Temperature and storage periods on the maintenance of chemical composition of medicinal plants

Priscila Mayara Rocha Leão[1], Leandro Camargo Neves[1], Ronan Carlos Colombo[2], Muhammad Shahab[2], Jane Maria Franco de Oliveira[3], Francisco Joaci de Freitas Luz[3] and Sergio Ruffo Roberto[2,*]

[1]Federal University of Roraima, Agricultural Research Center. BR 174 Road Km 12, CEP: 69310-270 Boa Vista, RR, Brazil. [2]State University of Londrina, Agricultural Research Center. Celso Garcia Cid Road, km 380, P.O. Box 10.011, ZIP 86057-970, Londrina, PR, Brazil. [3]Rodovia BR 174, Km 8, Distrito Industrial, Caixa Postal 133, ZIP 69301-970, Boa Vista, R, Brazil. [*]Corresponding author, E-mail: sroberto@uel.br

ABSTRACT

Determining the chemical composition of medicinal plants used for therapeutic purposes is of fundamental importance. These plants must meet quality standards for commercialization by ensuring pharmacological properties and efficacy. However, for the maintenance of their active compounds, it is very important to store them at a suitable temperature and ensure the safety of these compounds. The objective of this work was to evaluate the effect of different temperatures and storage periods in the maintenance of chemical composition of bushy lippia (*Lippia alba*), crajiru (*Arrabidaea chica*), eucalyptus (*Eucalyptus grandis*), lemon grass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*) and sage (*Lippia microphylla*) leaves. Leaves of these medicinal plants were stored for different periods (30, 60 and 90 days) at different temperatures (0, 17 and 24 °C). A complete randomized design was used with five replications, each one with 150g of leaves. For each combination of temperature and storage period, different variables were evaluated such as phenolic compounds, total anthocyanins, antioxidant activity (ORAC and DPPH), carotenoids and chlorophyll contents. Leaves stored at 0°C resulted in the most efficient preservation of chemical compounds at 30, 60 and 90 days. However, this temperature causes physiological damage to the leaves. Therefore, for commercial use, dried or crushed leaves in the form of powder is recommended. Storage at 17 °C keeps the chemical composition of the leaves at satisfactory levels, while at 24 °C there is a significant decrease over the periods of cold storage.

Key words: Conservation, bioactive compounds, pharmacological properties, antioxidant.

INTRODUCTION

Medicinal plants are all those plants with pharmacological properties, with active compounds that are beneficial to human health and used in different pharmaceutical forms defined as phytotherapy (Corrêa and Alves 2008).

The market for medicinal plants in the world has been expanding over the past years. The interest in the knowledge, use and commercialization of these plants and their phytotherapeutic products is increasing (Freitas et al., 2012). However, herbal medicines have some quality issues as compared with conventional medicines, mainly due to the variety of chemical compounds found in plants. Availability of these compounds depends on the nature of the plants, environmental factors, genetic factors, processing, drying, storage and extraction process. All these factors can modify the composition of these compounds, directly affecting their safety and efficacy (Argenta et al., 2011).

Environmental factors, postharvest management and storage period are pivotal to the quality and production of these active compounds. During postharvest storage, several desirable and undesirable changes occur in the plant metabolism, crucial for phytotherapeutic use. Thus, determining the most suitable temperature and storage period for medicinal species is crucial for keeping the chemical composition at a desirable level, for its use and commercialization (Silva et al., 2010).

The objective of this work was to evaluate the effect of different temperatures and storage periods on the maintenance of the chemical composition of bushy lippia (*Lippia alba*), crajiru (*Arrabidaea chica*), eucalyptus (*Eucalyptus grandis*), lemon grass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*) and sage (*Lippia microphylla*) leaves, six of the most produced and commercialized medicinal species of plants in Roraima State, Brazil.

MATERIAL AND METHODS

Plants of bushy lippia (*Lippia alba*), crajiru (*Arrabidaea chica*), eucalyptus (*Eucalyptus grandis*), lemon grass (*Cymbopogon citratus*), citronella (*Cymbopogon nardus*) and sage (*Lippia microphylla*) were collected from stock plants at Embrapa Roraima, Boa Vista, Roraima, Brazil (02°42'3''N; 47°38'0''W; elevation of 90 m.a.s.l.). The soil of this area is classified as Yellow Latosol with a sandy texture. The climate of the region is hot and humid, A_W type, tropical, with an average rainfall of 1,678 mm, with average annual temperatures of 28.6, 30 and 32 °C and relative air humidity of 79.8, 82.13 and 72.5%, respectively, in the years of 2013, 2014 and 2015. The weather of the area is hot and humid with an average rainfall of 1.678 mm, with a temperature of 38 °C max and 20 °C min and an average of 27.4 °C.

Plants for the experiment were collected in three consecutive years 2014-16 (during the summer season, from October to February - dry season in the Amazon Region), always in the early morning. All plants were re-cultivated at each season. Fifty-five plants of each species were used, discarding the border plants (10%). For the analyses, branches and leaves from the outside of the plant were used, as well as branches and leaves from the inner part of the plant. During the analysis, they were mixed for better standardization of the experiment. After the harvests, the samples were subjected to defoliation immediately, and healthy leaves were selected for evaluation, at the Laboratory of Food Technology, Federal University of Roraima, Brazil. Subsequently, the leaves were washed and sanitized with a disinfectant (sodium dichloroisocyanurate, containing 2.5% active chlorine), for 15 min. After, the leaves were dried until the total loss of humidity, avoiding the early deterioration and the decrease of the chemical compounds. The samples were placed to dry in a solar dryer under wooden trays with the bottom covered by the sombrite type screen, placing 1.0 kg of fresh matter in each one. Each replicate contained 150g of dried leaves, for each species. Then, the samples were packed in plastic bags and cold stored at different temperatures (0, 17 and 24 °C), for different periods (30, 60 and 90 days). The bags of LDPE presented measurements of 0.50 × 0.30m and, as specifications: plastic film of LDPE of 0.010mm thickness (TPO_2 of 11,234 cm^3 day^{-1}, at 25 °C and 1 atm; $TPCO_2$ of 36.705 cm m^{-2} dia^{-1}, at 25 °C and 1 atm, permeability area of 805 cm^2)

For each combination of temperature and storage periods, the chemical composition was evaluated by means of total anthocyanins, phenolic compounds, antioxidant activity (ORAC and DPPH), carotenoids and chlorophyll contents.

