









# The isolated effect of low oxygen and high carbon dioxide on yerba mate quality keeping

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**ABSTRACT:** The aim of this study was to evaluate the effect of controlled atmosphere storage on quality of native and cultivated yerba mate produced in Arvorezinha (RS) and São Mateus do Sul (PR) after 0, 3, 6 and 12 months of storage under ambient conditions, low oxygen (1.0 kPa) and high carbon dioxide (3.0 kPa) partial pressures. Total chlorophyll concentration reduced over the storage period regardless of the atmosphere condition, form of cultivation and production region. Total carotenoids, regardless of the form and place of cultivation, decreased until three months of storage under 1.0 kPa O<sub>2</sub>. Although, the O<sub>2</sub> reduction to 1.0 kPa maintains greener color and higher chlorophyll concentration after 12 months of storage. High carbon dioxide partial pressures (3.0 kPa) in the storage chamber increases the polyphenol concentration until six months of storage. The raw material originated from São Mateus do Sul (PR) has higher chlorophyll concentration and greener color, resulting in greater storage potential.

**Key words:** *Ilex paraguariensis*, carotenoids, coloration, polyphenol concentration, storage time.

## INTRODUCTION

The yerba mate (*Ilex paraguariensis*) is considered a very important crop in Argentina, Paraguay, Uruguay and the southern states of Brazil (Heck and Mejia 2007; Isolabella et al. 2010), especially due to its easy production in this region. In Latin America, the yerba mate growth occurs spontaneously in the nature but can also be cultivated. The southern states of Brazil, especially Paraná, are responsible for most of the yerba mate national production. This high production makes Brazil one of the greatest world producers of it (Giberti 2019). Nowadays, products of yerba mate have been quickly diffused into the United States of America (USA) and European countries (Valerga et al. 2012).

In the Latin-American countries, the yerba mate is consumed as tea and drinks called “chimarrão” and “tererê”. The consumption of these infusions can help prevent atherosclerosis and coronary heart disease (Heck and Mejia 2007; Menini et al. 2007). In addition to the healthy properties of the beverages, a recent report showed that the antioxidant extracts from yerba mate inhibited lipid oxidation of salami (Campos et al. 2007). Another work demonstrated that the antioxidant activity on *Saccharomyces cerevisiae* deficient in defense genes (Piovezan et al. 2016) and antioxidant activities with pharmaceutical and food industry potential (Kungel et al. 2018). This finding increased the importance of this crop even more, explaining the recent increment of yerba mate cultivation area. Nevertheless, in order to obtain the highest antioxidant level, yerba mate must be harvested at the correct development stage and stored in an appropriate condition until its processing and consumption takes place.

Nowadays, the yerba mate harvest is carried out over year due to the reduced storage time, especially when it is kept at an ambient condition. In Brazil, the appropriate yerba mate harvest period is from May until September. The reduced storage period, at ambient conditions, occurs due to the green color degradation, the reduction in phenolic compounds and the chlorophyll oxidation, which also culminate in a reduction of its benefits to health (Filip and Ferraro 2003; Prestes *et al.* 2014; Thewes *et al.* 2016). Furthermore, when the yerba mate harvest is carried out at incorrect maturity, its sensorial quality is changed, increasing the bitter flavor (Bastos *et al.* 2018). Another factor that can affect the postharvest quality is the region in which the yerba mate was produced or if it is native or cultivated. Recently, a research supported that the industrial processing also affects the chemical quality of the yerba mate (Isolabella *et al.* 2010). For this reason, it is necessary to develop technologies to maintain the postharvest quality and allow the harvest at the correct maturity/period.

One technique that can be used to prolong the postharvest life of yerba mate is the controlled atmosphere (CA) (Prestes *et al.* 2014; Thewes *et al.* 2016). The CA consists of oxygen partial pressure reduction and carbon dioxide partial pressure increasing in the storage environment. This technique is worldwide used for living products storage, such as apples (Hoang *et al.* 2011; Weber, *et al.* 2013), pears (Larrigaudiere *et al.* 2001; Pedreschi *et al.* 2008; Saquet 2019), peaches (Girardi *et al.* 2005; Murray *et al.* 2007; Sestari *et al.* 2008), persimmons (Burmeister *et al.* 1997; Brackmann *et al.* 2004), kiwis (Antunes and Sfakiotakis 2002; Brackmann *et al.* 2012), among other fruits. However, the application of CA on a processed product, such as yerba mate, is poorly studied. Therefore, it is necessary to evaluate the efficiency of CA on the maintenance of yerba mate's quality after harvest, allowing the harvest and processing at the correct time.

In a view of the above exposed, the present study aimed to evaluate the effect of CA storage on quality maintenance of native and cultivated yerba mate from two Brazilian production regions (Arvorezinha-RS and São Mateus do Sul-PR) after 0, 3, 6 and 12 months of storage under ambient conditions, low oxygen (1.0 kPa) and high carbon dioxide (3.0 kPa) partial pressures.

