these leaves in comparison to the environment.

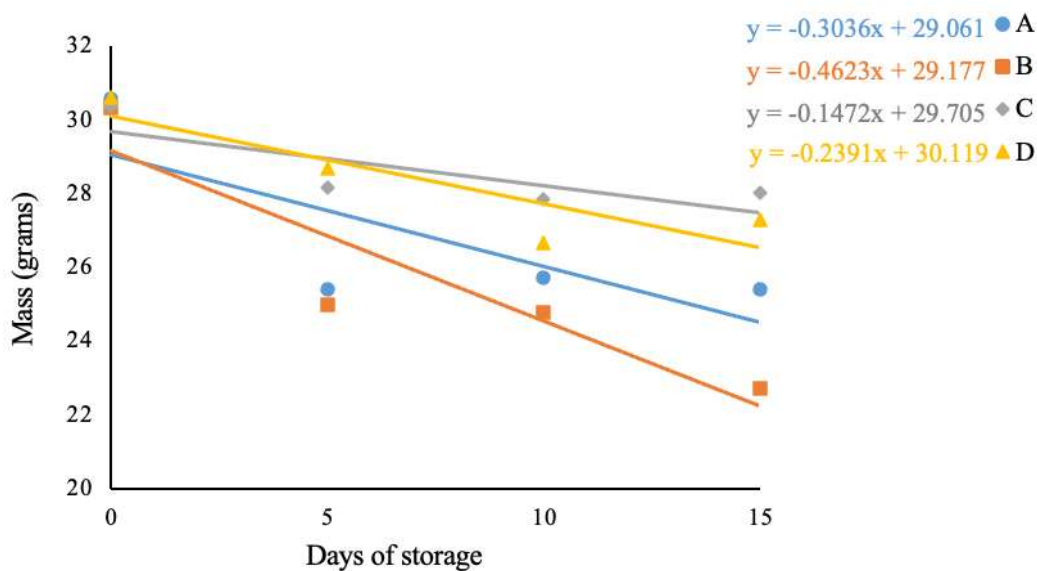
The mass loss percentage of lamb's-ear leaves was influenced by the storage time, regardless of the packaging system used. There was a significant reduction in leaf masses along the storage period, possibly in response to the imposed stress, resulting in increased respiratory activity.

This increasing mass loss in lamb's-ear leaves has also been observed in other vegetables, such as eggplant (Nasser et al., 2018) and yellow bell pepper (Kluge et al., 2014) stored under active modified atmosphere.

The packaging systems may have contributed to the gradual mass loss during storage because, according to Izumi et al. (1996), fresh mass loss is the sum of water loss due to transpiration and carbon loss through respiration.

The use of packages for storage purposes reduces the vapor pressure between the leaves and the storage environment and particularly creates a modified atmosphere inside the package. This consequently ensures a decrease in the rate of fresh mass loss, where the loss of water from the leaves to the storage environment occurs (Chitarra & Chitarra, 2005). This result was evidenced in the packaging systems D (Figure 4).

Figure 4. Mean values of mass of lamb's-ear leaves in response to different days of storage in different packaging systems under refrigeration at $7^{\circ}\text{C} \pm 2$.



(A) rigid polyethylene terephthalate (PET) package, used for protection against mechanical damage, (B) PET package containing an ethylene absorber sachet, (C) PET package wrapped with hermetically sealed high-barrier plastic film, and (D) PET package containing an ethylene absorber sachet and wrapped with high-barrier plastic film hermetically sealed with manual sealing machine.

Source: Authors.

