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A N   I N T E R N A T I O N A L   J O U R N A L

Assessment of Oxidation of Leaves of *Ilex paraguariensis* (St. Hil)

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ABSTRACT

This work aimed to study the oxidation of leaves of Ilex paraguariensis *St. Hil, in a process similar to the oxidation of* Camellia sinensis*, with the purpose of developing new products based on yerba maté. So far, no previous systematic study about the oxidation of yerba maté has been found elsewhere. A Plackett-Burman experimental design was used for the screening of the significant parameters that affected the oxidation. The oxidation was indirectly quantified by following the changes in the product color, water activity, and pH with the time of processing. The factors that most influenced the process were the age of the leaves and the relative humidity of the oxidation chamber. The differences between the treatments were more evident after 3, 6 12 and 24 h of oxidation. The results could contribute to the developing of new products based on* I. paraguariensis *St. Hil, increasing the profitability of cultivation of this native species.*

Key words: physicochemical parameters, yerba maté, leave, extract, experimental design

INTRODUCTION

The yerba maté (*Ilex paraguariensis* St. Hil.) is a native species from South America, used in the preparation of tealike beverages such as "chimarrão" in Southern Brazil, maté in Argentina and "tereré" in Paraguay (Costa, 1989; Mazuchowski and Rucker., 1997; Marx et al., 2003). The exploration of *I.paraguariensis* is an important agricultural activity in Brazil and Argentina due to socioeconomic issues. The yerba maté is able to stimulate the central nervous system due to its content of methylxanthine alkaloids, as caffeine, acids and phenolic compounds with antioxidant activity (Bassani and Campos, 1997; Saldanha et al., 1999; Deladino et al.; 2007; Bastos et al., 2007). Tealike infusions are worldwide popular and *Camellia sinensis* infusions (black tea, white tea, green tea, oolong tea) are the most consumed beverages after coffee. The *I. paraguariensis* and *C. sinensis* extracts present very similar active principles, although they belong to distinct species. The processing of *C. sinensis* for the production of different kinds of tea has been extensively studied (Muthumani and Kumar, 2007; Baruah and Mahanta, 2003; Obanda et al., 2004; Obanda et al., 2001; Bhattacharyya et. al., 2007). The basic process for tea production involves the drying and storage, followed by maceration and oxidation.

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This work aimed to study the oxidation of leaves of *Ilex paraguariensis* St. Hil in a process similar to the oxidation of *Camellia sinensis*, with the purpose of developing new products based on yerba maté. So far, no previous systematic study about the oxidation of yerba maté has been found elsewhere.

MATERIALS AND METHODS

Experimental design

The effect of five independent variables (temperature, relative humidity in the oxidation chamber, moisture and age of the leaves and presence/absence of light) on the oxidation of yerba maté leaves was evaluated by a Plackett-Burman experimental design (PB) with 12 runs and three repetitions in the central point (Rodrigues and Iemma, 2005). The investigated ranges are listed in Table 1. The monitored responses were instrumental color, water activity, and pH. The changes in color, water activity and pH were monitored with time. The variable time was not included in the experimental design, since its effect can mask the effect of other variables. Therefore, the statistical analysis was carried out at each single time.

Table 1 - Range of variables and levels investigated in the Plackett-Burman (PB12) experimental design.

Variable		-1	0^{a}	1
Temperature (ºC)	x_1	26	33	40
Relative humidity (%)	x_2	80	90	99
Age of the leaves[b](months)	x_3	1	6,5	12
Moisture of the leaves (%)	x_4	30-40	50-60	60-70
Fluorescent light (W)	x_5	Absent	150	200

[a]Central point.
[b]Age of the leaves were determined by following plant growth after the pruning.

Statistical analysis

Statistical analysis was performed with the results obtained with the Plackett-Burman experimental design using Statistica (StatSoft Inc., version 6.0). The statistical significance of the regression coefficients was 95%.

