Chlorophyll Stability in Yerba Maté Leaves in Controlled Atmospheres

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ABSTRACT

The objective of this research was to investigate the stability of chlorophyll in yerba maté leaves in controlled atmospheres of CO_2/air mixtures and different water activities at 25°C.Two levels of water activity were selected corresponding to saturated salt solutions of LiCl (a_w=0.113) and $MgCl_2$(a_w=0.330) and three levels of CO_2/air mixtures (0/100,20/80 and 40/60). The chlorophyll content was evaluated using a liquid chromatography HPLC technique. Experimental values varied between 2.16 and 0.61 mg/g of dry matter. For each sample, 5 determination were made during 58 days. Experimental values were fitted to an equation describing a first order reaction. In all cases, the agreement was good with $P<3\ 10^{-3}$. The initial concentration of chlorophyll dropped, in average, to 30.5% after 58 days. However, after comparing the velocity constants, no differences were found between them.

Key words: *yerba maté, chlorophyll stability, controlled atmosphere*

INTRODUCTION

When selecting yerba maté (*Ilex paraguarensis* St. Hil.), consumers judge color as one parameter of quality. Brazil and Argentina, as main yerba maté consumers, have different preferences for color. Argentinean consumers generally life a light olive green color, while Brazilians prefer a bright green one.

Yerba maté green color is due to chlorophyll presence. Its intensity has a direct relationship with the chlorophyll concentration. During processing, every stage is responsible for decreasing the level of chlorophyll.

In general, the yerba maté processing has four stages: *heat treatment, drying, grinding, and seasoning*. During the seasoning, yerba maté loses a high percentage of chlorophyll. Therefore, when the product is processed for the Brazilian market, the last stage is avoided.

Yerba maté packages for Brazilian market, containing between 5 and 8% of moisture (dry basis), are kept only three months on the supermarket shelves for selling. After that, packages are removed because the green color becomes lighter. Consequently, finding a method to preserve the green color in yerba maté is an issue of great importance for this market. Temperature, water activity (a_w), and inert gases are the main factors that influence chlorophyll degradation.

Temperature: Temperature influence in chlorophyll degradation was widely studied for different products. Researches were conducted by Lajollo *et al*. (1971) in spinach, Watada *et al*. (1987) in beans, spinach and pepper, Canjura *et al.* (1991) in spinach puree, and Steet and Tong (1996) in peas. They have shown that the chlorophyll degradation kinetics follows a pseudo-first order process. They also found that temperature behavior can be described by using the Arrhenius equation. Gomez Vara *et al*. (1979) studied chlorophyll stability in yerba

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maté at 60 and 80°C and Montiel and Avanza (1996) at 6, 30 and 50°C. All researchers reported a strong influence of the temperature on degradation rate. Gomez Vara *et al*. (1979) found that at 60°C, the initial concentration dropped to 25% in 11 days. Montiel and Avanza (1996) concluded that at 30°C chlorophyll content decreased to 60% from the initial concentration in 40 days.

Although temperature influences chlorophyll degradation, it is not economically feasible to control this factor. In addition, this would produce a change in the traditional commercialization approach.

Water activity (a_w): Lajollo *et al*. (1971) have studied how water activity and inert gases affect the chlorophyll degradation in spinach. They found that water activity starting from 0.52 begins to influence heavily the degradation process. Lower values of water activity produce less important effects. These authors concluded that by an a_w less than 0.52, in 120 days and a temperature of 37°C the chlorophyll concentration dropped only to a 70% of the initial content. However, in yerba maté the chlorophyll undergoes higher degradation. This was shown by Gomez Vara et al. (1979) who reported that for yerba maté naturally seasoned for less water activity and temperature (room humidity and temperature conditions) chlorophyll concentration was reduced to 50% of the initial value in 120 days.

Other researches were conducted for high values of a_w in fruits and fresh vegetables. Watada *et al*. (1987) studied the chlorophyll degradation of peas, pepper and spinach at 10 and 20° C, by packaging them in polyethylene bags and without packaging them. They found that without packaging, spinach lost more chlorophyll than the packaged one. However, the other two vegetables have not followed the same behavior.

Controlled atmospheres: Baarsdseth and Von Elbe (1989) studied the chlorophyll degradation in fresh spinach by using two atmospheres, one of pure air and the other a mixture of ethylene and air, during 5 days. They reported no difference between both atmospheres. Bastrash *et al.* (1993), using different mixtures of CO_2 and O_2 at 4°C, found that fresh broccoli retained more chlorophyll in a controlled atmosphere of 6% of CO_2, and 2 % of O_2 than for other combinations of both gases or the control—composed of 20% of O_2. These authors also reported an increase in chlorophyll concentration of packaged broccoli in atmospheres of CO_2/O_2 at 5°C after 144 hours. The control sample, air exposed, underwent a decrease in chlorophyll concentration of 60% from the initial content.