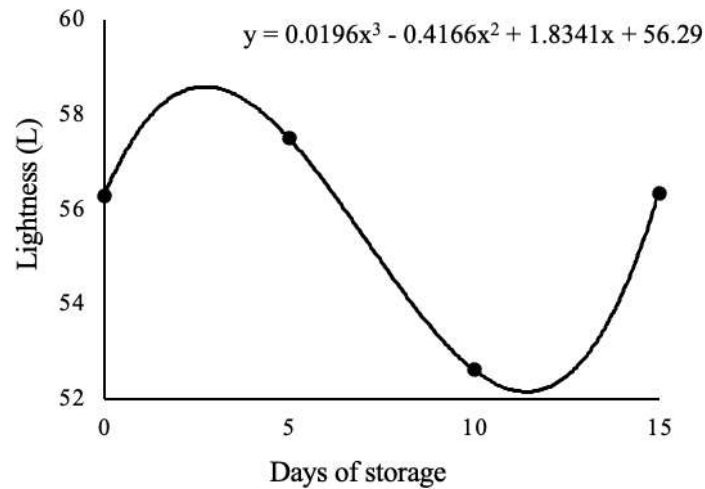
3.1.5 Colorimetric Parameters

3.1.5.1 Lightness (L^*)

Reduction in L^* means darkening of the product, i.e., the lower this value, the darker the leaf, and this darkening is related to various aspects, such as pigment oxidation and enzymatic and non-enzymatic browning (Arruda et al., 2003). The average values of L^* for the leaves showed great variation during the 15 days of storage, however, at the end of the storage period, the values were similar to those observed on the first day, as shown in Figure 5. In response to the tested packaging systems, the mean values of L^* ranged from 54.578 to 57.342, and only the systems A and D differed statistically from each other (Figure 5), but were similar to the other systems (B and C). The highest value was observed in system D.

During the 15 days of storage, the mean values of L^* for lamb's-ear leaves showed great variation, but at the end of the storage period, the values were similar to those observed on the first day, as shown in Figure 5.

Figure 5. Mean value of lightness (L^*) of lamb's-ear leaves in all packages during the days of storage under refrigeration at $7^\circ\text{C} \pm 2$.



Source: Authors.

Similar results were found by Nascimento (2005), who observed a decrease in L^* values for all treatments tested, when evaluating the physiological responses of cabbage leaves stored for 10 days subjected to application of silver nanoparticles at different concentrations. The initially found mean value of L^* was 45.51 on day 0, whereas the mean value between the treatments on the 10th day of storage was 39.36. According to Vilas Boas (2002), the change of appearance, usually related to darkening, is the most common alteration in fruits and vegetables during prolonged storage. This undesirable characteristic is accelerated by mechanical rupture of the cells, caused by the activity of cutting, which intensifies the enzymatic activity.

3.1.5.2 Chroma (C^*)

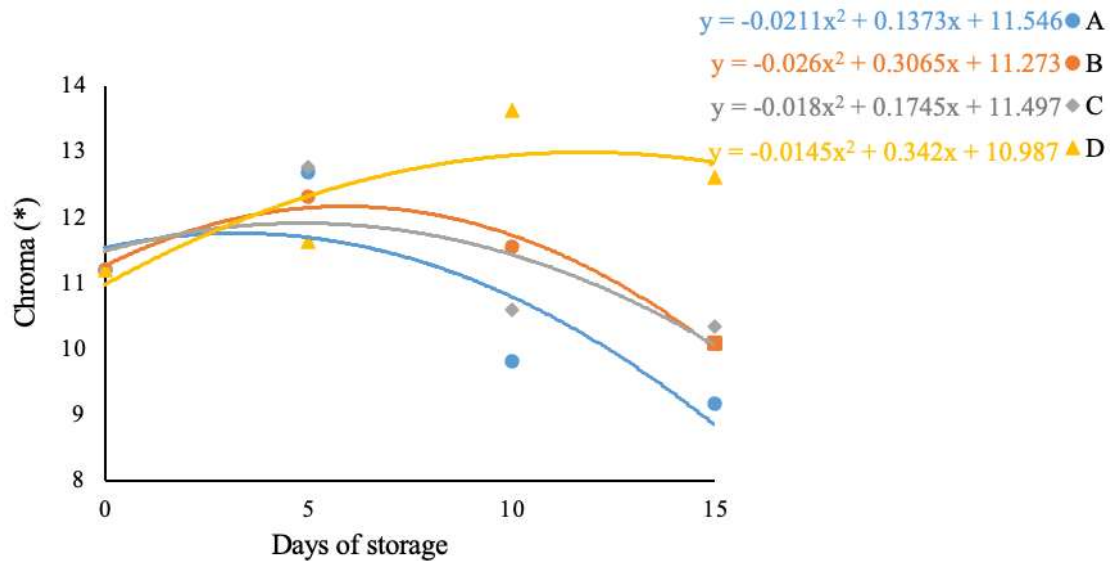
According to McGuire (1992), the purity or intensity of the color is determined by the chromaticity, with values ranging from 0, for neutral colors, to 60, for vivid colors. High values are related to higher color intensity and low values are related to neutrality. For the chromaticity parameter, there was a significant interaction between packaging systems and days of storage.