For total anthocyanins extraction, samples were grinded and homogenized with 200 mL of extracting solution (70 mL of ethanol 70% and 30 mL of HCl 0.1%, pH 2.0) for 2 min, in a blender, and kept in a beaker covered with parafilm and aluminum foil, in the dark, for 12 hours, at 4 ºC. Afterwards, the mixture was filtered in a 250 mL volumetric flask and filled with extracting solution. An aliquot of 2.0 mL was taken from the stock solution at 4 ± 0.5 ºC to a volumetric flask of 25 mL and filled again with the extracting solution then leaving it at room temperature, in the dark, for 2 hrs. The absorbance of each sample was determined at the wavelength of 535 nm, using a spectrophotometer (Shimadzu®, Model UV-3600-UV-Vis-NIR) and readings are expressed as milligrams of total anthocyanins per 100 g (mg 100 g^{-1}), on dry basis. The extracting solution was used as a blank (Clemente and Galli 2013).

Total phenolic compounds were calculated according to the methodology described by Wettasinghe and Shahidi (1999), using Folin-Ciocalteau reagent (Merck) and the gallic acid standard curve. A spectrophotometer was used for the sample evaluation and results are expressed as gallic acid equivalent (mg 100 g^{-1}), on a dry basis.

The antioxidant activity was measured by two methods: the ORAC (Ou et al., 2001) with some adjustments using microplates with fluorescein (Huang et al., 2002), and by the DPPH method (Brand-Willians et al., 1995). Samples were analyzed at three dilutions, considering the average as the final ORAC value. Antioxidant activity was calculated using the area under the fluorescence decay curve, as proposed by Prior et al. (2005). Thus, the remaining DPPH, at the end of the reaction, was determined and quantified using a standard curve of Trolox. The antioxidant activity is expressed in µmol Eq. Trolox per 100 g, on a dry basis.

For the extraction of total carotenoids, leaf samples were taken in test tubes covered with aluminum foil,

and 10 mL of hexane-acetone (6:4) extraction solution was added to it. The extract was shaken for 1 minute, using tube shaker. After waiting for 9 minutes, the extracts were filtered with cotton and immediately after that read using spectrophotometer at 450 nm, in triplicates. β-carotene was used as standard for making the calibration curve. Results were expressed in mg of β-carotene 100 g^{-1}, on dry basis (AOAC 2010).

The amounts of total chlorophyll were calculated according to Manfroi et al. (1996) methodology. From each sample 10 cm^2 of the epidermis from the equatorial region of the leaf (abaxial part) was removed as a strip measuring 1 cm wide and 10 cm long. These strips were then placed in sealed glass vials (220 mL) containing 20 mL of 80% acetone extraction solution. Next, flasks were covered with aluminum foil and kept at 4±1 °C for 72 h, absorbance of each solution was evaluated using spectrophotometer at wavelengths of 645 and 663 nm. The total chlorophyll contents were calculated using the following formula: total chlorophyll = 8.0 (absorbance at 663 nm) + 20.2 (absorbance at 645 nm). The results are expressed as mg. (10 cm^2)$^{-1}$, on dry basis.

A complete randomized design with five replications was used as statistical model. The data was submitted to analysis of variance and the means were fitted to regression models using the F test at 5% of probability for measuring the significance of the proposed model.

RESULTS AND DISCUSSION

All the data presented here represent an average performed during three study seasons (years). Over the three years, no statistically significant variations were detected among them, as mentioned above.

Bushy lippia (*Lippia alba*)

Results indicate that the total anthocyanin contents of bushy lippia, when kept at 24 °C, decreased as the storage period increased, since they are inconstant during the process, whereas temperature is one of the main reasons behind such behavior (Vargas et al., 2014). However, samples stored at 0 °C exhibited an increased concentration of anthocyanins due to the physiological stress caused by the low storage temperature. Results also show that storage of this product at 17 °C is the most suitable for the market, since at this temperature the anthocyanin contents remain at standard levels for up to 60 days and cause no cold injuries to the plant tissues (Figure 1A).

In regards to phenolic compounds (Figure 1B), the observed values for bushy lippia were lower than those reported by Morais et al. (2013) (203.60 mg g^{-1}). These low values can be related to the low amount of anthocyanin contents and total carotenoids found in bushy lippia, as these compounds are vital for the composition of the phenolic compounds.

Satisfactory level of antioxidant activity (DPPH and ORAC) was observed for bushy lippia at three different temperatures (Figure 1C). The antioxidant activity of these herbs inhibit or prevent the negative effect of oxidative stress, whereas, in this case, the presence of free radical scavengers is evident, the main ones being polyphenols, flavonoids and phenolic compounds (Azevedo et al., 2011). Using DPPH capture method, Morais et al. (2009, 2013) and Azevedo et al. (2011), recorded antioxidant activity of 0.54 mg mL^{-1}, 3.22 µg mL^{-1} and 27.29 mg mL^{-1} (EC 50), respectively. Antioxidant activity obtained by the DPPH method during this study was similar to that of other reported studies.

Total carotenoids of samples stored at 0 °C exhibited similar behavior (increased concentration) to that of anthocyanins and phenolic compounds due to the physiological stress caused by the low storage temperature (Figure 1D). Lins et al. (2015) observed the total carotenoid value of 1.81 mg 100 g^{-1} while evaluating bioactive compounds of bushy lippia in an open market, which are higher as compared with those observed in current research where total carotenoid contents decreased among samples stored at 17 and 24 °C along the storage periods. However, in the same way as in the present assay, the results found by Lins et al. (2015) were also lower, approximately 50% smaller, when compared with the carotenoid content of the samples stored at 0 °C.

The chlorophyll contents were higher at lower temperatures, showing a remarkable increase at 0 ºC during all the storage periods (Figure 1E). For bushy lippia, Lins et al. (2015) observed average chlorophyll contents of 4.52 mg 100g^{-1}, when evaluated its bioactive compounds in an open market. Considering the total chlorophyll contents (*a* and *b*) of bushy lippia noted by Alves (2015), chlorophyll levels found in this work can be considered lower, since he found values almost 25% higher than those presented here.