Working with an atmosphere rich in N_2, Lajollo *et al*. (1971) studied the influence of this inert gas on chlorophyll degradation velocity in spinach. At low a_w (0.11 and <0.01) and 55° C, they found that chlorophyll maintained a 56.2 and 58.8%, respectively of the initial value in 51 days. These values were higher than those found for yerba maté.

The objective of this research was to investigate the stability of chlorophyll in yerba maté leaves in controlled atmosphere of CO_2/air mixtures (0/100,20/80 and 40/60) and a_w (0.113 and 0.330) at 25°C.

MATERIALS AND METHODS

Yerba maté samples for Brazilian market were obtained from a local producer. Leaves were separated from twigs and ground until the particles passed a mesh number 80.

Moisture content determination
The moisture content was determined by heating the samples in an oven at 105° C during 6 h (IRAM 20503).

Chlorophyll content analysis
The chlorophyll content was evaluated using liquid chromatography HPLC technique

(Schwartz and Lorenzo, 1991). Ten ml of an acetone-water solution (80:20 in volume) was added to 1 g of the sample, and then introduced in an ultrasonic bath at 25°C for 5 min. Afterwards, a 3-ml solution was taken with a syringe, and filtered using a syringe filter with a pore size of 0.22 µm. The filtered solution was then injected into the chromatograph (Shimadzu LC6A, integrator CR3A, and detector Linear Uvis 200 with column C18, 5 Micron L 250mm x 4.6 mm). Assay conditions were: Mobile phase, ethyl acetate: methanol: water (55: 35: 10 in volume), Flow: 2 ml/min and detector: UV-Vis at 435 nm, 0.01 AUFS.

Experimental Method

Experiments were carried out by introducing the samples in modified atmospheres at three levels of CO_2 (0, 20 and 40%), and two levels of water activity (0.113 and 0.330).

The water activity was controlled using saturated solutions of LiCl (a_w=0.113), and $MgCl_2$ (a_w=0.330). The CO_2 level was maintained by injecting periodically mixtures of CO_2-air. Table 1 shows the combinations of the different levels of CO_2 concentration and water activity.

Samples were introduced in special containers, which had a grille to reserve a space in the bottom to locate the saturated solutions. The containers also had an orifice to allow the injection of the CO_2-air mixtures. The CO_2-air mixtures were obtained by mixing previously measured flows of CO_2 and air, in a mix chamber. The mixes were introduced into the containers by using a tube, whose diameter was the half of the container's orifice in order to remove the initial air. The amount injected was six times the container's volume.

The samples, previously prepared, were placed over the grille. Analysis of chlorophyll was made initially and then every 15 days. The containers were maintained at 25° C during the whole experience.

Table 1. Experimental design for studding chlorophyll stability in controlled atmospheres.

Sample	% CO_2	a_w
1	0	0.113
2	20	0.113
3	40	0.113
4	0	0.330
5	20	0.330
6	40	0.330

Statistical Analysis

The chlorophyll degradation was fitted to an equation describing a first order reaction:

$$\ln\left(\frac{C}{C_0}\right) = -k\,t \qquad (1)$$

Where,

C= Chlorophyll concentration at time t [mg/g]

C_0= Initial chlorophyll concentration [mg/g]

k= constant of velocity reaction [$days^{-1}$]

t= time [days]

RESULTS AND DISCUSSION

Moisture Content

After each sampling, the moisture content was determined, in duplicate, for each sample. For samples 1, 2, and 3, the moisture content varied between 4.67 and 4.82. For samples 4, 5 and 6, it varied between 7.29 and 8.13. The moisture content is expressed % dry basis.

Chlorophyll Content

Table 2 shows the total chlorophyll content (A + B) as a function of time. Experimental results were fitted to Equation 1 using linear regression. In all cases the fitting was significant with $P<3.10^{-3}$ and 3.10^{-5}. Figure 1 presents the predicted and experimental relationship between chlorophyll concentration (expressed as $\ln(C/C_0)$) and time for sample 1.

Table 2. Chlorophyll contents (mg/g dry basis) as a function of time.

	Sample					
Time (days)	1	2	3	4	5	6
0	2.16	2.16	2.16	2.16	2.16	2.16
14	1.62	1.57	1.53	1.87	1.68	1.79
28	1.31	1.45	1.14	1.35	1.26	1.15
43	1.01	0.98	0.85	0.88	0.89	0.89
58	0.63	0.72	0.61	0.64	0.71	0.64

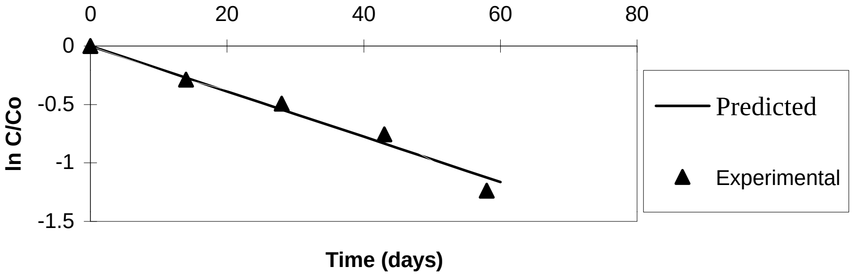

Figure 1. Experimental and predicted (obtained from Equation $\ln\frac{C}{C_0} = -1.94.10^{-2}t$) chlorophyll concentration vs. time for sample 1.