The packaging systems caused effect on the chroma only at 10 and 15 days of storage, and the system D had the highest value of chroma at both times.

This behavior can be explained, possibly, by the packaging system used; the system D, besides being made of PET like the system A was wrapped with high-barrier film and contained sachet with ethylene absorber. Lamb's-ear leaves stored in system A showed reduction of chroma (11.21 to 9.18), whereas the system D ensured the maintenance of the color of the leaves. This is one of the quality parameters for vegetables that directly interfere in the purchasing decision by the consumer.

According to the results found in the present study, lamb's-ear leaves stored in the system D showed green color for a longer period, especially in terms of chroma, with more intense colors (Figure 6), and lightness, responsible for maintaining cell turgor until the end of the experimental period.

Figure 6. Mean value of chroma (C) in lamb's-ear leaves in response to different days of storage in different packaging systems under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



(A) rigid polyethylene terephthalate (PET) package, used for protection against mechanical damage, (B) PET package containing an ethylene absorber sachet, (C) PET package wrapped with hermetically sealed high-barrier plastic film, and (D) PET package containing an ethylene absorber sachet and wrapped with high-barrier plastic film hermetically sealed with manual sealing machine.

Source: Authors.

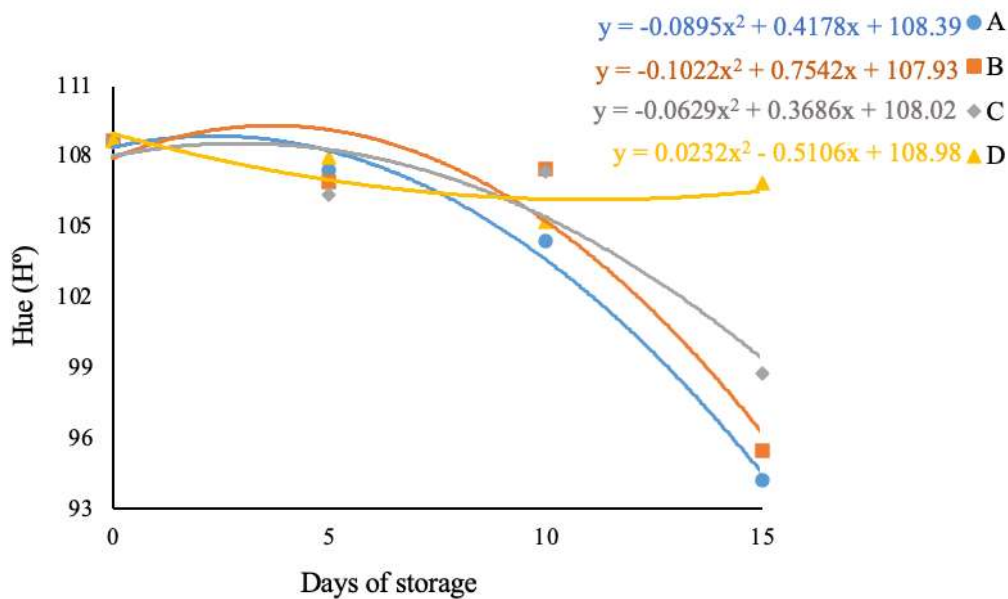
3.1.5.3 Hue (H°)

The analyzed lamb's-ear leaves showed significant difference in H° between the factors packaging systems and days of storage. The mean values of H° of the present study vary from 94.25 to 108.66 (Figure 7).