Sample collection and preparation

The yerba maté leaves used in this work were collected from an homogeneous cultivation under direct exposure to sunlight in the geographical coordinates 27º37'15" south, 52º 22' 47" west at 765 m altitude, after a growth pause (November, December). The leaves were dehydrated for 2 h using an oven with air circulation at 30°C and then were manually ground at room temperature (25°C) for 5 min (Fig.s 1 a), followed by ´´rolling´´ (Liang et al., 2007; Angayarkanni et al., 2002) (Fig. 1b). Figure 1 (c) shows the influence of process rooling on yerba mate leaves.

After rolling, the leaves were transferred to aluminum trays and submitted to the experimental conditions set by the experimental design. Samples were taken from the chamber at time 0, 3, 6, 12, 24 and 48h time intervals, subjected to drying and grinding for further physicochemical analysis.

Figure 1 - Dehydrated leaves (a).Rolling process (b).Leaves before rolling (up) and after rolling (below) (c).

Analytical determinations

Color

Instrumental color was determined using a colorimeter (Minolta CR400), with light source D65, expressing the results in the CIE Lab color space (L*a*b*). Equipment calibration was carried out using a standard white plate. Before measurements, the particle size of samples was standardized to be smaller than 2.35 mm. The samples were placed in a Petri dish (9 cm diameter) using the amount of sample necessary to cover completely the bottom of the dish. All the measurements were carried out in triplicates.

Water activity (a_w)

The determination of water activity was carried out with 1 g of sample in an Aqualab system (Decagon, Model CX-2) at 25°C. The equipment was previously calibrated with a sodium chloride solution with a_w 0.882, following the recommendations of the equipment's operations manual.

pH

For pH analysis, 10 g of ground leaves were mixed with 100 mL of deionized water. The pH of samples was determined with a digital pH meter (Digimed DMPH-2), calibrated with standard buffers.

RESULTS AND DISCUSSION

pH

Most of natural foods are acid, e.g., fruits that present pH between 3.0 and 4.5, and vegetables that present pH between 4.6 and 6.5. The pH of foods is one of the main factors that influence the growth, survival and/or destruction of native and contaminant microorganisms (Silva, 2000). The matrix of the PB12 experimental design with the real and coded factors with the respective pH of the samples after the different time of oxidation is shown in Table 2. Figure 2 showed that the major changes in pH occurred after 6, 12 and 24 h of oxidation. No significant (p<0.05) differences between the samples were observed at 0, 3 and 48h of processing. The pH of *I. paraguariensis* leaves varied from 5.2 to 6.4. This variation showed a strong effect on the activity of the enzymes in the leaves, since even a decimal variation in pH could cause a large variation in enzyme activity (Riegel, 2006). Nakasaki et.al. (2008), studying the oxidation of *Camelia sinensis*, observed that the pH value was maintained at a weakly acidic level of 5 to 6 throughout the fermentation.

Table 2 – Matrix of the experimental design (coded and real values) with responses in terms of pH.