## MATERIAL AND METHODS

The experiment was conducted at the Postharvest Research Center of the Department of Plant Sciences of the Federal University of Santa Maria (UFSM). The experimental material comprised raw material (yerba mate), proceeding from two macro production regions, located at Arvorezinha (28°51'00.6"S 52°08'11.2"W) and São Mateus do Sul (25°53'57.3"S 50°21'45.4"W), from the states of Rio Grande do Sul and Paraná, respectively. According to Wrege *et al.* (2012), the region of Arvorezinha has an annual temperature of 17.4 °C and precipitation of 1,855.1 mm, and the region of São Mateus do Sul has an annual temperature of 17.2 °C and precipitation of 1,612.5 mm. According to Köppen, Arvorezinha and São Mateus do Sul have a Cfb climate classification (temperate, humid without defined dry season). Both locations are situated in Brazil, South America. Two types of yerba mate were harvested: native and cultivated. The main difference between native and cultivated yerba mate is that the native grows spontaneously under shade of other plants and the cultivated one is grown in open field. Immediately after harvest, the raw material was transported to the industry Vier (yerba mate industry, Santa Rosa, RS, Brazil). At this industry, the raw material was processed according to Prestes *et al.* (2014). The yerba mate was processed and transported to the Postharvest Research Center immediately after processing, then samples were done and the storage conditions applied.

The yerba mate samples were homogenized and put into experimental chambers of 0.23 m<sup>3</sup> that were hermetically closed. Then, the following storage conditions were applied: (1) 20.9 kPa O<sub>2</sub> + 0.04 kPa CO<sub>2</sub> (Ambient storage); (2) 1.0 kPa O<sub>2</sub> + 0.04 kPa CO<sub>2</sub> and (3) 20.9 kPa O<sub>2</sub> + 3.0 kPa CO<sub>2</sub>. All storage conditions were carried out at a room temperature of 20 ± 0.5 °C. For each treatment, three replications of 1 kg each were used.

The O<sub>2</sub> reduction was obtained by flushing the chamber atmosphere with nitrogen until it reached the pre-established partial pressure. The CO<sub>2</sub> level was obtained by its injection from a high-pressure cylinder up to the pre-established level. During the storage period, the gases partial pressures (O<sub>2</sub> and CO<sub>2</sub>) were monitored and corrected once a week, using a Isolcell, Oxycarb 6 (Italy) analyzer.

The quality parameters were evaluated after 0, 3, 6 and 12 months of storage, as follows:

a) Total chlorophyll and total carotenoid concentrations, the extraction of these compounds was made with acetone 80% (v/v). Then, the absorbance was obtained by spectrophotometry at 647, 663 and 470 nm. The concentration of chlorophyll and carotenoid was calculated according to the equations described in Lichtenthaler (1987). The solution absorbance was obtained with a spectrophotometer (Femto, 700 plus, SP, Brazil) at 470, 647 and 663 nm. The following expressions (Eq. 1 and 2) were used for quantification:

$$\text{Total chlorophylls} = 7.15 (\text{Absorbance } 663 \text{ nm}) + 18.71 (\text{absorbance } 647 \text{ nm}) \quad (1)$$

$$\text{Total carotenoids} = [1,000 (\text{absorbance } 470 \text{ nm}) - 1.82 \text{ Chlorophyll 'a'} - 85.02 \text{ Chlorophyll 'b'}]/198 \quad (2)$$

The chlorophyll and carotenoid concentrations were expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  of fresh weight (FW);

b) Total phenolic compounds were determined by the Folin–Ciocalteu colorimetric method, described by Singleton et al. (1999), the phenolic compounds were extracted from 20 g of yerba mate per replicate, according to Prestes et al. (2014);