The Hue angle refers to the value in degrees corresponding to the three-dimensional color diagram, in which: 0° -red, 90° -yellow, 180° -green and 270° -blue (Mendes et al., 2011; Chitarra & Chitarra, 2005). During the 15 days of storage, the H° values for lamb's-ear leaves stored in the four packaging systems fluctuated, but at the end of the storage period the observed values were lower than those obtained on the first day (Figure 7). Silva et al. (2007) observed similar behavior when evaluating the conservation of minimally processed lettuce leaf and scallion kept under refrigeration. These authors detected a reduction in the value of H° for both vegetables evaluated along 7 days of storage. Imahori et al. (2004) found a reduction in H° values when analyzing the physiological and quality responses of Chinese scallion leaves under modified atmosphere and refrigeration.

Products with strong and bright colors positively attract the consumer, and color is one of the main characteristics analyzed in the purchase of processed products (Miguel et al., 2010).

Figure 7. Mean value of chroma (C) in lamb's-ear leaves in response to different days of storage in different packaging systems under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



(A) rigid polyethylene terephthalate (PET) package, used for protection against mechanical damage, (B) PET package containing an ethylene absorber sachet, (C) PET package wrapped with hermetically sealed high-barrier plastic film, and (D) PET package containing an ethylene absorber sachet and wrapped with high-barrier plastic film hermetically sealed with manual sealing machine.

Source: Authors.

3.2 Antioxidant activity and bioactive compounds

There was significant interaction between the factors packaging systems and storage time for antioxidant activity. The bioactive compounds (phenolic compounds, flavonoids, anthocyanins and carotenoids) were significantly influenced only by the storage times.

Plants react to stress before and during storage, through the production of bioactive compounds and antioxidants that involve the activation of secondary metabolism in order to maintain balance with reactive oxygen species (ROS) (Stagnari et al., 2018).

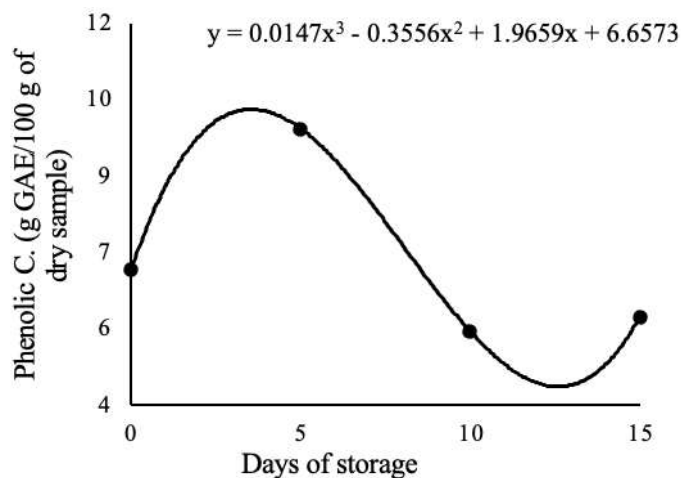
3.2.1 Bioactive Compounds

3.2.1.1 Phenolic compounds

The contents of phenolic compounds were not affected by the packaging systems used. However, these levels fluctuated significantly along the days of storage. In relation to the mean values, there was a reduction from the beginning to end of the storage period.

According to the data presented, during the 15 days of storage, the phenolic contents of compounds in lamb's-ear leaves stored in the four different packages fluctuated, but at the end of the storage period the values were lower than the respective values detected on the first day of storage, as shown in Figure 8. Such variation in the concentration of these compounds, which exhibit antioxidant properties (Souza et al., 2021), can be explained by the fact that in fruits and vegetables the total phenolic content is strongly influenced by genetic factors, storage (considering temperature, relative humidity and atmosphere inside chambers or packages), environmental conditions, degree of maturity and variety of the plant, mechanical injuries, besides the type of cultivation (Gobbo-Neto & Lopes, 2007). At 5 days of storage, the leaves had higher levels of phenolic compounds than on the first day, showing that it is an influence of storage time on this variable, regardless of the type of packaging (Figure 8).

Figure 8. Mean content of phenolic compounds (g GAE 100 g⁻¹ dry sample) in lamb's-ear leaves along the days of storage under refrigeration at 7 °C ± 2.