Run	Factors					pH					
	X_1	X_2	X_3	X_4	X_5	0h	3h	6h	12h	24h	48h
1	1(40)	-1(80)	1(12)	-1(30-40)	-1	5.9	5.9	5.3	5.7	5.8	5.7
2	1(40)	1(99)	-1(1)	1(60-70)	-1	6.2	5.7	6.4	6.4	6.5	6.5
3	-1(26)	1(99)	1(12)	-1(30-40)	1	5.6	5.7	5.6	5.6	5.6	5.8
4	1(40)	-1(80)	1(12)	1(60-70)	-1	5.9	6.1	5.7	5.3	5.8	6.3
5	1(40)	1(99)	-1(1)	1(60-70)	1	6.1	6.5	6.6	6.5	6.4	5.7
6	1(40)	1(99)	1(12)	-1(30-40)	1	5.6	6.2	5.9	5.8	5.8	5.5
7	-1(26)	1(99)	1(12)	1(60-70)	-1	5.9	5.9	5.8	5.9	5.8	5.7
8	-1(26)	-1(80)	1(12)	1(60-70)	1	5.9	6.0	5.3	5.3	6.0	6.3
9	-1(26)	-1(80)	-1(1)	1(60-70)	1	6.4	6.3	5.7	5.7	6.0	6.5
10	1(40)	-1(80)	-1(1)	-1(30-40)	1	6.4	5.8	6.4	6.3	6.2	5.8
11	-1(26)	1(99)	-1(1)	-1(30-40)	-1	6.1	6.2	6.2	5.7	5.8	5.6
12	-1(26)	-1(80)	-1(1)	-1(30-40)	-1	6.0	6.0	6.4	6.5	6.3	5.8
13*	0(33)	0(90)	0(6,5)	0(55)	0	5.2	6.0	5.5	5.3	6.0	5.8
14*	0(33)	0(90)	0(6,5)	0(55)	0	5.6	5.7	6.0	5.9	6.2	6.3
15*	0(33)	0(90)	0(6,5)	0(55)	0	5.5	5.8	5.7	6.0	6.0	6.2

*Central point. X_1 = Temperature (°C), X_2 = Relative humidity of chamber (%), X_3 = Age of the leaves (months), X_4= moisture of the leaves (%), X_5 = light power (W).
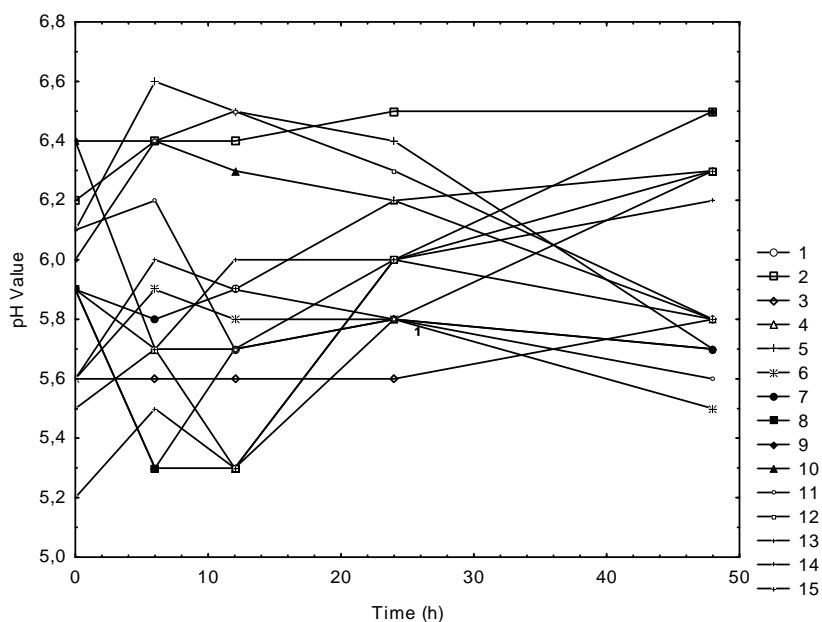
Figure 2 - Kinetic behavior of pH during oxidation in the different experimental conditions presented in Table 2.

The Pareto chart of effects for pH after 6 h of oxidation is presented in Figure 3. The relation between the pH and moisture of the leaves showed that younger leaves presented an increase in pH during oxidation, while the pH of mature leaves tended to decrease during the process. The behavior of pH at 6, 12, 24 and 48 h of processing was similar to that reported in Figure 3. pH is an important factor influencing enzyme activity (Lehninger, 2006) and can affect the oxidative enzymes in vegetables (peroxidases and polyphenoloxidases).