Crajiru (*Arrabidaea chica*)

Anthocyanin content for crajiru kept at 24 °C decreased along storage time. At 17 ºC, the anthocyanin content remained constant up to 60 days, showing a gradual decrease from that period on. However, when stored at 0 °C, these values increased along with the storage time (Figure 2A). According to Vargas et al. (2014), during the processing and manufacturing of anthocyanins derived products, color deterioration is higher, mainly during storage, where the color of the products degrades even more.

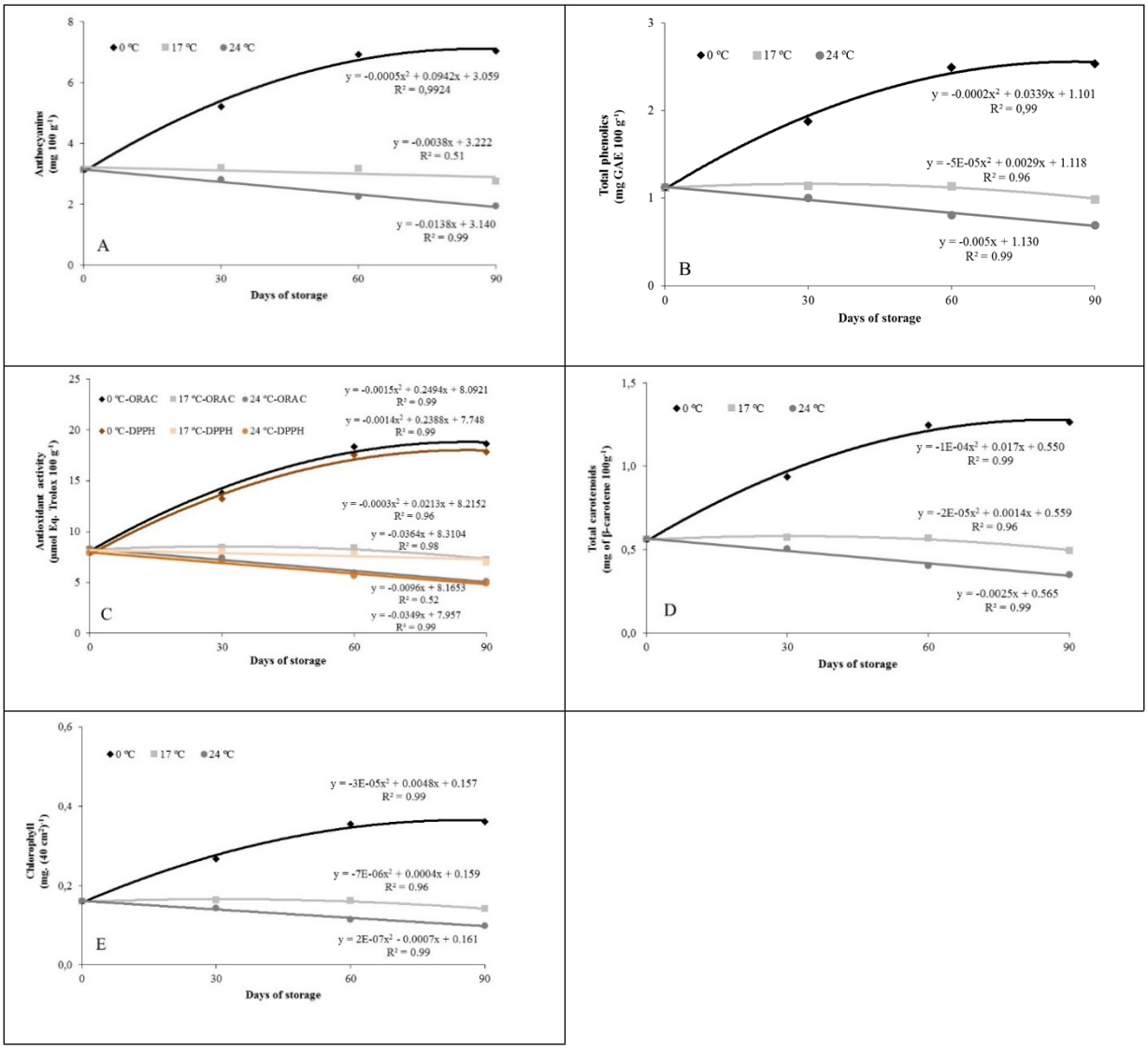

Figure 1. A: Total anthocyanins; B: Phenolics; C: Antioxidant activity; D: Carotenoids and chlorophyll contents of bushy lippia (*Lippia alba*) stored at different temperatures and for different periods.

While evaluating the pigmentation of crajiru in relation to its biomass, Taffarello (2008) observed anthocyanin contents between 1.25 and 1.7 mg g^{-1}, whereas during this study higher values were recorded for samples stored at 24 °C for up to 30 days, at 17 °C for up to 60 days and at 0 °C for up to 90 days. Thus, the treatments to which the crajiru was submitted seem to be feasible for keeping anthocyanin contents at an acceptable level for commercialization.

When samples were kept at 24 °C, the phenolic compounds decreased gradually along the storage periods. However, at 17 °C, the phenolic compounds of the samples remained constant until 60 days, presenting a gradual decrease after that period. Whereas at 0 °C, samples showed a progressive increase along the storage periods (Figure 2B).

While evaluating a mixed extract of crajiru, Port's (2011) observed phenolic compounds at the level of 159.22 mg g^{-1}, while Silva et al. (2007) reported 10.2 mg g^{-1} under the same conditions. During the current study, higher values were recorded when the samples were kept at 0 °C, regardless of the storage time.

Sousa (2013) studied and characterized crajiru extracts, and found that phenolic compounds of supercritical extracts were between 48.93 and 88.62 mg g^{-1}, while among conventional extracts the values were 37.63 (water) and 80.54 mg g^{-1} (ethanol), which are lower than those found in the current study.