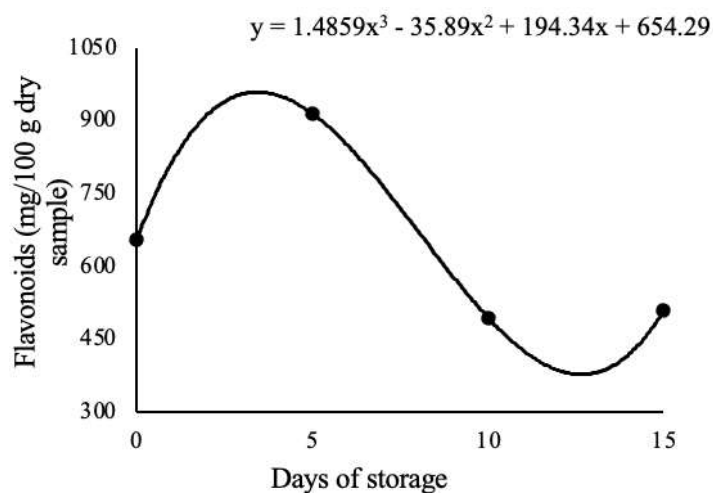


Source: Authors.

3.2.1.2 Flavonoids

The mean contents of flavonoids in lamb's-ear leaves (Figure 9) did not differ significantly between the packaging systems, but significant difference was found for the storage time factor.

Figure 9. Mean contents of flavonoids (mg 100 g⁻¹ of dry sample) in lamb's-ear leaves along the days of storage under refrigeration at 7 °C ± 2.



Source: Authors.

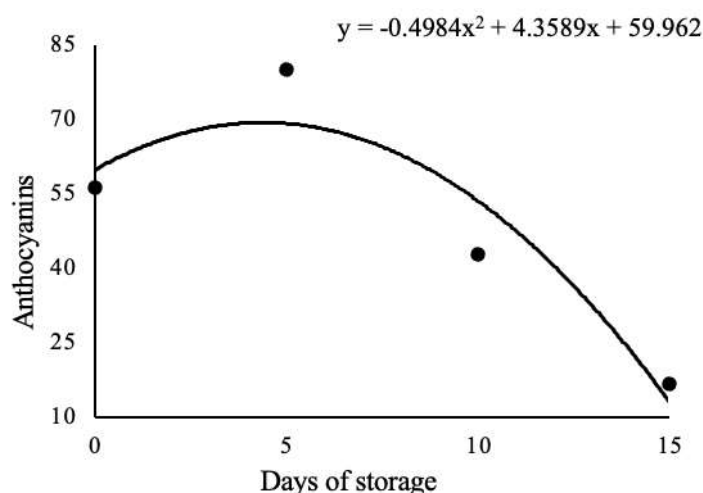
From the fifth day of storage, the contents of flavonoids decreased significantly and such trend continued for more 5 days (Figure 9). The packaging systems did not significantly influence the content of total flavonoids. However, the storage time caused a significant reduction in this phytochemical from the 5th day (Figure 9), and there was no significant alteration in the contents of these compounds in the stored leaves until the 5th day of storage. In this case, there was no significant influence of the packaging system. Chu et al. (2000) reported loss of 67% in the content of flavonoids in sweet potato leaves after storage for 4 days at room temperature (25 °C), whereas after storage at refrigeration temperature (4 °C) the loss was equal to

20%, increasing to 55% after 9 days storage. In the present study, the loss in the content of total flavonoids in lamb's ear leaves was 44.33% from the 5th to the 15th day of storage.

3.2.1.3 Anthocyanins

From the beginning of the storage period until approximately the 4th day (4.37 days), the contents of anthocyanins increased, with subsequent reduction in the four packaging systems tested (Figure 10).

Figure 10. Contents of anthocyanins (mg of cyanidin 3-glucoside per 100 grams of dry sample) in lamb's-ear leaves along the days of storage, under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



Source: Authors.