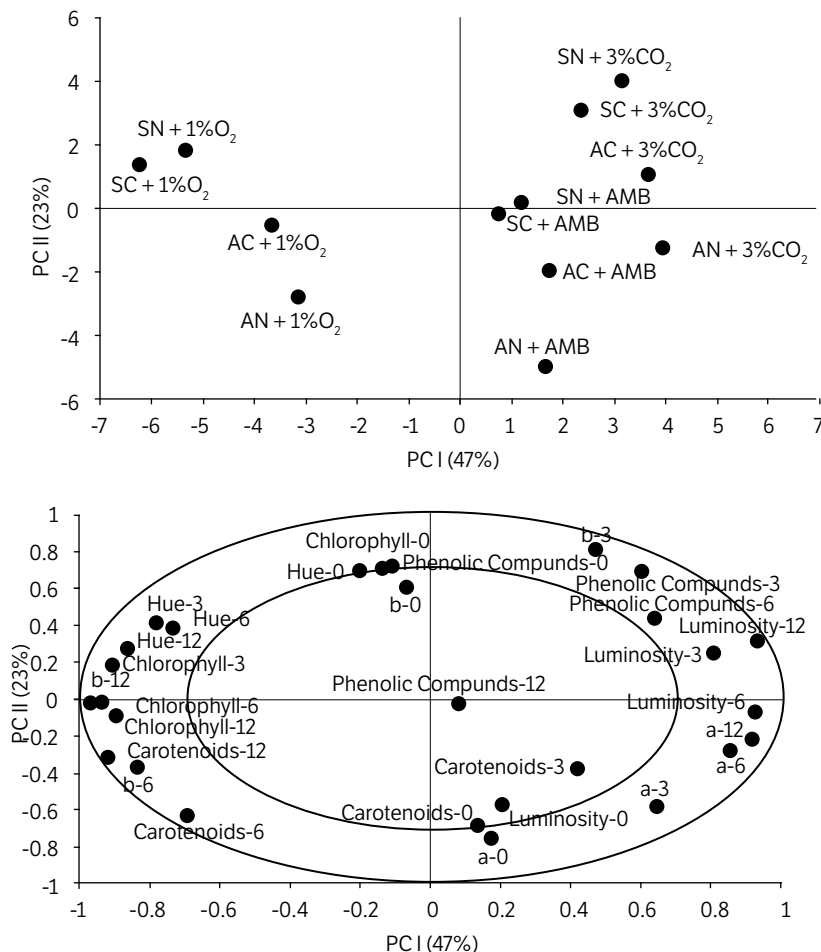
c) Yerba mate coloration was determined with a colorimeter (Minolta CR 310). Previously to the color determination, yerba mate samples were manually sieved, with the use of test strainer with an 800  $\mu\text{m}$  mesh aperture, to eliminate white sticks left from the branches milling. Then the samples were stowed and compacted in Petri dishes. The color values were expressed in terms:  $L^*$  (Luminosity) indicates black/white coordinate (values of 0 indicate black and values of 100 indicate white);  $a^*$ : red/green coordinate (+  $a^*$  indicates red and –  $a^*$  indicates green);  $b^*$ : yellow/blue coordinate (+  $b^*$  indicates yellow and –  $b^*$  indicates blue) and hue angle.

The experiment was conducted in a completely randomized scheme. All data were submitted to a Principal Component Analysis (PCA) using The Unscrambler X software (version 9.7, CAMO A/S, Trondheim, Norway) to show an overview of the results. Before PCA, the data matrix was auto scaled for each variable in order to obtain the same weight for all variables (mean = 0 and variance = 1). Additionally, an analysis of variance (ANOVA) was performed for all the parameters evaluated. When ANOVA was significant, the parameters were compared by least significant difference (LSD) test at 5% of error probability ( $p < 0.05$ ). A regression analysis was also undertaken ( $p < 0.05$ ) to study the effect of the storage period (months). The software Sisvar and Action for Excel were used to run the statistical analysis.

## RESULTS AND DISCUSSION

The multivariate analysis was carried to show an overview of storage conditions and its correlation with the variables evaluated (Fig. 1). The principal component one (PCI) separated the yerba mate stored under 1.0 kPa  $\text{O}_2$  from the ones stored under 20.9 kPa  $\text{O}_2$  + 0.03 kPa  $\text{CO}_2$  and 20.9 kPa  $\text{O}_2$  + 3.0 kPa  $\text{CO}_2$ , regardless of the form and place of cultivation (Fig. 1a). Chlorophyll concentration, hue angle and carotenoids evaluated after 6 and 12 months of storage are correlated to 1.0 kPa  $\text{O}_2$ . (Fig. 1b). On the other hand, luminosity, phenolic compounds, carotenoids at 0 and 3 months and, color coordinate  $a^*$ , are correlated to yerba mate stored under 20.9 kPa  $\text{O}_2$  + 0.03 kPa  $\text{CO}_2$  and 20.9 kPa  $\text{O}_2$  + 3.0 kPa  $\text{CO}_2$ . Concerning PCII, yerba mate produced in Arvorezinha were separated from those produced in São Mateus do Sul (Fig. 1a). Yerba mate produced in São Mateus do Sul are correlated to hue angle and phenolic compounds, regardless of the form of cultivation (Fig. 1b). On the other hand, Arvorezinha yerba mate is correlated with carotenoids and  $a^*$  values of color.

The green color of the yerba mate has a close relationship with the presence of chlorophyll concentration. On the present work the total chlorophyll concentration reduced over the storage period, regardless of the storage condition, form of cultivation and place in which the yerba mate was cultivated (Table 1). Concerning the effect of the storage condition in each evaluation time, a higher chlorophyll concentration was obtained on cultivated yerba mate from São Mateus do Sul stored at 1.0 kPa  $\text{O}_2$  (Fig. 2). The lower chlorophyll degradation of yerba mate under this storage condition is correlated to the lower oxidation process due to the low oxygen partial pressure in the storage environment. The use of high carbon dioxide levels to reduce the chlorophyll degradation was not efficient and did not present any benefits in comparison to the yerba mate stored at ambient conditions, otherwise high  $\text{CO}_2$  generally reduced chlorophyll content.