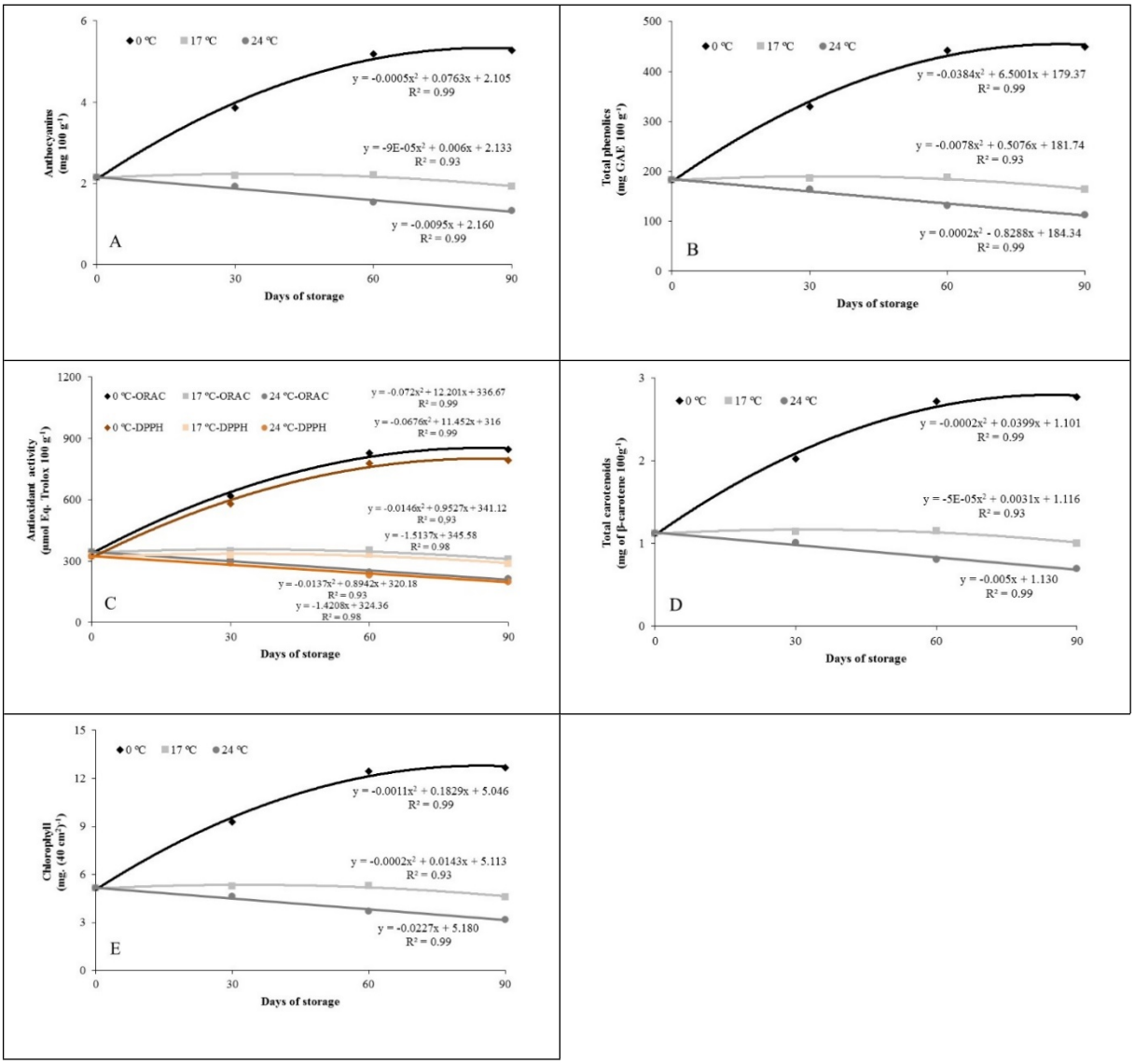

Figure 2. A: Total anthocyanins; B: Phenolics; C: Antioxidant activity; D: Carotenoids and chlorophyll contents of crajiru (*Arrabidaea chica*) stored at different temperatures and for different periods.

The antioxidant activity of crajiru is similar to that of other analyzed variables, such as anthocyanins, carotenoids and phenolic compounds. Since this activity is directly associated with these other variables, the antioxidant activity was higher when samples were kept at 0 °C. When samples were stored at 17 °C, the antioxidant activity increased up to 60 days, with a slight decrease at 90 days. At 24 °C, the antioxidant activity presented a gradual decrease at 30, 60 and 90 days of storage (Figure 2C).

While evaluating crajiru extracts using DDPH method, Santos (2015) found that antioxidant activity of supercritical extracts ranged between 38.34 EC50 (water) and 86.13 µg mL^{-1} (ethanol), while conventional extracts reached up to 167.34 (water) and 42.58 (ethanol) mg mL^{-1} (EC_{50}), which are lower when compared with the results of the current work. By using ORAC method, the antioxidant activity of crajiru was lower than that described by Sousa (2013), who reported mean values of 1066.9; 1363.8 and 1026.2 µM g^{-1} when evaluating antioxidant activity of extracts obtained using spray drying process. Samples stored at 0 °C for 90 days showed similar results to those described by Sousa (2013).

In regards to the total carotenoid content, similar behavior was observed in crajiru samples as of anthocyanins and phenolic compounds, when stored at 0 °C. This behavior can certainly be associated with the physiological stress caused by the low storage temperature, thus resulting in high carotenoid contents and increasing antioxidant activity. As for samples stored at 17 and 24 °C, similar pattern like anthocyanins

and phenolic compounds was observed (Figure 2D).

The chlorophyll contents of crajiru (Figure 2E) were higher as compared with those of bushy lippia. At 0 °C, the chlorophyll content increased along the storage periods, while at 17 and 24 °C the values decreased over time. Chlorophyll plays a key role in keeping the plant at a desirable physical shape, whereas this quality attribute is very important for consumer in selecting plant products (Von Elbe 2000).

Eucalyptus (*Eucalyptus grandis*)

Total anthocyanins contents among the eucalyptus samples increased at 0° C along the storage periods, whereas at 17 and 24 °C the effect is reverse (Figure 3A).

Vargas et al. (2014) observed anthocyanin values of 38.33; 13.38 and 12.65 mg $100g^{-1}$, when evaluating total anthocyanin contents in dried eucalyptus leaves at 50 and 70 °C, and verified that the leaves drying process causes significant loss to the anthocyanin contents. However, in the current study, recorded values at 0° C indicates that lower temperatures conserved anthocyanin contents in eucalyptus more effectively.

Phenolic compounds demonstrated the same behavior as to that of anthocyanins. Lower values were only observed when the samples were stored at 24 °C for 90 days (Figure 3B). Haida et al. (2011) recorded phenolic compounds values at the level of 8.06 mg g^{-1} in dry leaves of eucalyptus kept at room temperature, in a shaded place, and, finally, in an oven, at 45 ºC for 48 hours.