Studies with brussels sprouts found as pH 9 the optimal for the activity and stability of polyphenoloxidases (Nagai and Suzuki, 2003).The effect of pH on the activity of polyphenoloxidases has been extensively studied for fruits, juice and vegetables with preservative agent inhibiting the oxidation of color modifications and browning of explants in tissue culture (Niu et al., 2010; Lin et al., 2010; Wu et al., 2010; Dziki et aL., 2008; Dziki et al.,2007; Delicado et al., 2007; Mayer et al., 2006). However, the relationship between the pH and polyphenol oxidase activity in the oxidation process the leaves of yerba mate to be utilized in the form of tea is still a recent study.
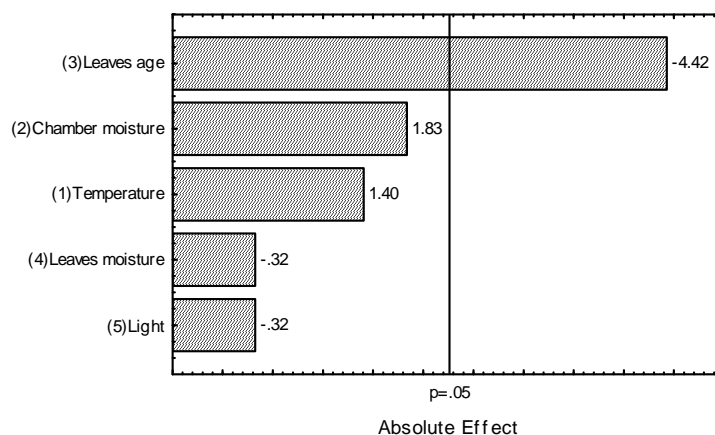

Figura 3 - Pareto chart of effects for pH after 6 h of oxidation.

Water activity (a_w)

The water activity varied between 0.623 and 0.996. The main effects of the variables studied was observed in the leaves subjected to 6 h of oxidation as shown in Figure 4. Similar results were observed to 24 and 48 h. The statistical analysis of the results of a_w at 3 and 12 h did not show statistically significant effects (p<0.05) on a_w. It was observed that both the relative humidity of the oxidation chamber and the initial moisture of the leaves positively influenced the water activity, as it would be expected. The magnitude of the effects of both factors were similar (2.58 and 2.43), indicating that they influenced the water activity in the same way. The other factors did not significantly influence this variable.

The relative humidity of the chamber affects the water activity of the leaves, since it is proportional to the partial pressure of water in the air, and the higher the difference in the partial pressure of water between the air and the leaves, the higher the transfer rate of water from the air to the leaves, increasing the water activity in the equilibrium (Ramallo and Albani, 2004).

The water activity directly influences the enzyme activity, and the decrease in a_w can reduce the action of enzymes (Court, 2006). Lipid oxidation is also affected by the a_w. When a_w varied between 0.2 and 0.8, the oxidation rate was low when compared to the rates that were obtained when a_w tended to 1. Some values found in the present work could be considered high and could affect the browning of the leave particles.

The water activity also affects the microbial growth. Most of bacteria and fungi cannot grow in water activity lower than 0.91 and 0.80, respectively (Fennema, 2000). However, microbiological analyses of the samples studied in the present work did now show any growth of such microorganisms during oxidation (date not showed).
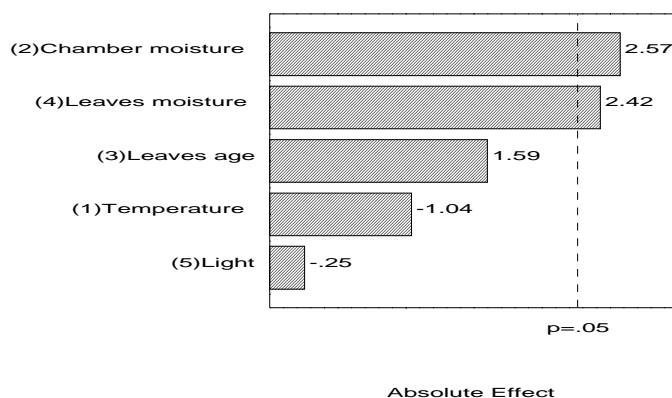

Figure 4 - Pareto chart of effects for a_w after 6 h of oxidation.