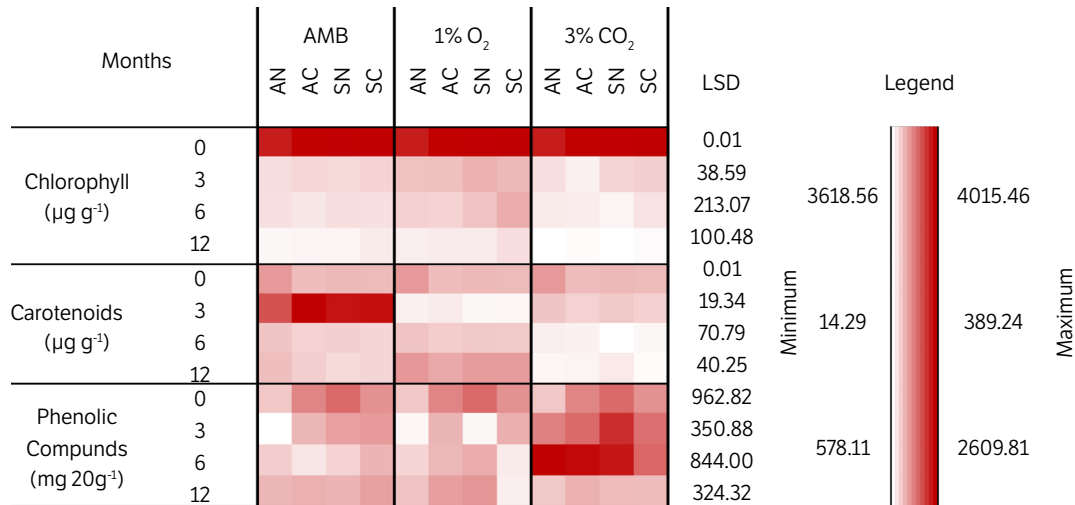
**Figure 1.** Scores of treatments (a) and correlation loadings (b) of variables plots showing the principal components of yerba mate stored under the conditions of: ambient storage (AMB); 1.0 kPa O<sub>2</sub> and 3.0 kPa CO<sub>2</sub> after 0, 3, 6 and 12 months of storage. AN - Arvorezinha native, AC - Arvorezinha cultivated, SN - São Mateus do Sul native, SC - São Mateus do Sul cultivated.

**Table 1.** Regression analysis of chlorophylls, carotenoids and phenolic compounds concentration change over the 12 months of storage under ambient storage (AMB), 1.0 kPa O<sub>2</sub> and 3.0 kPa CO<sub>2</sub>.

Storage conditions	Chlorophylls		Carotenoids		Phenolic compounds		
	Equation	r <sup>2</sup>	Equation	r <sup>2</sup>	Equation	r <sup>2</sup>	
AMB	AN <sup>*</sup>	$y = 2233.9e^{-0.146x}$	0.74	ns <sup>**</sup>	-	$y = 6.9629x^2 - 61.844x + 930.84$	0.53
	AC	$y = 2369.3e^{-0.15x}$	0.72	ns	-	$y = 15.017x^2 - 212.84x + 1580.3$	0.95
	SN	$y = 2438.9e^{-0.149x}$	0.74	ns	-	$y = 14.056x^2 - 221.59x + 1806.7$	0.97
	SC	$y = 2368.3e^{-0.131x}$	0.66	ns	-	ns	-
1% O <sub>2</sub>	AN	$y = 2623.9e^{-0.135x}$	0.85	$y = 2.6273x^2 - 29.439x + 147.59$	0.67	$y = 6.3007x^2 - 66.318x + 957.11$	0.54
	AC	$y = 2739e^{-0.137x}$	0.80	$y = 1.5243x^2 - 14.736x + 102.63$	0.75	$y = 9.2337x^2 - 124.09x + 1522.6$	0.93
	SN	$y = 3042.6e^{-0.14x}$	0.89	$y = 1.9546x^2 - 18.252x + 103.46$	0.72	$y = 17.712x^2 - 223.44x + 1608$	0.47
	SC	$y = 2942.1e^{-0.112x}$	0.77	$y = 1.8738x^2 - 17.057x + 99.05$	0.74	$y = -63.484x + 1362.1$	0.79
3% CO <sub>2</sub>	AN	$y = 2230.7e^{-0.174x}$	0.80	$y = 1.4717x^2 - 29.414x + 167.96$	0.99	$y = -37.935x^2 + 470.23x + 879.98$	0.86
	AC	$y = 1975.8e^{-0.158x}$	0.60	$y = 0.9005x^2 - 17.932x + 116.24$	0.97	$y = -26.804x^2 + 306.82x + 1423.2$	0.81
	SN	$y = 2450.8e^{-0.188x}$	0.81	$y = 1.4444x^2 - 24.348x + 128.15$	0.82	$y = -26.224x^2 + 262.66x + 1754.8$	0.99
	SC	$y = 2651.8e^{-0.172x}$	0.84	$y = 0.8468x^2 - 18.53x + 118.26$	0.94	$y = -14.036x^2 + 140.26x + 1447.8$	0.99

<sup>\*</sup> AN - Arvorezinha native, AC - Arvorezinha cultivated, SN - São Mateus do Sul native, SC - São Mateus do Sul cultivated. <sup>\*\*</sup> Not significant at 5% of error probability.