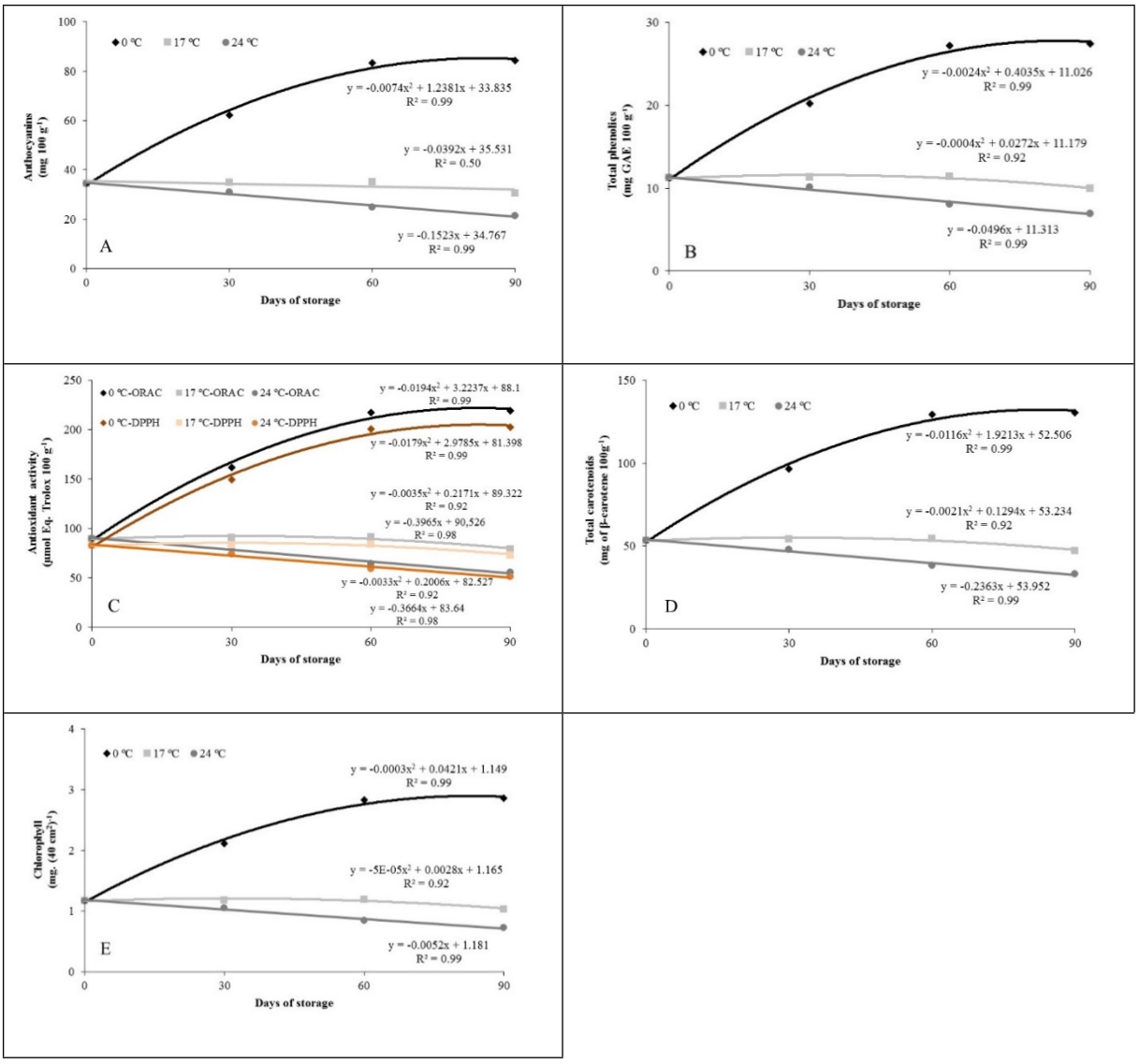

Figure 3. A: Total anthocyanins; B: Phenolics; C: Antioxidant activity; D: Carotenoids and chlorophyll contents of (*Eucalyptus grandis*) stored at different temperatures and for different periods.

There are still very few studies on phenolic compounds and antioxidant activity in eucalyptus. Yoo et al. (2008) compared several common commercial herbs and reached an average value of 6.21 mg 100 g^{-1} for eucalyptus. Recorded values of phenolic compound in the current work exceed that of Yoo et al. (2008).

Antioxidant activity in eucalyptus (Figure 3C) showed the same response as that of bushy lippia and crajiru; however, higher than that for bushy lippia and inferior to that for crajiru. Recorded values for antioxidant activity in eucalyptus were higher, except when samples were stored at 24 °C for 90 days.

So far, very few works have been done on the antioxidant activity and phenolic compounds for this specie. Haida et al. (2011) found that antioxidant activities in eucalypt ranged from 81.89 to 88.82%, and did not present any significant difference in the concentrations of methanoic extract. Inhibition percentages higher than 70% are considered high antioxidant activity, between 60 and 70% is considered moderate, and values below 60% are considered low (Melo 2007). Therefore, values observed during the current work are considered high antioxidant activities, except when samples were stored at 24 °C for more than 60 days.

High amounts of total carotenoids were observed in eucalyptus samples (Figure 3), highlighting their high antioxidant properties, being much higher than the amounts found for bushy lippia and crajiru (Figures 1D and 2D). The behavior of carotenoid contents is similar to that observed for the bushy lippia and crajiru, but with higher averages at lower temperatures and storage periods.

High contents of carotenoid may be a plant protection mechanism against oxidative stress damage. Marques et al. (2011) subjected leaves of *Eucalyptus camaldulensis* to high doses of cadmium in a nutrient solution and observed different response in young leaves which were not yet exposed to this treatment, with a reduction of 60% on the 20th day of exposure (21.44 µg g^{-1}). Results from the current study are same as those of Marques et al. (2011), even higher when samples were stored at 0 °C and 17 °C.

Results for chlorophyll content varied among eucalyptus samples like in the other species, with values slightly above those found for bushy lippia and below those found for crajiru, with an increase in mean values along storage periods at 0 °C (Figure 3E).