Color (L*a*b*)

The effects of the studied factors on color, estimated by the parameters L*, a* and b* may be observed in Figures 5, 6 and 7, respectively, in the oxidation times of the 3 h, 6h and 48h. The parameter L* represented the lightness of the sample, in a scale from 0 (black) to 100 (white). The samples from the experimental runs of the PB 12 design presented a variation from 12 to 39, considering all the experimental conditions and time of oxidation. black tea infusions for instance may show L* from 13 to 47 (Liang et al., 2002). The color of the oxidized leaves was directly related to the quality of the final product, since this parameter changed according to the content of pigments, nitrogen and phenolic compounds.

The age of the leaves showed a significant negative effect (p<0.05) on the color of the samples after 3 and 6 h of oxidation. The negative effect meant that younger leaves were darker than the older leaves, after the above mentioned time of oxidation. The relative humidity of the chamber also influenced negatively the L* (Fig. 5).
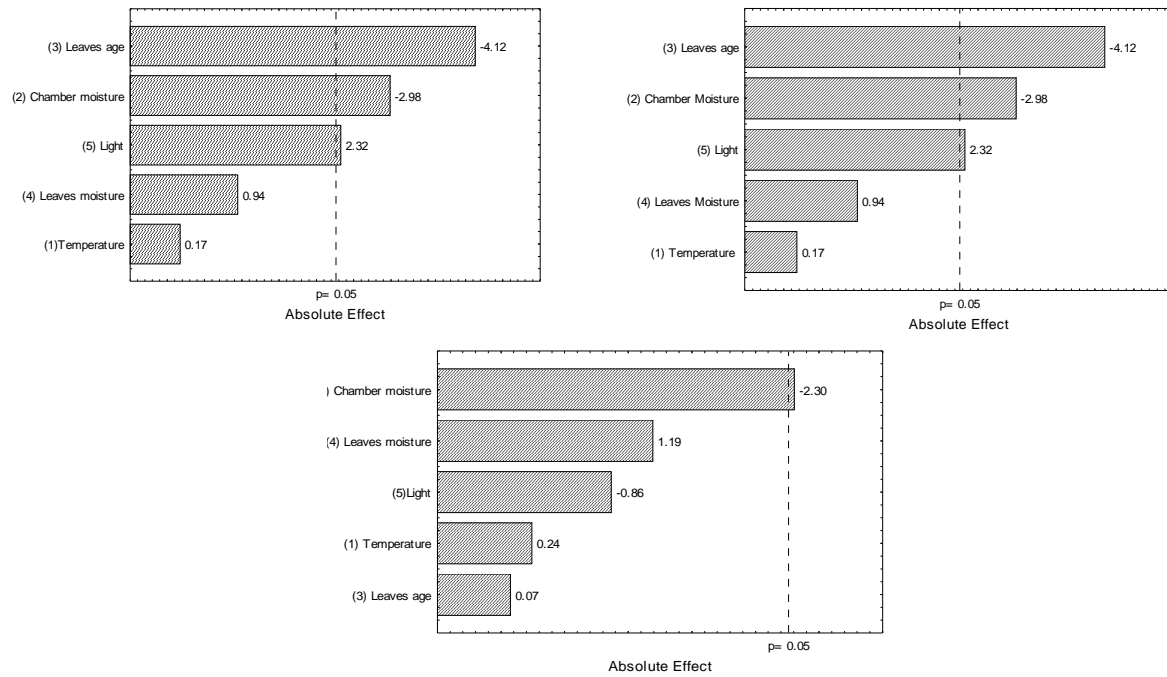
Figura 5 - Pareto chart of effects for color parameter L* after (a) 3 h, (b) 6 h and (c) 48 h of oxidation.
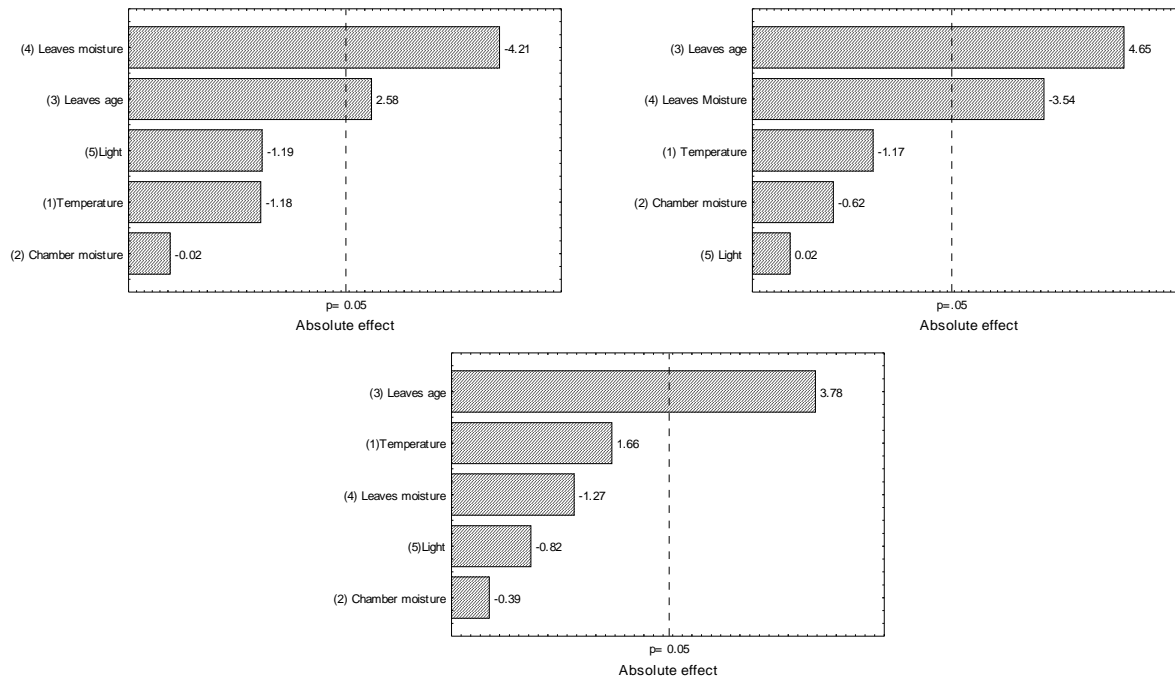