No significant difference was observed after 12 months of storage between the native and cultivated yerba mate originated from Arvorezinha, whereas the cultivated yerba mate, obtained from São Mateus do Sul, maintained a higher chlorophyll concentration in ambient storage and CA with 1.0 kPa O<sub>2</sub>. These results suggest that the cultivated yerba mate from São Mateus do Sul has a higher storage potential as compared to the native one. Comparing the two places, a noteworthy fact is that yerba obtained from São Mateus do Sul showed an equivalent or a higher chlorophyll concentration, but never lower, in relation to the yerba mate from Arvorezinha (Fig. 2).



**Figure 2.** Heat map showing the difference between the storage conditions in relation to the total chlorophylls, carotenoids, phenolic compounds of yerba mate stored under the conditions of: ambient storage (AMB); 1.0 kPa O<sub>2</sub> and 3.0 kPa CO<sub>2</sub> after 0, 3, 6 and 12 months of storage. AN - Arvorezinha native, AC - Arvorezinha cultivated, SN - São Mateus do Sul native, SC- São Mateus do Sul cultivated.

When comparing the three storage conditions, after a period of three months of storage, the yerba mate stored at ambient conditions showed the highest carotenoids concentration, differing from the other treatments, but at six months of storage, its concentration was already higher than yerba mate stored in a CA with 3.0 kPa CO<sub>2</sub>. After 12 months of storage, CA with 3.0 kPa CO<sub>2</sub> had the lower carotenoids concentrations as compared to ambient and CA with 1.0 kPa O<sub>2</sub> (Fig. 2).

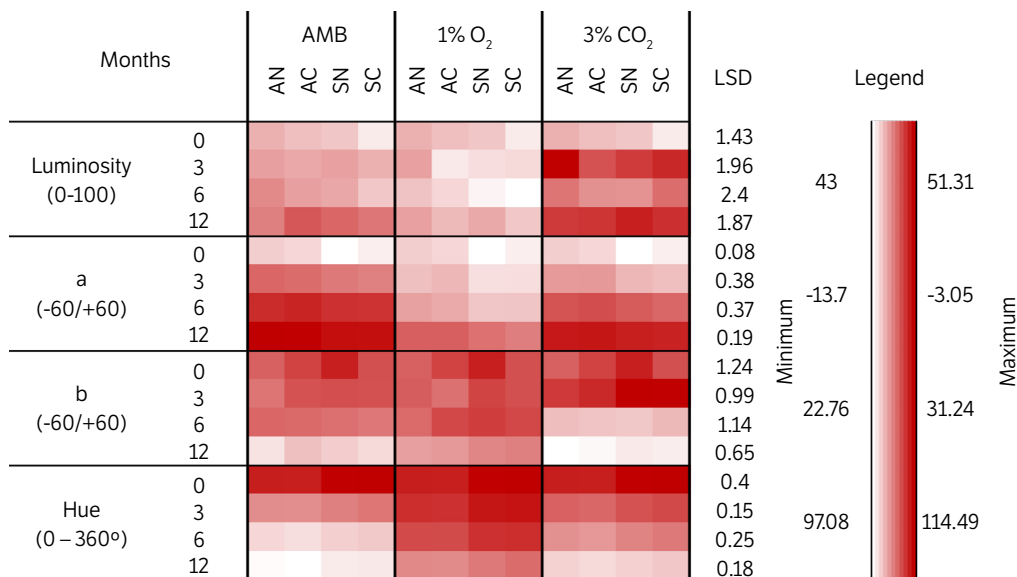
No significant difference between native and cultivated yerba mate from São Mateus do Sul was observed at the harvest, but when the raw material was originated from Arvorezinha the native yerba mate demonstrated a higher carotenoids concentration in relation to the cultivated one (Fig. 2). An inverse result was observed in the yerba mate stored at ambient condition and under CA with 1.0 kPa O<sub>2</sub>, after three months of storage. When comparing the native yerba mate from both places, a higher carotenoids concentration was observed in samples obtained from Arvorezinha, whilst the cultivated yerba mate did not present a significant difference at harvest (Fig. 2). After three months of storage, yerba mate in ambient conditions, regardless of the cultivation form, showed the highest carotenoids concentration when compared to CA conditions.

Regarding the total phenolic compounds, a remarkable fact is that the carbon dioxide application increased the phenolic compounds until six months of storage and then a reduction happens after 12 months of storage (Table 1). The yerba mate cultivated from São Mateus do Sul in an ambient storage condition did not show change in phenolic compounds during the storage period (Fig. 2). Comparing the storage conditions, the samples stored in CA with 3.0 kPa CO<sub>2</sub> showed higher phenolic concentration at three and six months of storage in all types of yerba mate. No considerable difference was observed between ambient storage and CA with 1.0 kPa O<sub>2</sub>. This result suggests that the storage in low O<sub>2</sub> levels did not bring significant benefits to the maintenance of the phenolic compounds.