Lemon grass (*Cymbopogon citratus*)

Keeping lemon grass samples at 0 °C leads to higher anthocyanins contents and phenolic compounds over the storage periods, while at 17 and 24 °C these values were reduced (Figures 4A and 4B). Sena et al. (2012) reported an average value of 0.4305 µg g^{-1} for phenolic compounds, which is similar to the results observed during the current study.

Port's (2011) observed an average value of 0.27 mg g^{-1}, when evaluated phenolic and flavonoids profile in lemon grass tea, a value higher the value found by Lima et al. (2004), which was 0.16 mg g^{-1}. Average values recorded for most of the evaluated treatments during the present experiment are higher than the values found by other researchers, except for 24 °C at 60 and 90 days, where values are lower than 0.27 mg g^{-1}.

Antioxidant activity (by ORAC and DPPH methods) in lemon grass was similar to that found for bushy lippia, crajiru and eucalyptus, but with lower averages (Figure 4C). These results can be attributed to the low levels of phenolic compounds among the samples, since these compounds present high antioxidant activity.

Sena et al. (2012) observed antioxidant activity in lemon grass between 84.92% and 105.21%, while Morais et al. (2009) reported 17.36 mg mL^{-1} (EC_{50}) in teas and condiments, using the DPPH method.

The carotenoid contents of lemon grass at different temperatures and storage periods were similar to those found for other evaluated species, with values close to bushy lippia and crajiru, while inferior to eucalyptus (Figure 4D). Total carotenoid contents observed in this study are higher than those reported by Lins et al. (2015) (0.96 mg $100g^{-1}$), however, when stored at 24 °C for more than 60 days, results were inferior to those of Rêgo (2001) (1.4 mg 100 g^{-1}).

The chlorophyll contents of lemon grass were lower than those found for other medicinal plants, which were subjected to the same treatment (Figure 4E). Lins et al. (2015) when measured the compounds of this specie in an open market, recorded 4.52 mg 100 g^{-1} of chlorophyll contents, which are higher compared with the results of the current work.

Citronela (*Cymbopogon nardus*)

The anthocyanin contents of citronella are higher in comparison to those found for crajiru and lemon grass, except for eucalyptus, which presented higher anthocyanin contents (Figure 5A). Only the bushy

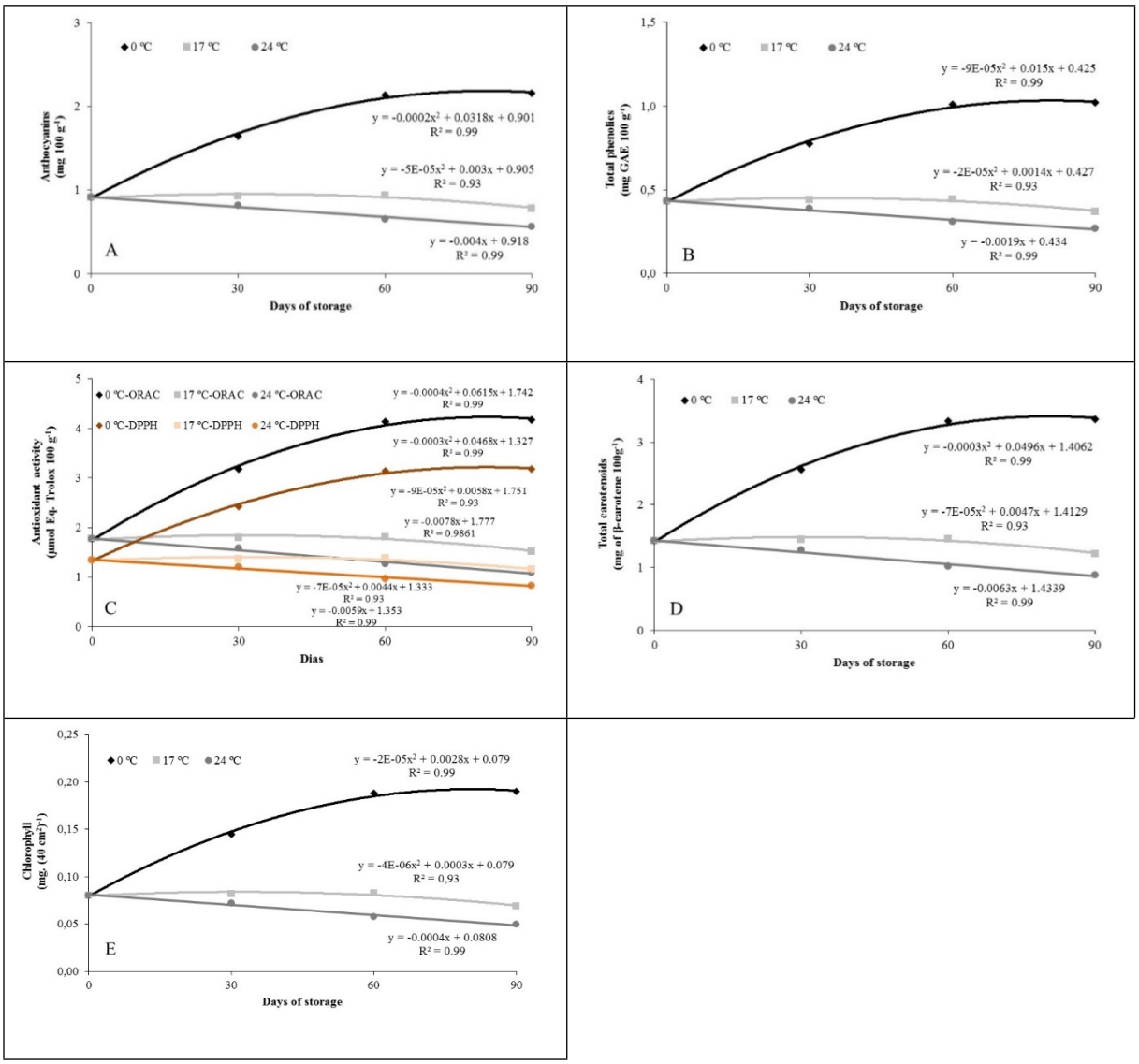

Figure 4. A: Total anthocyanins; B: Phenolics; C: Antioxidant activity; D: Carotenoids and chlorophyll contents of lemon grass (*Cymbopogon citratus*) stored at different temperatures and for different periods.

lippia showed values similar to those of citronella, showing the important phytotherapeutic potential of these species. Phenolic compounds followed the same pattern of other species, such as crajiru (Figure 5B).