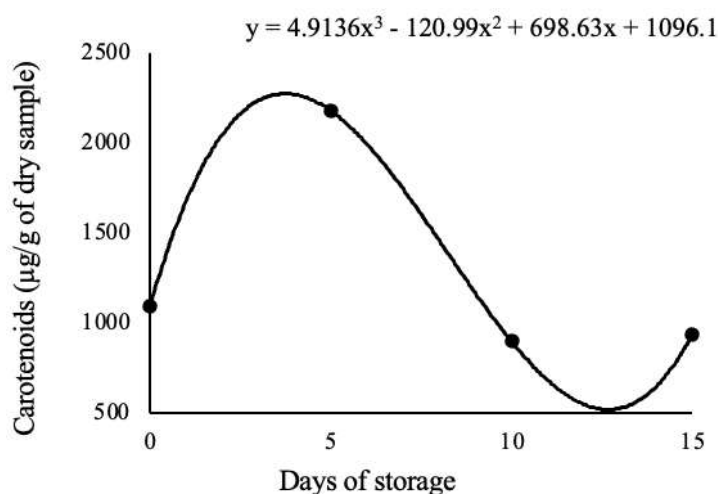
There was an increase in the total contents of anthocyanins, which changed from 56 mg of cyanidin 3-glucoside per 100 grams of leaves on dry basis at the beginning of the storage to 70 mg of cyanidin 3-glucoside per 100 grams of leaves on dry basis on the 5th day. From this point on, the behavior of this component was altered, characterizing a possible degradation, evidenced by a significant reduction until the 15th day, reaching 10.5 mg of cyanidin 3-glucoside per 100 grams of sample on a dry basis at the end of this period, regardless of the type of packaging used. The biosynthesis of anthocyanins and flavonoids can continue after harvest and during storage, even at low temperature, which explains the increase in the concentration of this analyte until the 5th day. Concellón et al. (2007) observed a 30% reduction in the contents of anthocyanins in eggplant, stored at 0 and 10 °C at the end of 15 days of storage.

3.2.1.4 Carotenoids

Carotenoids are pigments widely disseminated in nature and arouse interest due to their preventive action against certain types of disease, such as cancers, cardiovascular diseases and cataracts (Della Lucia et al., 2008). Given the importance of these compounds, Figure 11 relates the content of these natural antioxidants in minimally processed lamb's-ear leaves as a function of storage time. Intermediate content of carotenoids was observed between 8 and 9 days of storage (8.21).

There was a reduction in the pigments, components of carotenoids in the lamb's-ear leaves during the storage period, with a small increment in the end. According to Viana et al. (2015), this reduction can be explained by the fact that these compounds are very sensitive to temperature and acidity. Hence, it is possible to note the importance of proper storage, using packages that prevent the degradation of this class of natural antioxidants for a longer period of time, allowing the consumption of vegetables with similar properties to those found shortly after harvest.

Figure 11. Content of carotenoids (mg 100 g⁻¹ of dry sample) in lamb's-ear leaves along the days of storage under refrigeration at 7 °C ± 2.



Source: Authors.

In this study, leaves stored for five days had high contents of phenolic compounds (9.43 g GAE 100 g⁻¹ of sample), flavonoids (914.5 mg 100 g⁻¹ dry sample), anthocyanins (80.22 mg of cyanidin 3-glucoside per 100 grams of dry sample) and carotenoids (2178.74 µg g⁻¹ dry sample), demonstrating that the storage, regardless of the packaging used, was efficient in this period, enabling the transport and commercialization, so the product can be consumed with the same quality observed shortly after harvest. Considering that the consumption of foods rich in carotenoids is related to the reduction of risks of chronic diseases, prevention of cataract formation and reduction of macular degeneration related to aging, these storage conditions, regardless of the packaging, contributed to the conservation of this important bioactive component until 5 days of storage.

3.2.2 Antioxidant Activity

Bioactive compounds are characterized by their antioxidant function, due to their interaction with free radicals, acting in the prevention of cancerous and cardiovascular diseases (Grune et al., 2010).