No substantial difference in phenolic compounds were verified when comparing the native and the cultivated yerba mate at the harvest from both locations (Fig. 2). However, after three months of storage in ambient conditions, the cultivated yerba mate from Arvorezinha showed a higher phenolic compound concentration when compared to the native one. Native yerba mate from São Mateus do Sul exhibited a higher phenolic concentration after harvest and at three months of storage (Fig. 2). On the other hand, the

cultivated yerba mate did not present any significant difference at harvest (Fig. 2). However, after six months of storage, cultivated yerba mate from Arvorezinha (RS) showed a higher phenolic compounds concentration in CA with 1.0 kPa O<sub>2</sub> or 3.0 kPa CO<sub>2</sub>.

One of the main quality characteristics of the yerba mate is its color. The luminosity of the color increased or remained the same during the storage for all CA treatments, form of cultivation and location (Table 2). This fact demonstrated that, during the storage period, the yerba mate acquires a whiter color than at the harvest. When comparing the CA storage conditions, yerba mate stored with 1.0 kPa O<sub>2</sub> showed lower luminosity than the other treatments in almost all periods of evaluation, proving that low oxygen partial pressures maintain the color of the yerba mate similarly to the harvest (Fig. 3). However, CA with a high carbon dioxide level (3.0 kPa) did not preserve the original color (harvest color) and increased the white color formation of the yerba mate over the storage period (Fig. 3).



**Figure 3.** Heat map showing the difference between the storage conditions in relation to the luminosity of color, hue angle of color and coloration of yerba mate stored under the conditions of: ambient storage (AMB); 1.0 kPa O<sub>2</sub> and 3.0 kPa CO<sub>2</sub> after 0, 3, 6 and 12 months of storage. AN - Arvorezinha native, AC - Arvorezinha cultivated, SN - São Mateus do Sul native, SC - São Mateus do Sul cultivated.

When examining the native and the cultivated yerba mate from Arvorezinha, only one significant difference was observed at three months of storage at CA with 1.0 kPa O<sub>2</sub> and 3.0 kPa CO<sub>2</sub>: the native yerba mate showed higher luminosity of color. Concerning the raw material obtained from São Mateus do Sul, a significant difference was found only in CA with 3.0 kPa CO<sub>2</sub>, after six months of storage the cultivated yerba mate exhibited higher luminosity than the native one. The luminosity of color was higher in raw material, either native or cultivated, originated from Arvorezinha (RS), and native differed from cultivated from São Mateus do Sul (PR) (Fig. 3).

The green color of the yerba mate decreased over the storage period in all analyzed conditions (Table 2). Although, at ambient storage condition, green color loss was more pronounced (Fig. 3). These results indicated that the yerba mate stored in ambient conditions has a faster degradation of its green color in relation to the other treatments. Another noteworthy result is that the yerba mate stored in a CA with 1.0 kPa O<sub>2</sub> presented the highest green color in all evaluation. Perhaps this is correlated to the chlorophyll concentration, seeing that the yerba mate stored in CA with 1.0 kPa O<sub>2</sub> also exhibited a high concentration of this molecule until the 6<sup>th</sup> month storage. The yerba mate stored under CA with 3.0 kPa CO<sub>2</sub> demonstrated an intermediate green color maintenance when compared to the ambient storage and CA with 1.0 kPa O<sub>2</sub> (Fig. 3).

The cultivated yerba mate from São Mateus do Sul presented a greener color after three months of storage under CA with 3.0 kPa CO<sub>2</sub> and after 12 months of storage in CA with 1.0 kPa O<sub>2</sub>. Comparing the two places from which the yerba mate was obtained, it can be observed that yerba mate from São Mateus do Sul (PR) exhibited a greener color than the one from Arvorezinha. The greener color of this yerba mate is correlated to its higher chlorophyll concentration (Fig. 1).

**Table 2.** Regression analysis of the yerba mate color change over the 12 months of storage under ambient storage (AMB), 1.0 kPa O<sub>2</sub> and 3.0 kPa CO<sub>2</sub>