Figure 6 - Pareto chart of effects for color parameter a* after (a) 3 h, (b) 6 h and (c) 48 h of oxidation.
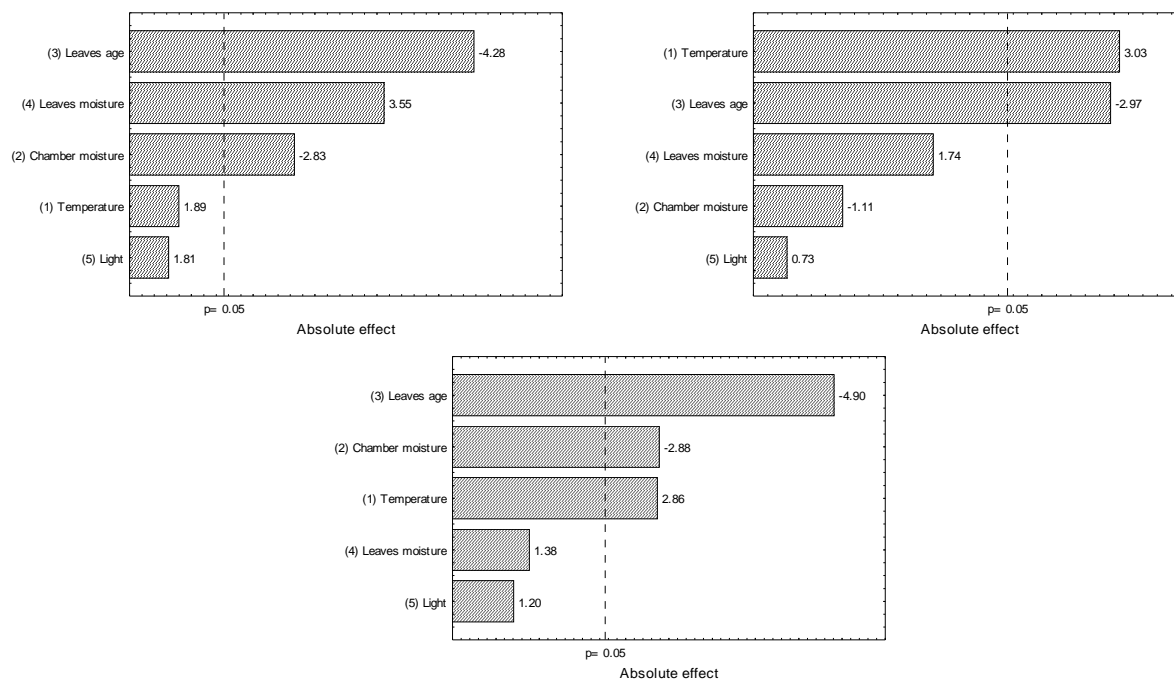
Figure 7 - Pareto chart of effects for color parameter b* after (a) 3 h, (b) 6 h and (c) 12 h of oxidation.