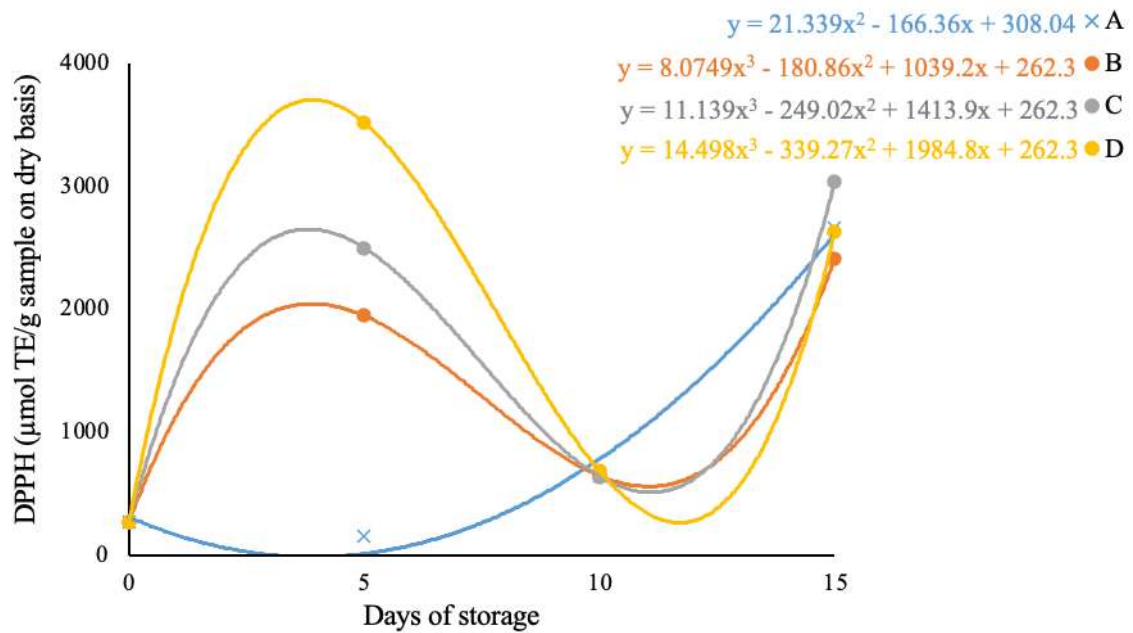
3.2.2.1 DPPH method

Regarding the quantification of antioxidant activity, the results obtained through the DPPH test showed that there was significant interaction between the packaging systems and days of storage. The values of antioxidant activity in lamb's-ear leaves stored in the four packaging systems varied along the 15 days of storage and, on the fifth day, the leaves stored in the packaging system D had the highest value for this parameter (Figure 12).

Along the storage period, lamb's-ear leaves showed a significant difference between the packaging systems tested only on the fifth day of storage. Leaves in the systems B, C and D had higher antioxidant activity than leaves in system A. This behavior can be explained by the initial stress after harvest because, according to Vieites et al. (2012), the increase in antioxidant activity during storage may be associated with loss of fresh mass.

Although the average values fluctuated along the storage period, leaves stored in the four packaging systems tested had significantly higher values of antioxidant activity than those found at the beginning of the experiment (Figure 12).

Figure 12. Antioxidant capacity measured by the DPPH method of lamb's-ear leaves in response to different days of storage in different packaging systems under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



(A) rigid polyethylene terephthalate (PET) package, used for protection against mechanical damage, (B) PET package containing an ethylene absorber sachet, (C) PET package wrapped with hermetically sealed high-barrier plastic film, and (D) PET package containing an ethylene absorber sachet and wrapped with high-barrier plastic film hermetically sealed with manual sealing machine.
Source: Authors.