Storage conditions	Luminosity		A		b		hue		
	Equation	r <sup>2</sup>	Equation	r <sup>2</sup>	Equation	r <sup>2</sup>	Equation	r <sup>2</sup>	
AMB	AN	$y = 0.1355x + 45.691$	0.91	$y = -0.0733x^2 + 1.5884x - 11.578$	0.99	$y = -0.0443x^2 + 0.1947x + 27.803$	0.95	$y = 0.1421x^2 - 2.974x + 112.56$	0.99
	AC	$y = 0.2742x + 44.939$	0.95	$y = -0.0826x^2 + 1.7335x - 12.005$	0.99	$y = -0.0212x^2 - 0.0904x + 29.017$	0.99	$y = 0.1474x^2 - 3.0566x + 112.52$	0.99
	SN	$y = 0.2459x + 44.905$	0.92	$y = -0.104x^2 + 2.0778x - 13.616$	0.99	$y = -0.4777x + 30.229$	0.99	$y = 0.1683x^2 - 3.3424x + 114.35$	0.99
	SC	$y = 0.2877x + 43.851$	0.83	$y = -0.0873x^2 + 1.8302x - 13.057$	0.99	$y = -0.0308x^2 - 0.0215x + 28.679$	0.99	$y = 0.1604x^2 - 3.2474x + 114.56$	0.99
1% O <sub>2</sub>	AN	ns**	-	$y = 0.0146x^2 + 0.2164x - 11.697$	0.99	$y = -0.0218x^2 + 0.084x + 28.062$	0.99	$y = -0.0185x^2 - 0.4024x + 112.62$	0.99
	AC	$y = 0.0316x^2 - 0.3474x + 44.936$	0.78	$y = 0.0114x^2 + 0.2676x - 11.889$	0.98	$y = -0.2034x + 28.945$	0.61	$y = -0.0188x^2 - 0.4021x + 112.5$	0.99
	SN	$y = 0.0491x^2 - 0.5185x + 44.943$	0.95	$y = 0.0129x^2 + 0.3296x - 13.627$	0.99	$y = -0.2721x + 30.222$	0.91	$y = -0.025x^2 - 0.3784x + 114.39$	0.99
	SC	$y = 0.0213x^2 - 0.1883x + 43.903$	0.51	$y = 0.0167x^2 + 0.1892x - 13.008$	0.99	$y = -0.0253x^2 + 0.1815x + 28.51$	0.96	$y = -0.0171x^2 - 0.4099x + 114.51$	0.99
3% CO <sub>2</sub>	AN	ns	-	$y = 0.636x - 11.261$	0.96	$y = -0.5199x + 28.999$	0.79	$y = -1.0061x + 111.58$	0.97
	AC	$y = 0.301x + 45.912$	0.58	$y = 0.6733x - 11.43$	0.95	$y = -0.5833x + 29.731$	0.81	$y = -1.0314x + 111.36$	0.96
	SN	$y = 0.3566x + 45.889$	0.53	$y = -0.0501x^2 + 1.4088x - 13.907$	0.99	$y = -0.6584x + 30.844$	0.74	$y = 0.0561x^2 - 1.8294x + 114.24$	0.99
	SC	$y = -0.07x^2 + 1.251x + 44.526$	0.63	$y = 0.7297x - 12.792$	0.96	$y = -0.5568x + 29.981$	0.66	$y = -1.1036x + 113.56$	0.97

\* AN - Arvorezinha native, AC - Arvorezinha cultivated, SN - São Mateus do Sul native, SC - São Mateus do Sul cultivated. \*\* Not significant at 5% of error probability.

High CO<sub>2</sub> concentration seems to intensify the yellow color up to six months of storage at all yerba mate sources. The higher yellowing at 3 kPa CO<sub>2</sub> concentration has a direct relation to chlorophyll and carotenoids lower concentration up to the 6<sup>th</sup> month storage of this treatment and lower chlorophyll concentration at ambient storage after 12 months at both yerba mate sources (Fig. 3).

The CA with 3.0 kPa CO<sub>2</sub> maintained higher hue angle when compared to ambient storage over the storage period at both raw yerba mate source, but lower than CA with 1.0 kPa O<sub>2</sub> (Fig. 3). There was no discrimination among native and cultivated yerba mate at both production region sources. Only when comparing the production regions, there was a tendency of higher hue angle of São Mateus do Sul compared to Arvorezinha.

The intense green color of yerba mate is a reflection of the presence of chlorophyll (Cabral-Malheiros et al. 2010). The present study proved that chlorophyll concentration decreases during storage, congruently to results found by previous reports (Steet and Tong 1996; Morawicki et al. 1999; Cabral-Malheiros et al. 2010). However, during storage, the yerba mate's green coloration does not follow this behavior observed in the chlorophyll concentration, with the exception of the raw material stored in ambient conditions, which showed a faster green color loss. Perhaps, the lower chlorophyll degradation during storage at both CA conditions may be related to the different pathways by which the chlorophyll is degraded. The exchange of Mg<sup>2+</sup> by a hydrogen molecule in the chlorophyll molecule results in the pheophytin formation (Langmeier et al. 1993; Ahmed et al. 2002). The pheophytin changes the yerba mate color from a brilliant green to an olive-green color. This alteration may occur during the processing and storage of the yerba mate (Schwartz and Lorenzo 1990; Heaton et al. 1996) and is the fastest reaction, being considered the most important pathway for chlorophyll degradation during these steps (Martins and Silva 2002; Streit et al. 2005). Nevertheless, there is another significant pathway for chlorophyll degradation on yerba mate, in which phytol is removed from the chlorophyll molecule and then Mg<sup>2+</sup> is removed from the center of the

porphyrin molecule, resulting in the formation of a molecule called pheophorbide, which exhibits greenish-brown coloration. This pheophorbide molecule is the last green colored compound formed during chlorophyll degradation (Thomas *et al.* 2001). Posteriorly, the pheophorbide is transformed into colorless products and the appearance of associated pigments, such as carotenoids, occurs (Streit *et al.* 2005). Thus, it can be inferred that yerba mate stored in ambient conditions followed the first way, due the fastest chlorophyll degradation and yerba mate stored in a CA with 1.0 kPa O<sub>2</sub> and at CA with 3.0 kPa CO<sub>2</sub>, the second way cited previously.