In Figure 2, the expected curves for all trials are plotted. In addition, Table 3 presents the specific velocity reaction (day^{-1}) and the confidence limits at 95%.

From Table 3, no significant difference among the constants of specific velocity reaction were observed, since the confident limits at 95% overlapped.

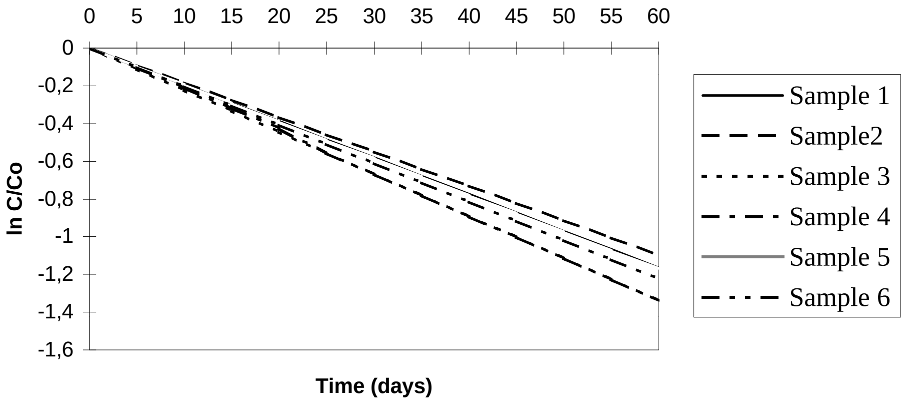

Figure 2. Predicted chlorophyll concentration vs. time, according to Equation 1, for samples 1 to 6.

Table 3. Specific velocity reaction (day^{-1}), and confidence limits at 95%.

Sample	K ± CL (day^{-1})
1	$1.94.10^{-2} \pm 0.58.10^{-2}$
2	$1.83.10^{-2} \pm 0.54.10^{-2}$
3	$2.23.10^{-2} \pm 0.17.10^{-2}$
4	$1.90.10^{-2} \pm 0.75.10^{-2}$
5	$1.95.10^{-2} \pm 0.21.10^{-2}$
6	$2.04.10^{-2} \pm 0.48.10^{-2}$

It was seen that the chlorophyll content after 58 days dropped to 30.5% with respect to the initial concentration, which was lower than that one found by Gomez Vara *et al*. (1979) (although they did not control temperature). Montiel and Avanza (1996) reported 60% chlorophyll content after 40 days at 30° C (the moisture content was not specified). The specific velocity constants obtained by the present research were higher than those reported by these authors.

RESUMO

O objetivo deste trabalho foi pesquisar a estabilidade da clorofila em folhas de erva mate em misturas atmosféricas controladas de CO2/ar e diferentes atividades de vapor de água a 25ºC. Dois níveis de atividade de vapor de água foram selecionadas, correspondendo a soluçoes saturadas de LiCl (a_w=0.113) e MgCl2 (a_w=0.330) e três níveis de misturas CO_2/ar (0/100,20/80 e 40/60). O conteúdo de clorofila foi avaliado usando a técnica de cromatografia líqüida HPLC. Os valores experimentais variaram entre 2.16 e 0.61 mg/g de matéria seca. Para cada amostra foram realizadas 5 determinaçoes durante 58 dias. Os valores experimentais foram ajustados para uma eqüação descrevendo uma reação de primeiro ordem. Em todos os casos houve boa concordância P < 3 10^{-3}. A concentração inicial de clorofila ficou reduzida em média um 30.5% depois de 58 dias. Porém, depois da comparação das constantes de velocidade, não foram achadas diferenças entre elas.

CONCLUSIONS

Studies of chlorophyll degradation in yerba maté under controlled atmospheres were carried our by controlling CO_2 level and water activity.

The relative amounts of CO_2/air used in the experiment were 40/60, 20/80 and 0/100. The water activity was adjusted at 0.113 and 0.330.

The chlorophyll concentrations were fitted to an equation describing a first order reaction. In all cases the fitting was significant with $P<3.10^{-3}$ and 3.10^{-5}. The initial concentration of chlorophyll dropped, in average, to 30.5% after 58 days. However, after comparing statistically the velocity constants, significant differences among them were not found. Therefore, we conclude that the chlorophyll's degradation velocity is not affected by the relations of CO2/air and water activity at the tested levels.

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