Citronella presented high antioxidant activity, which can be attributed mainly to the phenolic compounds of this species, since the higher values were observed among samples stored at 0 and 17 °C for 60 days, and at 24 °C for 30 days (Figure 5C). The antioxidant activity of citronella essential oils derived by Andrade (2010) using the DPPH method was 517.40 µg mL^{-1} (IC_{50}). Thus, results for samples stored at 0°C and 17 °C for 60 days, and 24 °C for 30 days can be considered exceptional.

The total carotenoid content of citronella had a similar behavior to that observed among other evaluated species (Figure 5D). Andrade (2010) reported carotenoids contents of 20.65 µg.mL^{-1} (IC_{50}) for citronella, which is higher than the recorded values of this study.

In regards to chlorophyll contents, higher values were observed for citronella than those found for the other species, with a similar behavior under different temperatures and storage periods, except at 24 °C for 90 days (Figure 5E), and they were also higher than those reported by Lins et al. (2015) (4.25 mg 100 g^{-1}).

Sage (*Lippia microphylla*)

The anthocyanin contents observed for the sage samples are close to those found in bushy lippia, lemon grass and citronella (Figure 6A). Morais et al. (2013) observed phenolic compounds of 292.13 mg g^{-1} among sage plants. During the current study, the contents of phenolic compounds recorded among all the species

were lower as compared with other studies, except for eucalyptus, which presented higher values (Figure 6B).

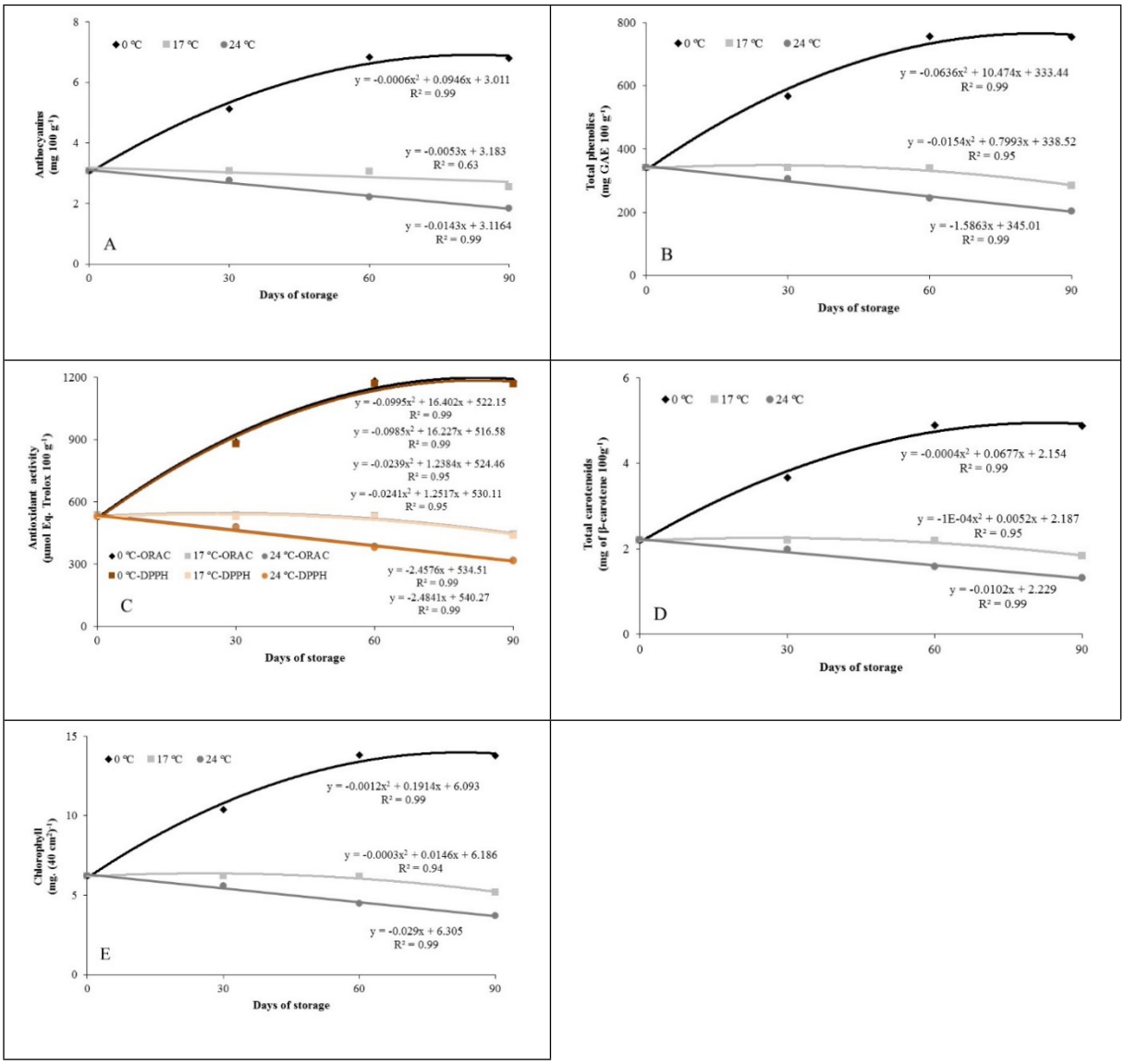

Figure 5. A: Total anthocyanins; B: Phenolics; C: Antioxidant activity; D: Carotenoids and chlorophyll contents of citronella (*Cymbopogon nardus*) stored at different temperatures and for different periods.

The antioxidant activity of sage samples were at satisfactory levels when compared with total phenolic compounds (Figure 6C). Similar effect of temperature and storage periods was observed on other species as well. Morais et al. (2013) reported antioxidant activity of 2.39 mg mL^{-1} (EC_{50}) for sage using the DPPH method, whereas the values obtained by the current experiment can be considered sufficient when leaves were stored at 0 °C, 17 °C and 24 °C for 60 days, with high antioxidant potential.

The carotenoids and chlorophyll behavior of sage followed the same pattern as the other evaluated medicinal species (Figures 6D and 6E).