Another research suggests that yerba mate stored in ambient conditions can be a favorable environment for the development of enzymatic darkness (Cabral-Malheiros *et al.* 2010). However, on the present study, an opposite result was observed, considering that the luminosity of color increased during the storage time and a whiter coloration was obtained after 12 months of storage in relation to harvest (Table 2). The increase of the luminosity during the storage is possibly related to the increase of colorless products, generated by the chlorophyll degradation. According to the studies previously cited, the chlorophyll degradation allows the appearance of some associated pigments, such as carotenoids. This molecule is an important fraction of the antioxidant capacity of the yerba mate and is essential to prevent the oxidative process and human diseases, such as cancer (Shami and Moreira 2004; Candelas-Cadillo *et al.* 2005; Zeb and Murkovic 2011).

It was pointed out in the present study that the low oxygen level in the storage chamber preserves the carotenoid concentration after 12 months of storage at CA with 1.0 kPa O<sub>2</sub>. This fact is due to the low oxygen concentration, since when yerba mate stored in CA with 3.0 kPa CO<sub>2</sub> and the oxygen partial pressure at 20.9 kPa, the higher oxygen concentration may have increased the oxidation in the yerba mate submitted to this treatment and decreased the carotenoids concentration. The main degradation pathway of dried vegetables are lipid oxidation, non-enzymatic darkness, chlorophyll and carotenoids oxidation (Streit *et al.* 2005; Campos *et al.* 2007). The results found suggest that yerba mate stored in a CA with 1.0 kPa O<sub>2</sub> brings more benefits to human health than those stored in the other conditions, since carotenoids can be effective antioxidants and maintain one's health (Woodall *et al.* 1997).

Antioxidant activity of the yerba mate increases and has a positive relationship with the total polyphenol concentration (Heck *et al.* 2008; Valerga *et al.* 2012). Among the total phenolic compounds, quercetin is the greatest contributor of the yerba mate leaves antioxidant activity (Valerga *et al.* 2012). Studies previously conducted have demonstrated that a significant increase in polyphenol concentration occurs with the process of "sapeco", that is, a heat treatment of the green leaves (Isolabella *et al.* 2010; Valerga *et al.* 2012). The present study demonstrates that the yerba mate stored in high carbon dioxide levels suffers an increase of phenolic compounds of the yerba mate after harvest. Perhaps, the increase in phenolic compounds is related to the formation of secondary compounds, since the polyphenol compounds are a type of secondary metabolites (Ross and Kasum 2002). Among these secondary metabolites are the phenolic acids, tannins, proteins, alkaloids.

Concerning the yerba mate color, previous reports already found a strong correlation between green color loss and total chlorophyll content loss (Prestes *et al.* 2014; Thewes *et al.* 2016). This can be observed at ambient and CA with 3.0 kPa CO<sub>2</sub>, where the lower hue angle was reached after three months storage and lower total chlorophyll concentration along all storage period.

## CONCLUSION

The O<sub>2</sub> reduction to 1.0 kPa maintains a greener color and a higher chlorophyll concentration after storage of yerba mate. The increase of carbon dioxide in the storage chamber increases the polyphenol concentration until six months of storage. Further studies should associate low oxygen and high carbon dioxide partial pressures on the yerba mate quality.

Chlorophyll concentration decreased over the storage, but the green color did not present the same behavior, demonstrating different pathways for chlorophyll degradation and yerba mate color degradation.

Cultivated yerba mate presented a higher storage potential due to its higher chlorophyll concentration.

The raw material obtained from São Mateus do Sul presented a higher chlorophyll concentration and greener color, which maintained a greater storage potential of this yerba mate.



## AUTHORS' CONTRIBUTION

Conceptualization: Thewes, F.R., Brackmann, A. and Prestes, S.L.C.; Methodology: Thewes, F.R., Prestes, S.L.C., Schultz, E.E., Ludwig, V., Wendt, L.M.; Writing – Review and Editing: Thewes, F.R., Brackmann, A., Prestes, S.L.C., Schultz, E.E., Ludwig, V., Wendt, L.M., Thewes, F.R., Berghetti, M.R.P.; Supervision: Brackmann, A.

## DATA AVAILABILITY STATEMENT

All dataset were generated or analyzed in the current study.

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