Sinnecker et al (2002) showed that the parameter L* for soybeans presented negative correlation with the concentration of total chlorophyll in the grains stored at 25°C. The increase in the L* followed the loss of chlorophyll. The loss of chlorophyll might be due to different factors such as pH, enzymes, oxygen, temperature and light. Furthermore, the reaction of degradation of this pigment is determined by the water activity of the sample (Malheiros, 2007).

Obanda et al (2001) showed that the lightness (L*) of black tea was affected by the temperature rise and the time of oxidation. These authors showed that the lightness decreased as the oxidation develops. Muthumani and Kumar (2007) showed that the L* for *Camellia sinensis* was affected by the content of theoflavines, while the content of taurobigines were responsible for changes in the color of the teas and alcoholic beverages.

In the present study, as the oxidation progressed, the L* parameter was less little affected by the studied factors, indicating that the browning of the leaves was achieving its maximum. The color parameter a* indicated the variation from green to red. This parameter ranged from -0.110 (12 h) to -8.080 (3 h) for the conditions studied in the PB12 design. The negative values denoted that the green color was predominant in the ground leaves, except after 12 h. When this parameter approached zero, the less saturated was the green color of the sample (Lawless; Heymann, 1999).

The age of the leaves presented a significant positive effect (p<0.05) for the three oxidation times (3 h, 6h and 48 h), showing that the green color was lost in mature leaves (Fig. 6). The mature leaves presented a higher content of chlorophyll, and thus a more reddish color associated with the degradation of the pigment, when compared to the younger leaves.

The parameter b* denoted the variation of color between yellow and blue. Most of samples analyzed in the present work showed positive b*, characteristic of yellow color. The factors that significantly influenced b* varied as the oxidation advanced. The older age of the leaves, however, always showed a negative effect on b*, indicating that the mature leaves were less yellow. The older the leaves, the smaller was the rate of degradation of chlorophyll during oxidation, decreasing the intensity of yellow color of the product.

The temperature of incubation positively influenced the yellow color of the leaves after 6 h of incubation (Fig. 7). This suggested that higher temperatures led to a higher degradation of chlorophyll, turning the leaves more yellow.

CONCLUSIONS

The factors that most influenced the oxidation of yerba maté were the relative humidity of the incubation chamber and the age of the leaves. However, these variables did not influence the pH and the color parameter a*. The statistically relevant results were obtained at 3, 6, 12 and 24 h of incubation. Young leaves presented higher enzymatic browning than mature leaves.

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