At a high storage temperature (24 °C), quality and life of the studied samples reduced, and loss of active compounds was much higher, compared with samples stored at lower temperatures (17 °C). Loss of color, dryness, burns and tissue softening may have occurred due to enzymatic action, which occurs more rapidly at higher temperatures.

There is higher loss of carotenoids, anthocyanins and phenolic compounds at higher temperatures during storage time for all the evaluated species. The high contents of anthocyanins, carotenoids and phenolic compounds at 0 °C among the plant species may have occurred due to the physiological stress caused by the temperature, where the cells rupture due to ethanol and acetaldehyde accumulation, the inability of enzymes to metabolize such compounds upon rupture and cellular contents overflow.

Chlorophyll is an important characteristic that needs to be kept at satisfying levels in leaf samples for commercialization, since there is no loss of color among these products. Results from this study show that lower temperatures are more suitable for chlorophyll contents of leaves. Carotenoids are also much desired pigments during the storage of these species, as they are not only important for the pharmaceutical industries but they are also the precursors of vitamins A. Studies have confirmed that eucalyptus presents the highest carotenoid contents among the analyzed species, especially when stored at 0 °C.

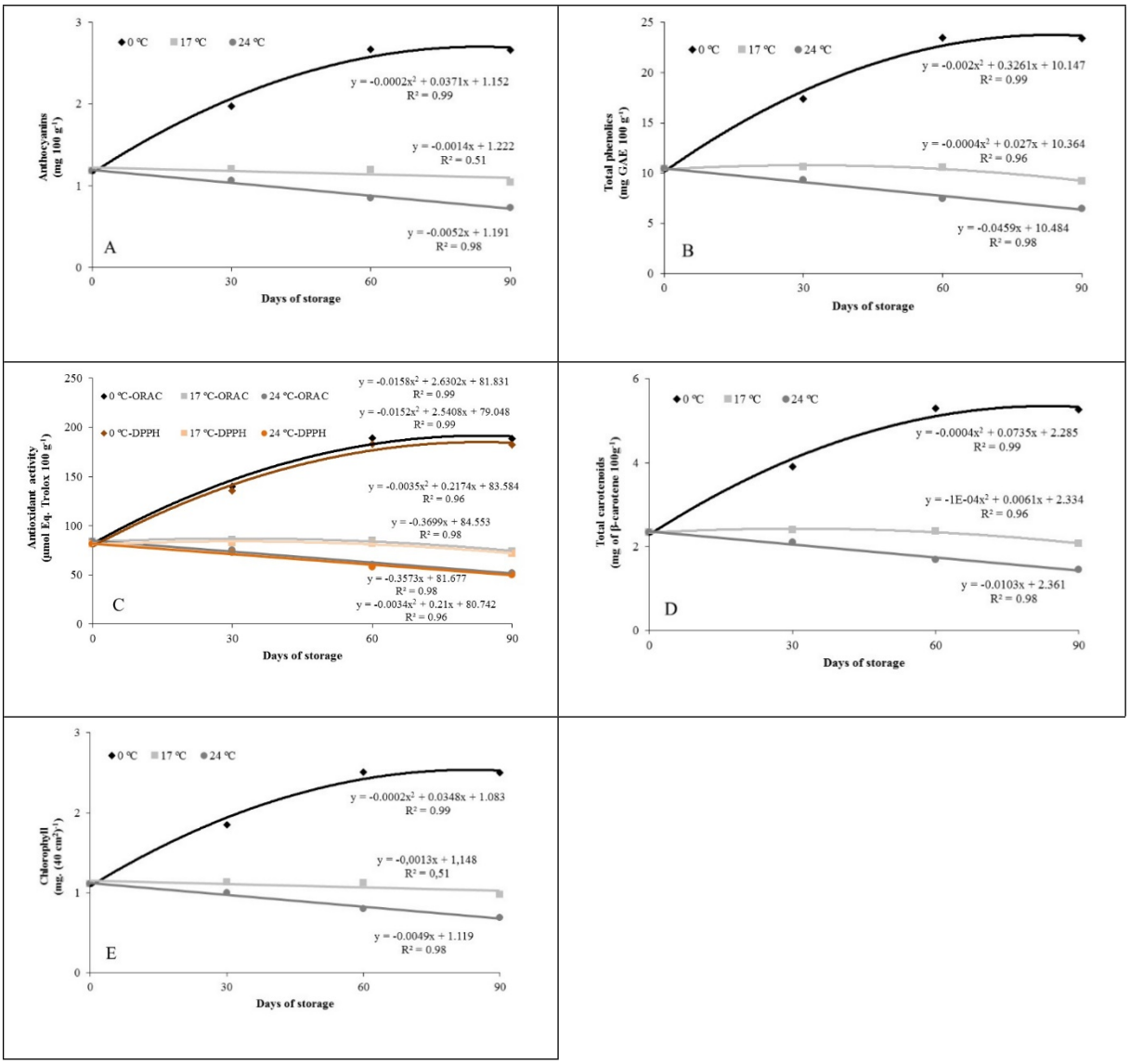

Figure 6. A: Total anthocyanins; B: Phenolics; C: Antioxidant activity; D: Carotenoids and chlorophyll contents of sage (*Lippia microphylla*) stored at different temperatures and for different periods.

Cold injuries suffered by leaf samples when stored at lower temperatures cause physiological disturbances with subsequent release of metabolites, such as amino acids, sugars and secondary metabolites. This explains the high contents of secondary compounds found at 0 °C storage.

All samples of the medicinal species stored at 0 °C showed better conservation and composition of chemical compounds and no fungal contaminations. A remarkable increase in anthocyanins, phenolic compounds, antioxidant activity, carotenoids and chlorophyll was observed, along the storage periods, mainly due to the physiological stress caused by the freezing temperature. In regards to storage at 17 and 24° C, different behavior was observed where anthocyanins, phenolic compounds, antioxidant activity, carotenoids and chlorophyll showed opposite response to those stored at 0 ºC. With increasing storage time to 24ºC, these contents decreased gradually.

CONCLUSION

The medicinal plant species investigated in this study showed better maintenance of their chemical composition, when stored at 0 °C, than the other samples stored at 17 and 24 °C, during the experimental time. However, this temperature causes physiological damage to the leaves. Thus, the commercialization of dried or crushed leaves in powder form is recommended.

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