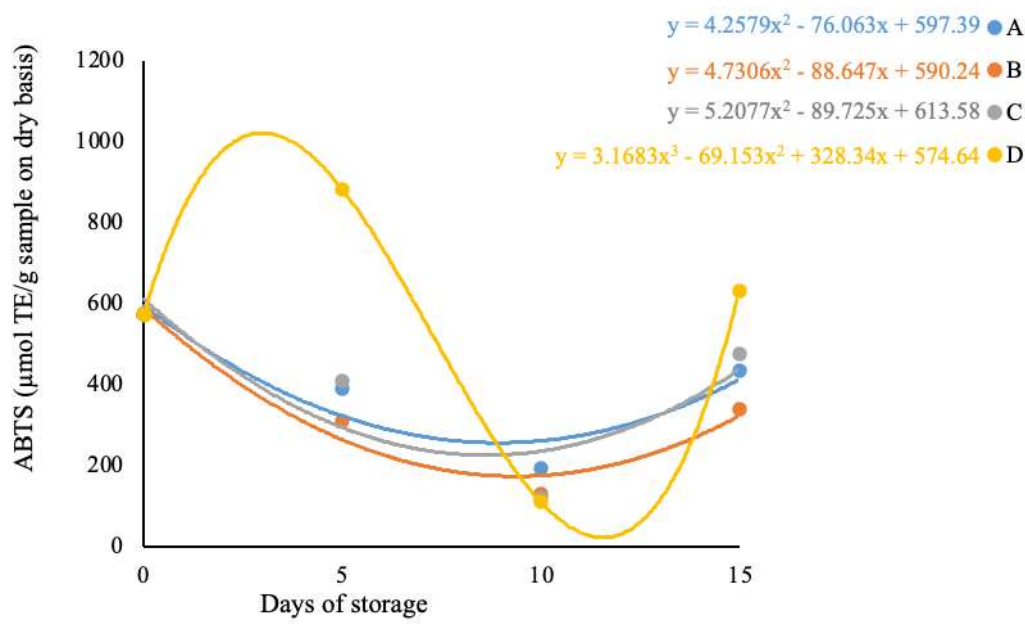
Due to its association with health, antioxidants have been actively explored, since consuming high amounts of different bioactive compounds reduces the risk of degenerative diseases (Carvalho et al., 2018).

3.2.2.2 ABTS method

For the ABTS test, the results obtained for the analysis of the antioxidant activity in lamb's-ear leaves show that there was significant interaction between the types of packaging in storage function.

As observed in the DPPH test, during the fifteen days of storage, the antioxidant activity measured by the ABTS test varied, but at the end of the experiment the values were significantly lower than those found at the beginning, except for the product stored in the packaging system D (Figure 13).

Figure 13. Antioxidant capacity evaluated by the ABTS method in lamb's-ear leaves in response to different days of storage in different packaging systems under refrigeration at $7\text{ }^{\circ}\text{C} \pm 2$.



(A) rigid polyethylene terephthalate (PET) package, used for protection against mechanical damage, (B) PET package containing an ethylene absorber sachet, (C) PET package wrapped with hermetically sealed high-barrier plastic film, and (D) PET package containing an ethylene absorber sachet and wrapped with high-barrier plastic film hermetically sealed with manual sealing machine.

Source: Authors.

4. Conclusions

The quality attributes of lamb's-ear leaves stored in the four packaging systems (A, B, C and D) were maintained until the end of the 15 days of storage, but the highest contents of bioactive compounds and antioxidant activity (ABTS and DPPH) occurred until the 5th day of storage at $7\text{ }^{\circ}\text{C} \pm 2$. The packaging system which led to obtaining passive modified atmosphere, without ethylene absorber (system C) preserved the mass of the leaves. Leaves stored in the packaging systems containing ethylene absorbers (sachets) showed greater loss of fresh mass, which negatively influenced their physical characteristics and antioxidant capacity, but not the contents of bioactive compounds. Lamb's-ear leaves stored in the packaging system A had quality attributes similar to those of leaves stored in the packaging systems B, C and D, characterized by the presence of ethylene absorber and/or high-barrier film. Considering the economic issues and practicality in handling, the package A is the most indicated for refrigerated storage ($7\text{ }^{\circ}\text{C} \pm 2$) and commercialization of minimally processed lamb's-ear leaves. The results found in the present study point to the viability of the utilization and distribution of lamb's-ear leaves ready for consumption, a vegetable that has functional potential and that can contribute to the improvement of the population's health and reduction of food monotony. For future studies it would be interesting to evaluate other types of packaging, the purchase intention and acceptance of the product ready for consumption.

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